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22 Role of adenylosuccinate lyase in purine biosynthesis and interconversion: Molecular biology and genetic defects

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Abstract

Adenylosuccinate lyase catalyses two steps in the synthesis of purine nucleotides leading to non-hydrolytic cleavage of succinyl groups to yield fumarate: the conversion of 5-aminoimidazole-4-(N-succinylocarboxamide) ribonucleotide (SAICAR) into 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) along the de novo pathway and the formation of adenosine monophosphate (AMP) from adenylosuccinate (S-AMP) in the conversion of inosine monophosphate into adenine nucleotides. In addition to its role in purine biosynthesis, the enzyme is involved, together with AMP deaminase and S-AMP synthetase, in the purine nucleotide cycle.

The reaction mechanism involves a general base catalyst, removing a proton from the succinyl group of the substrate, acting in concert with a general acid catalyst that protonates the amino group left on

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AICAR or AMP. The enzyme shows four active-site clefts, located on the boundaries between three subunits of the tetramer, which contain two highly-conserved histidine residues and a glutamate-histidine relay that are involved in catalysis.

Human adenylosuccinate lyase gene is located in chromosome 22 and spans over 23 kb with 13 exons of 146 to 4536 bp. Two adenylosuccinate lyase mRNA isoforms, which are produced by an alternative splicing of exon 12, have been identified in human tissues. Exon 12 deletion results in the translation of a protein missing 59 amino acids, which is completely inactive.

The deficiency of adenylosuccinate lyase causes variable degrees of psychomotor retardation, often accompanied by epileptic seizures and/or autistic features. The existence of genetic heterogeneity for the adenylosuccinate lyase deficiency could account for variability of the clinical presentation. The defect is characterised by the appearance of succinylpurines in cerebrospinal fluid and urine. Deficiency of purine nucleotides, toxic effects, and impairment of energy metabolism are potential mechanisms of cerebral damage.

1. Introduction

Adenylosuccinate lyase (adenylosuccinase, ADSL, EC 4.3.2.2) catalyses the nonhydrolytic cleavage of the C-N bond linking the succinate moiety to the nucleotide part of the substrate to yield fumarate. The enzyme is involved in two distinct points of purine biosynthesis. The first reaction is the cleavage of 5-aminoimidazole-4-(N-succinylocarboxamide) ribonucleotide (SAICAR) to produce 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) and fumarate along the *de novo* pathway. The second reaction is the formation of AMP and fumarate from adenylosuccinate (S-AMP) in the conversion of IMP into adenine nucleotides (Fig. 1).

Both reactions are freely reversible. A value of 2.3 mM has been reported for the equilibrium constant of the reaction involving SAICAR at 37°C and pH 7.2 [1]. As far as S-AMP is concerned, values around 6.8 mM have been obtained by direct measurement of the equilibrium constant at 25 - 35°C and pH 7.0 [2, 3], while Haldane relationships from the steady state analysis of the enzymatic reaction gave values of 7.3 - 9.8 mM at 25°C and pH 7.8 [4].

The stereochemistry of the reactions was investigated by Miller and Buchanan [5], using SAICAR and S-AMP in which the *erythro* hydrogen on the β-carbon of the succinate moiety was replaced with ^3H. They showed that the stereospecificity of the substrate cleavage with respect to both the α- and β-carbon atoms of the aspartate moiety was identical with that exhibited in the addition of ammonia and water to fumarate by aspartase and fumarase, respectively. Since the mechanism of these reactions occurs via a *trans*-addition [6], they suggested that the cleavage of SAICAR and S-AMP involves the abstraction of the *erythro* proton of the β-carbon of the succinate moiety, followed by the elimination of fumarate and the protonation of the nitrogen on the nucleotide residues.

The conclusion that both SAICAR and S-AMP are cleaved by the same enzyme is based on the observation (reviewed in reference [7]) that (a) the ratio of activities with both substrates remains the same along all purification procedures of the enzyme, (b) both substrates exhibit mutual competitive inhibition, and (c) mutants lacking of S-AMP

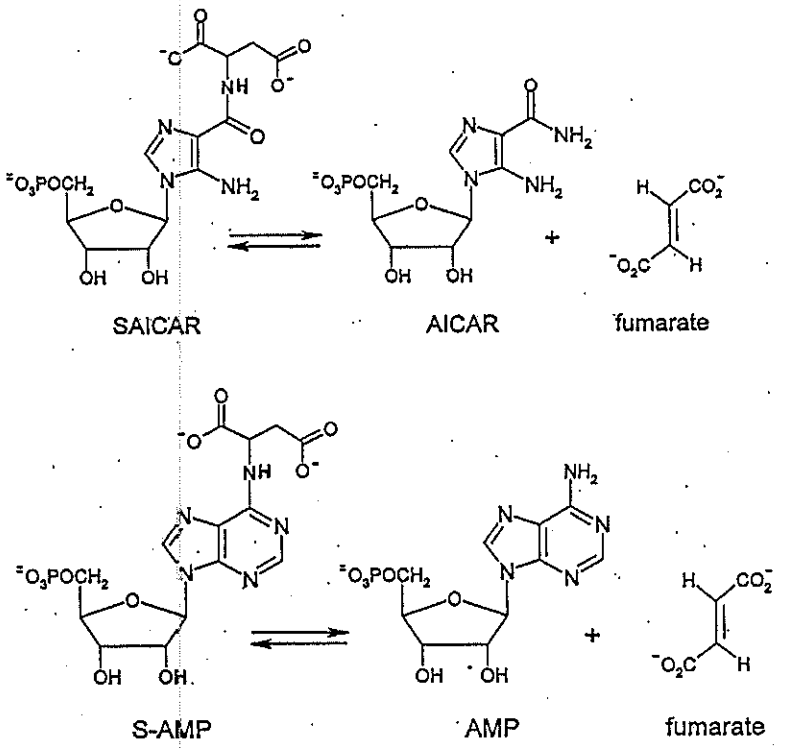

Figure 1. Adenylosuccinate lyase catalysed reactions in *de novo* synthesis and interconversion of purine nucleotides.

2. Enzyme structure and function

Adenylosuccinate lyase activity was found in nearly all organisms and tissues examined [8-16]. Enzyme activity is present in cultured mammalian cells [17-21], including fibroblasts and lymphoblasts [22]. In humans, adenylosuccinate lyase activity was measured in erythrocytes, granulocytes, lymphocytes, liver, kidney, and muscle [23, 24]. The question of whether there are many isoenzymes of adenylosuccinate lyase in vertebrate tissues still is not resolved [23, 25]. Isoelectrophocusing of rat muscle adenylosuccinate lyase showed three isomeric forms in similar amounts that underwent no further characterisation up to now [26]. Characterisation of the cDNA encoding murine adenylosuccinate lyase revealed only a single predominant gene [27]. An abundant mRNA of approximately 1.7 kb and two minor messages of 1.2 and 3.0 kb were identified from chicken liver [20]. Expression of cDNA clones in an epidermoid carcinoma and two colon carcinoma revealed two mRNAs of 1.8 and 2.5 kb [28]. As far as the enzyme from human samples is concerned, two mRNA isoforms resulting by alternative exon 12 skipping have been identified in all the tissues studied [29], as it will be discussed below. No indication of tissues-specific isoenzymes have been obtained by

Adenylosuccinate lyase from bacteria [31, 32], yeast [2, 4, 5], and *Artemia* embryos [33] has been purified to homogeneity and characterised. The enzyme from mammalian tissues is particularly unstable and has resisted attempts at extensive purification. Adenylosuccinate lyase has been purified 10,000-fold from human erythrocyte lysates [34] and to apparent homogeneity from rat skeletal muscle [35]. Recombinant adenylosuccinate lyase of chicken [20], mouse [27] and human [36] has been purified to homogeneity from engineered *Escherichia coli*.

Adenylosuccinate lyase from most species and tissues [32, 34, 35, 37, 38] has a molecular weight of about 200,000 and is composed of four subunits. The enzyme from *Artemia* embryos exhibits molecular forms of 200 kDa at pH 8.7, larger than 500 kDa at pH 6.5, and an unique form of 200 kDa, regardless of the pH, in the presence of 1 M NaCl [33]. The enzyme from *Neurospora crassa* exhibits predominant molecular forms with sedimentation coefficient of 10 S (that corresponds to about 200 kDa) at pH 5.2, 4 S at pH > 7, and 2.8 S at pH 3, which may be interpreted as hexameric, tetrameric and monomeric structures, respectively [31].

The amino acid sequences of adenylosuccinate lyase from up to 44 sources have been determined to date. Most of the data refer to enzymes from *Bacillus subtilis* [39], *Escherichia coli* [40], *Methanococcus jannaschii* [41], *Thermotoga maritima* [37], *Pyrobaculum aerophilum* [38], chicken [20], mouse [27], and human [29, 42]. The human monomeric subunit contains 484 amino acids and shows 95% identity to murine enzyme, 85% identity to chicken enzyme and 25% identity plus 18% similarity to *Bacillus subtilis* enzyme. Adenylosuccinate lyase also bears sequence homology to a group of enzymes that generate fumarate from different substrates, namely argininosuccinate lyase, aspartase, and class II fumarase (Fig. 2). Moreover, it displays sequence similarity with δ-crystallin, the major structural component of the lens of most birds and reptiles that likely evolved from argininosuccinate lyase by gene sharing and still retains its enzymatic activity [43]. Sequence comparison shows that an 11-amino acid span (from Gly^{261} to Pro^{271} in the sequence of *Bacillus subtilis*) is a region of high homology among these proteins and constitutes the "signature sequence" of the fumarase superfamily of enzymes.

The first crystal structure of an adenylosuccinate lyase (from *Thermotoga maritima*) was published in 2000 [37] and is consistent with four active sites. Adenylosuccinate lyase monomer contains three structural domains arranged in an elongated manner, which can easily be distinguished (Fig. 3). Domain **I** and domain **III** form the two lobes at each end of the dumbbell-like structure. Domain **II** contains five long α-helices connecting domain **I** and domain **III**. The association of the monomers to form a tetrameric structure with D_2 symmetry mainly involves hydrophobic interactions through domain **II** of each subunit. Subunit A has a head-to-head arrangement with subunit B and a head-to-tail arrangement with subunit C and D. The 20 α-helices form a bundle tethering the tetramer, while the active sites locate at the four corners of the structure and are formed by residues from three subunits. Differences in the overall structure have been noticed among enzymes from different sources. One unique feature of adenylosuccinate lyase from the hyperthermophilic archaebacterium *Pyrobaculum aerophilum* is the presence of three intra-chain disulfide bonds, which very likely increases its stability to extreme environmental conditions [38]. The first two disulfide bonds, Cys37-Cys50 and Cys49-Cys87, appear to tether sequentially helix 3 of domain I

Region 1

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B. subtilis ADSL :  89 His-Tyr-Gly-Leu-Thr-Ser-Thr-Asp-Val-Val-Asp-Thr-Ala-Leu-Ser
   human ADSL : 107 His-Leu-Gly-Ala-Thr-Ser-Cys-Tyr-Val-Gly-Asp-Asn-Thr-Asp-Leu
    human AOS : 108 His-Thr-Gly-Arg-Ser-Arg-Asn-Asp-Gln-Val-Val-Thr-Asp-Leu-Arg
duck δ-crystallin : 108 His-Thr-Gly-Arg-Ser-Arg-Asn-Asp-Gln-Val-Val-Thr-Asp-Leu-Lys
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Region 2

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B. subtilis ADSL : 137 Met-Gly-Arg-Thr-His-Gly-Val-His-Ala-Glu-Pro-Thr-Thr-Phe-Gly
   human ADSL : 155 Leu-Gly-Phe-Thr-His-Phe-Gln-Pro-Ala-Gln-Leu-Thr-Thr-Val-Gly
    human AOS : 156 Pro-Gly-Tyr-Thr-His-Leu-Gln-Arg-Ala-Gln-Pro-Ile-Arg-Trp-Ser
duck δ-crystallin : 156 Pro-Gly-Tyr-Thr-His-Leu-Gln-Lys-Ala-Gln-Pro-Ile-Arg-Trp-Ser
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Region 3

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B. subtilis ADSL : 261 Gly-Ser-Ser-Ala-Met-Pro-His-Lys-Arg-Asn-Pro-Ile-Gly-Ser-Glu
   human ADSL : 288 Gly-Ser-Ser-Ala-Met-Pro-Tyr-Lys-Arg-Asn-Pro-Met-Arg-Ser-Glu
    human AOS : 280 Gly-Ser-Ser-Leu-Met-Pro-Gln-Lys-Lys-Asn-Pro-Asp-Ser-Leu-Glu
duck δ-crystallin : 280 Gly-Ser-Ser-Leu-Met-Pro-Gln-Lys-Lys-Asn-Pro-Asp-Ser-Leu-Glu
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Figure 2. Amino acid sequence of three highly conserved regions in the fumarase superfamily. The sequences refer to adenylosuccinate lyase (ADSL), argininosuccinate lyase (AOS), and δ-crystallin. His^{141} of *B. subtilis* ADSL is conserved across the superfamily. In a side-directed mutagenesis study on duck δ-crystallin [44] in which the corresponding histidine (i.e. His^{162}) was converted to asparagine, the protein completely lost enzymatic activity. The region from Gly^{261} to Pro^{271} (in the sequence of *B. subtilis* ADSL) constitute the "signature sequence" of the fumarase superfamily and most of the residues, including Lys^{268} and Glu^{275}, are conserved in almost all these enzymes. Pro^{271} is conserved in most but not all ADSL. Positions on human ADSL cDNA are according the corrected sequence reported by Kmoch et al. [29]; initial reports [42] showed a sequence 25 amino acids shorter at the N-terminus because of misinterpretation of the initiation codon.

to the remaining secondary structure units of that domain, while the third disulfide bond, Cys167-Cys403, tethers the last residue of the protein in the C-terminal extension of domain **III** to helix 8 of domain **II**.

Adenylosuccinate lyase is capable of efficiently cleaving a variety of analogues. Monoanions (Cl^-, F^-, I^-, NO_3^-) and dianions (PO_4^{2-}, SO_4^{2-}) activate the enzyme at low concentration under V_{max} conditions, but four of them, namely I^-, NO_3^-, PO_4^{2-}, and SO_4^{2-}, inhibit enzyme activity at concentrations higher than 25 - 200 mM [35]. The cleavage of SAICAR and S-AMP displays hyperbolic kinetics [4, 36]. Initial rate and product inhibition patterns and equilibrium kinetics indicate that all nucleotide substrates and products bind to the free enzyme form, while fumarate is the first product released. Because of the similarities between the kinetic constants and the alternative substrate inhibition pattern displayed by SAICAR and AMP [36, 45], it seems highly likely that the enzyme has a single active site for binding either substrates. Further support for an essentially ordered mechanism of the reaction is provided by the observation [46] that AMP decreases the reactivity of the enzyme to *p*-chloromercuriphenyl sulfonate, whereas fumarate has no effect unless AMP is also present. An alternative path, in which

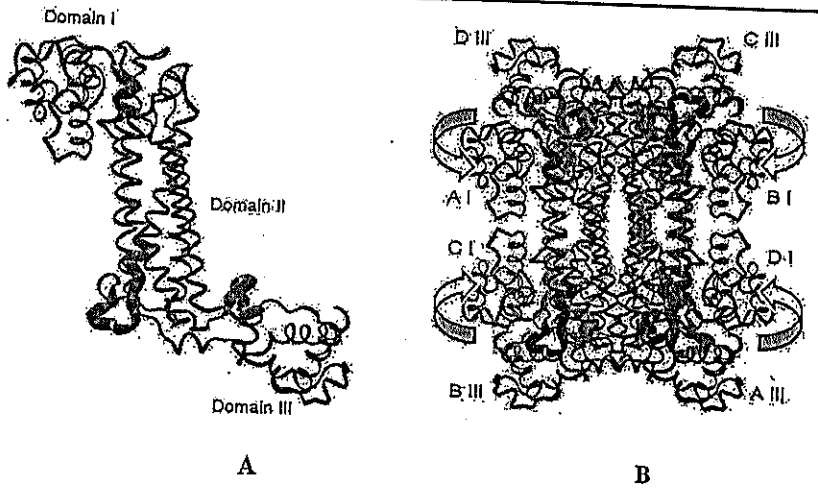

Figure 3. Ribbon structure of adenylosuccinate lyase from *B. subtilis*. (A) Diagram of subunit A. (B) Subunit association. Arrows indicate the active site clefts. Modified from Lee et al. [3].

exchange at saturating levels of fumarate and S-AMP, but this exchange is less than one-tenth the rate of fumarate/S-AMP exchange [4].

It has been suggested [4] that AMP enables fumarate to be attached by inducing a conformational change in the enzyme by which the necessary binding groups are brought into position in the active center. The failure of free enzyme to bind fumarate at kinetically significant concentrations is supported by failure of dicarboxylic acid analogues (fumarate, malate, aspartate, N-acetylaspartate, succinate, mesaconate, thiomalate) to inhibit the enzyme. Because the only enzyme form in which the dicarboxylate-binding site is available to a significant extent is the E-AMP complex, this enzyme-substrate form might well be able to distinguish between fumarate and nonplanar dicarboxylate that contain groups which would be expected to collide with amino group of AMP.

The small degree of inhibition by ATP or adenosine [4] indicates that the monophosphate group is important in the binding of substrate to the enzyme. By contrast, the integrity of the ribose ring is not necessary for substrate binding. The nucleotide analogs S-AMP 2',3'-acyclic dialdehyde and S-AMP 2',3'-acyclic dialcohol undergo enzymatic cleavage as well as the natural substrates, while S-AMP 2',3'-acyclic (N,N-ethyl)diamine is a weaker competitive inhibitor that is not subjected to catalysis [47]. Adenylosuccinate lyase is also able to convert 2',3'-dideoxyadenylosuccinate [48, 49], arabinosyladenylosuccinate [50] and xylosyladenylosuccinate [51] into the respective AMP derivatives, although the enzyme activity toward the 2',3'-dideoxy analog is only 1.85% of that toward S-AMP.

The differences in the association constants for each pair of nucleotide substrates and products have been used to calculate the binding free energy (ΔG = 1.2 kcal/mol) contributed by the succinate moiety of each substrate, which suggests that the binding is most likely accomplished through the carboxylate groups by means of one or two hydrogen bonds [36]. Replacement of the aspartate moiety with various amino acid

yields analogs without measurable activity as substrates, which behave as competitive inhibitors with respect to S-AMP and/or SAICAR in the standard assay system. Analogues containing monocarboxylic acids on the 6-amino group of AMP are very weak inhibitors [52]. Alanosine derivatives (K_i = 1.3-1.5 μM), *erythro*-β-fluoroaspartate derivatives (K_i = 0.1-1.0 μM), and *threo*-β-fluoroaspartate derivatives (K_i = 15-140 nM) are stronger inhibitors of the enzyme [35, 53]. Condensation of DL-β-phosphonoalanine on the purine nucleotide ring yields adenylophosphonopropionate, the most potent of these inhibitors (K_i = 22-65 nM) in which the β-carboxy group of adenylosuccinate is replaced by a phosphonate group [52]. Comparison of the inhibition constants of pairs of substrate analogues differing only by substitution of a phosphonate for a carboxyl group shows that the enzyme has a much higher affinity for phosphonate than for β-carboxyl. Substitution of the dianionic alkyl phosphonate group with the monoanionic alkyl sulfonate group and analysis of the pH dependence of the inhibition by the phosphonate analogues gave further indication that the dianion phosphonate group contribute to the binding. On the whole, these data suggest that the strong interaction of adenylosuccinate lyase with adenylophosphonopropionate is due to the presence of two positive charges in the β-carboxyl binding domain of the enzyme. It has been hypothesised [52] the two positive charges at the β-carboxyl binding site would facilitate proton abstraction from the β carbon of adenylosuccinate. Interaction of these charges with a phosphonate dianion would be much stronger than with the carboxylate monoanion, but would not facilitate proton abstraction from the β carbon.

It has been suggested [54] that the cleavage of the carbon-nitrogen is facilitated by an electron deficiency that already exists on the departing nitrogen atom. The catalytic mechanism would involve a general base catalyst, removing a proton from the succinyl group of the substrate, acting in concert with a general acid catalyst that protonates the amino group left on AICAR or AMP (Fig. 4). Support for this model has been provided by detailed kinetic study of the argininosuccinate lyase, which catalyses the β-elimination of fumarate from argininosuccinate, another example of a carbon-nitrogen lyase in which the departing nitrogen atom has a "built-in" electron deficiency [55]. As far as adenylosuccinate lyase is concerned, the pH-dependence of V_{max} and V_{max}/K_m has provided evidence that general acid/base catalysis may be involved in the reaction mechanism. A bell-shaped V_{max}/K_m *vs* pH profile revealed the presence of a group with pK ≈ 6.4 that must be deprotonated and a group with pK ≈ 8.2 that must protonated for binding of substrate to the enzyme [35]. This profile is essentially the same as that obtained for the argininosuccinate lyase reaction [55].

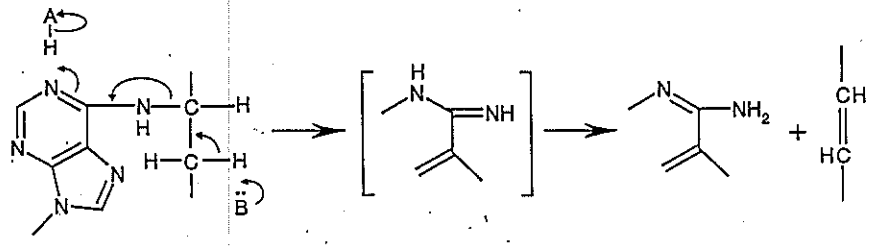

Figure 4. Hypothetical concerted catalytic mechanism of adenylosuccinate lyase. A and B

Identification of critical residues in the active site of adenylosuccinate lyase has been achieved by studying the crystal structure and the kinetic properties of mutants prepared by site-directed mutagenesis [3, 37, 38, 56-58]. His^{141} and His^{68} (the numbers refer to the enzyme sequence from *Bacillus subtilis*) are positioned to serve as general base and acid, respectively. These residues are located on the opposite sides of the monomeric subunit, so that each subunit provides His^{141} and His^{68} to two active sites, and each active site contains the two histidines from different subunits. His^{141} is at the conjunction of domains II and III, projecting out of the loop, and can be modified by affinity labeling with 6-(4-bromo-2,3-dioxobutyl)thioadenosine 5'-monophosphate [43]. The ε-N of His^{141} is only 2.78 Å from the β-H of the succinyl moiety of the substrate, while the δ-N of His^{141} is separated from δ-O of Glu^{275} by 2.85 Å, a distance sufficient for hydrogen bonding and for Glu^{275} to influence the orientation of His^{141}. His^{68} is in domain I and located 3.20 Å from N1 or 6-NH_2 of the adenine ring. Asn^{270}, Lys^{268}, and His^{89} contribute to substrate binding. Asn^{270} potentially interacts with the carboxylate group, His^{89} interact with the ribose hydroxyls, while the positively charged Lys^{268} undergoes significant electrostatic attraction by the phosphoryl oxygen of the substrate. His^{68} and His^{89} belong to the same subunits, while Lys^{268}, Asn^{270}, and Glu^{275} come from a third distinct subunit (Fig. 5)

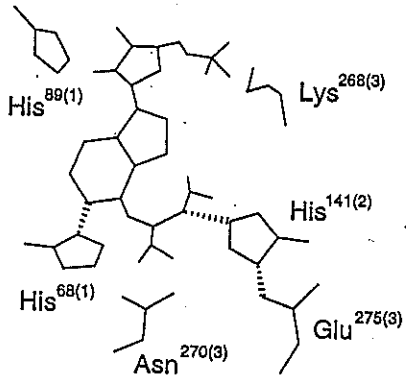

Figure 5. Schematic representation of the active-site region of adenylosuccinate lyase from *B. subtilis*.

3. Molecular biology of the gene

Human adenylosuccinate lyase gene was assigned to chromosome 22 by hybridising human fibroblasts with Chinese hamster auxotrophs lacking of adenylosuccinate lyase activity under selective conditions in which retention of the complementing gene on the human chromosome was necessary for survival [17]. Gene assignment was confirmed by Southern blot analysis with a DNA probe that had been isolated from a human fetal brain library and previously mapped to chromosome 22. Using both a somatic cell hybrid mapping panel and fluorescence in situ hybridization, adenylosuccinate lyase gene was localised to 22q13.1-q13.2 [18], while Southern blotting techniques using somatic cell

[19]. Hybridisation of sheep white blood cells with hamster auxotrophs mapped sheep adenylosuccinate lyase on chromosome 12 [59].

Using low stringency hybridation conditions with the avian cDNA serving as a probe [20], cDNA of human liver adenylosuccinate lyase was cloned and its sequence of 1377 nucleotides was deduced [42]. However, the high degree of identity of the nucleotide upstream sequence on the cDNAs cloned from both sources with the mouse sequence and the correction from C to A of the third nucleotide of the cDNA sequence revealed an error in the starting point in the gene sequence and an alternative ATG located in-frame, 75 nucleotides from the originally reported initiation codon [29]. The open reading frame thus comprises 1452 nucleotides instead of 1377 nucleotides and encodes a protein that is 25 amino acids longer at the N-terminus. Expression of the full-length enzyme in engineered *Escherichia coli* gave rise to a soluble stable protein, while the truncated enzyme was insoluble and inactive under standard experimental conditions. The kinetic parameters of the full-length enzyme were identical to those of the native enzyme isolated from human erythrocyte and rat muscle, and of the truncated enzyme kept soluble under specific conditions [36], suggesting that the 25 amino acid N-terminal sequence was probably not essential for the catalytic activity, but important for the structural stability of the protein.

The genomic organization of human adenylosuccinate lyase gene showed identity to that of the murine gene (Table I). The murine gene is about 27 kb long and contains 13 exons that vary in the size from 45 to 204 bp [27], while the human gene spans over 23 kb with 13 exons of 146 to 4536 bp [29]. Two adenylosuccinate lyase mRNA isoforms, which are produced by an alternative splicing of exon 12, have been identified in human tissues (brain, heart, kidney, liver, lung, pancreas, placenta, and skeletal muscle). Exon 12 deletion results in the translation of a protein missing 59 amino acids (from Gln^{397} to Gln^{456}) that is completely inactive. The functional relevance of the spliced isoform remains to be elucidated. Mixed tetramers with spliced and full-sized subunits could account for the different enzyme activities in various tissues.

Table I. Genomic organization of human adenylosuccinate lyase. Position and length of exons and introns.

Exon	Position in cDNA	Size (bp)	Intron	Size (bp)
1	1-208	208	1	3115
2	209-412	204	2	3038
3	413-457	45	3	1130
4	458-537	80	4	4536
5	538-709	172	5	224
6	710-756	47	6	1095
7	757-847	91	7	780
8	848-917	70	8	146
9	918-1065	148	9	1345
10	1066-1156	91	10	1204
11	1157-1246	90	11	514
12	1247-1423	177	12	1379

The promoter region [60, 61] has features typical of a house-keeping gene, i.e. high G + C content, high CpG nucleotides, and no TATA and CAAT boxes. Several potential binding sites for nuclear factors have been found in the 5' flanking region of the adenylosuccinate lyase gene. The -49T→C mutation provokes a reduction to 25% of wild-type control of promoter function, as evaluated by luciferase activity and mRNA level in transfection experiments. The mutation also affects the binding of nuclear respiratory factor 2 (NRF-2), a known activator of transcription, suggesting a role for NRF-2 in the gene regulation of the purine biosynthetic pathway [61]. No motifs suggesting tissue-specific expression regulation were found [29].

Molecular analysis of 31 affected families has revealed the existence of 30 different mutations in human adenylosuccinate lyase gene (Table II). Most of them are found in compound heterozygous form. The large majority are missense mutations; among these

Table II. List of mutations in human adenylosuccinate lyase

Base Change	Exon	Amino Acid Change	Domain	Homozygote patients	Compound heterozygotes	References
3A→T	1	M1L	I		1 with R374W	*
7C→T	1	A2V	I		1 with S395R	[62, 63]
10C→T	1	A3V	I		1 with R337X	[29]
78A→T	1	M26L	I		1 with R426H	[62, 63]
216A→G	1	I72V	I		1 with K246E	[62]
300C→G	2	P100A	I		1 with D422Y	[30]
342T→C	2	Y114H	II		2 with R190Q	[29]
423C→T	4	R141W	II		1 with Δ 206–218	[62, 63]
571G→A	5	R190Q	II		2 with Y114H 1 with K246E	[29] [62]
582C→T	5	R194C	II		1 with D268N 1 with R396C	[29] *
620C→A	5	Δ 206-218	II		1 with R141W	[62, 63]
738A→G	7	K246E	II		1 with R190Q 1 with I72V	[62] [62]
804G→A	8	D268N	II		1 with R194C	[29]
909C→T	9	R303C	II	2		[62, 63]
933C→G	9	L311V	II		1 with R396H	[67]
955C→T	9	P318L	II		1 with -49T→C	[61]
1011C→T	9	R337X	II		1 with A3V	[29]
1092G→A	10	V364M	II		2 with R452P	[67]
1122C→T	11	R374W	III		1 with M1L	*
1187C→A	11	S395R	III		1 with A2V	[62, 63]
1188C→T	11	R396C	III		1 with R194C	*
1189G→A	11	R396H	III		1 with L311V	[67]
1266G→T	12	D422Y	III		1 with P100A	[30]
1269C→G	12	L423V	III		1 with R426H	*
1279G→A	12	R426H	III	11	1 with M26L 1 with D430N 2 with T450S 1 with -49T→C 1 with L423V	[29, 62, 63] [62, 63] [29] [63] [61] *
1290G→A	12	D430N	III		1 with R426H	[29]
1314T→C	12	S438P	III	4		[42]
1341T→C	12	S447P	III		1 with -49T→C	[61]
1351C→G	12	T450S	III		2 with R426H	[63]
1357G→C	12	R452P	III		2 with V364M	[67]

the predominant mutation, R426H, is found in 13 families. A splicing error [62] and a nonsense mutation [29] have also been found. Hallmark of the defective enzymes is the marked accumulation of succinyladenosine and SAICA riboside, the dephosphorylated derivatives of the two substrates of adenylosuccinate lyase, in the biological fluids. Diminished levels of enzyme activity have been attributed to loss of catalytic activity or to protein instability, presumably followed by degradation.

Expression of mutant enzymes in *Escherichia coli* [29, 63] showed that A2V, A3V, R141W, R303C, and S395R substitutions give rise to thermostable proteins. A2V and A3V mutations caused negligible loss in enzyme activity, in line with the observation (discussed above) that the N-terminal part of the enzyme is not involved in catalysis. R141W, R303C, and S395R mutations affected enzyme activity with S-AMP to a markedly larger extent than that with SAICAR. The R141W mutation is peculiar because of its proximity to His^{159} which behaves as general base in the catalysis. R303C is the mutation which is located most closely to the charge relay pair Glu^{302}-His^{159}. Ser^{395} is located near the conjunction of domains II and III and should play a critical role in the enzyme function, since S395R mutation, when associated with A2V mutation, led to a very severe clinical presentation with early death. It is conceivable that mutations cause structural changes leading to a different handling of the two substrates. Indeed, (1) contact amino acids for SAICAR and S-AMP are not exactly the same; (2) mutation may cause structural changes which modify the aperture of the catalytic cleft and thereby its accessibility for the substrates; (3) S-AMP molecule may be less flexible than SAICAR and, thus, more sensitive to structural changes of the catalytic site.

Thermolabile proteins have been obtained by expressing enzymes with M26L, R426H, S438P, and T450S mutations [42, 63]. Remarkable susceptibility to heat denaturation, with a T_m value of 39°C, is elicited by the S438P mutant enzyme that shows, on the other hand, kinetic profiles indistinguishable from the wild-type enzyme [36]. Most thermolabile proteins involve mutations in domain III. Intrinsic instability of the peripheral domain might explain why mutations in this region have deleterious effect. As discussed above, a strategically placed disulfide bridge severely limits the mobility of this region in the hyperthermophilic archaebacterium *Pyrobaculum aerophilum* and stabilises the enzyme structure [38].

The significance of protein destabilization is unclear in the case P100A/D422Y compound heterozygosis [30, 64]. Crude P100A/D422Y enzyme preparations from a patient with mental retardation showed kinetic constants very similar to those of the wild-type enzyme and a T_m value that was decreased by only 8°C (to 51°C, compared to 59°C of control samples). D422Y mutation involves a cluster of helices in domain III, on the outskirts of the tetrameric enzyme and far away from the active site, which is lost in the *Pyrobaculum aerophilum* enzyme and, thus, is not required for catalysis or protein integrity [38]. The helix cluster of domain III is present in the *Bacillus subtilis* enzyme, where it is a candidate for binding to glutamyl-tRNA synthetase (as it will be discussed in more detail below) and very likely serves as a recognition element for response to some environmental stimulus [38]. P100A substitution distorts the amino acid chain of domain I in the proximity of His^{86}, which behaves as general acid in the catalysis. Correct geometry of this region may be essential to preserve enzyme function, since the

101 in the human sequence) to the helix 3-helix 4 pair [38]. The hypothesis is also in line with the finding that expression of Y114H mutant in *Escherichia coli* gives rise to a completely inactive enzyme [29]. Distortion of amino acid chain by P100A substitution may expose Cys^{98} and Cys^{99} to oxidising agents. It has been shown [65] that P100A/D422Y enzyme was strongly inhibited by 4-hydroxy-2-nonenal, a major product of membrane peroxidation, which is belived to cause some of the tissue damage that occurs *in vivo* under conditions of oxidative stress [66]. Kinetic analysis was consistent with the view that 4-hydroxy-2-nonenal reacts with both the free enzyme and the enzyme-substrate complex (though with different affinity) by means of a mixed-type cooperative mechanism, leading to kinetically ineffective enzyme species. Inhibition of P100A/D422Y enzyme by 4-hydroxy-2-nonenal is slightly reversed by dithiothreitol suggesting a thio-ether linkage between 4-hydroxy-2-nonenal and the enzyme.

4. Metabolic role

Adenylosuccinate lyase is involved in two closely analogous sequences of reactions whereby the amino nitrogen atom of aspartate is incorporated into AICAR and AMP along the purine biosynthesis *de novo* and the nucleotide interconversion pathway, respectively (Fig. 6). The synthesis of AICAR occurs in two steps involving an initial condensation of 5-aminoimidazole-4-carboxylate ribonucleotide with aspartate to form SAICAR that is subsequently cleaved to AICAR and fumarate. The sequence requires ATP as energy source. The synthesis of AMP involves the condensation of IMP with aspartate to form S-AMP and the cleavage of S-AMP to AMP and fumarate. Energy for this reaction is derived from GTP [68]. A regulatory role for adenylosuccinate lyase in purine biosynthesis *de novo* has been suggested by the observation that prolonged starvation causes loss of adenylosuccinate lyase activity as well as a decrease in the rate

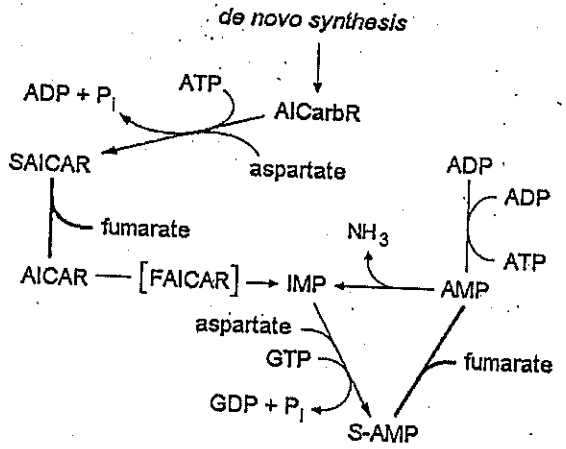

Figure 6. General scheme showing the role of adenylosuccinate lyase (bold lines) in purine metabolism. AICAR: 5-aminoimidazole-4-carboxamide ribonucleotide; AICarbR: 5-aminoimidazole-4-carboxylate ribonucleotide; FAICAR: 5-formamidoimidazole-4-carboxamide ribonucleotide; SAICAR: 5-aminoimidazole-4-(N-succinylocarboxamide) ribonucleotide

of AMP synthesis in rat liver [14]. The conversion of IMP to AMP is limited by the concentration of aspartate [69]. *In vitro* experiments with CHO-Ade I (adenylosuccinate lyase partially deficient) cell lines and human fibroblasts indicate that adenylosuccinate synthetase is the rate-limiting enzyme in the two-step conversion of IMP into AMP and that adenylosuccinate lyase deficiency is overcome by up-regulation of the synthetase activity [21].

Adenylosuccinate lyase also functions in ammonia production from aspartate *via* the purine nucleotide cycle that is formed by the coupled reactions catalysed by AMP deaminase (EC 3.5.4.6), adenylosuccinate synthetase (EC 6.3.4.4), and adenylosuccinate lyase. Each turn of the cycle results in the utilisation of one molecule of aspartate and GTP and the production of one molecule of GDP, fumarate, inorganic phosphate and ammonia. The operation of this cycle has been demonstrated in extracts of skeletal muscle [70], kidney [71], and brain [72]. The cycle also occurs in extracts of liver, where it is a source of free ammonia for ureogenesis [73], and may be involved in nitrogen elimination by ammonotelic teleosteans and invertebrates [74].

Several studies indicate that flux through purine nucleotide cycle is substantially increased during exercise of skeletal muscle. IMP that accumulates during the work is continuously recycled to AMP and back to IMP. Ammonia concentration increases in blood, but it cannot be accounted for by the stoichiometric conversion of ATP to IMP, suggesting that a substantial proportion of NH_3 released into the blood is derived from aspartate [75]. It has been shown [76] that citrate, isocitrate, and succinate concentrations increase following exercise, and that about 70% of this expansion of the pool of citric acid cycle is derived from aspartate *via* the adenylosuccinate lyase-catalysed reaction. Inhibition of adenylosuccinate lyase activity is conversely associated with rapid onset of fatigue [77].

Several mechanisms have been proposed to explain the role of the purine nucleotide cycle in muscle function. One hypothesis is that the increase in flux through the purine nucleotide cycle during exercise prevents AMP accumulation following ATP catabolism, and this in turn displaces the adenylate kinase reaction toward ATP formation [76]. A second hypothesis is that local production of ammonia acts in conjunction with the decrease in ATP to stimulate the activity of phosphofructokinase and enhance the rate of glycolysis [78]. A further enhance of glycolysis may be due to the increase in IMP level that contribute to the activation of glycogen phosphorylase [79]. Still another hypothesis is that the generation of fumarate, malate, and citrate from aspartate provides a mechanism by which intermediates of citric acid cycle could be replenished during a time of increased demand for ATP production [76]. Finally, accumulation of IMP may preserve the pool of purine nucleotides during exercise, and this reservoir of IMP may be used to replenish the ATP pool rapidly during recovery [80].

Another important metabolic role of adenylosuccinate lyase is exerted in the regulation of activity of AMP-activated protein kinase (EC 2.7.1.109). This kinase is activated by the reaction products of adenylosuccinate lyase, AICAR and AMP, both *via* direct allosteric activation [81-83] and *via* increased phosphorylation by an upstream kinase kinase [84]. Mammalian AMP-activated protein kinase phosphorylates a number of key enzymes, including 3-hydroxy-3-methylglutaryl-CoA reductase [85], acetyl-CoA carboxylase [86], malonyl CoA decarboxylase [87], glycogen synthase [81], and glycogen phosphorylase kinase [81]. When the energy state of the tissues is

compromised, AMP-activated protein kinase stimulates fatty acid oxidation by inhibiting acetyl CoA carboxylase and activating malonyl CoA decarboxylase [88]. This results in a decrease in the concentration of malonyl CoA, an inhibitor of carnitine palmitoyl transferase I, the enzyme that controls the transfer of long-chain fatty acyl CoA's into the mitochondria where they are oxidized. Activation of glycogen phosphorylase and inhibition of glycogen synthase increase glycogenolysis and anaerobic glucose oxidation [89].

Finally, it has been shown [90] that adenylosuccinate lyase from *Bacillus subtilis* interacts with glutamyl-tRNA synthetase, forming an heterologous enzyme-enzyme complex. Considering that tRNA synthetases and adenylosuccinate lyase share a common product (AMP), the interaction of these two proteins may coordinate purine metabolism and protein biosynthesis by regulating AMP formation. There are hints that at least another aminoacyl-tRNA synthetase interacts with adenylosuccinate lyase, since in *Escherichia coli* a polypeptide of 46 kDa was found to be copurified with glutaminyl-tRNA synthetase [90, 91], which has a close evolutionary linkage with several glutamyl-tRNA synthetases.

5. Medical aspects

Adenylosuccinate lyase deficiency (succinylpurinemic autism; McKusick 103050) is the first enzyme deficiency reported on the *de novo* pathway of purine synthesis in humans [23]. The inherited defect has been diagnosed up to now in approximately 60 patients from a number of ethnic groups. A great number of patients have been found in Belgium and The Netherlands, while the defect seems to be extremely rare in other countries, such as United Kingdom, Ireland, and Scandinavia. Clinical presentation is remarkably heterogeneous [92-94]. Seizures are observed in 60% of the cases, start to manifest themselves within the first month of life in 35% of the cases, and represent the first sign of the disease in many patients. The epileptic phenotype is characterised by erratic myoclonias, subtle partial seizures, generalised tonic-clonic seizures, spasms, and status epilepticus. Severe infantile epileptic encephalopathy with hypsarrhythmic pattern is the first manifestation of the enzyme defect in some patients. Electroencephalograms are abnormal in 85% of the cases, showing sometimes a pattern of burst suppression. Neurologically, symptoms include marked axial hypotonia with peripheral hypertonia, muscle wasting, fasciculations of the tongue or distal extremities, and strabismus. Delayed motor milestones are evident in all patients ranging from mild to severe. In the first years of life, growth retardation has been observed in 30% of the cases, mainly related to feeding problems. Although autistic features were initially reported as one of the clinical manifestations, subsequent findings showed that autism was present in only one third of patients. Some children show an unusual behavioural phenotype characterised by inability to fix, several stereotypies (hand washing movements, repetitive manupulation of toys, grimacing, clapping hands, rubbing feet, and inappropriate laughter), aggressive behaviour, temper tantrums, marked impulsivity, hyperactivity, short attention span, and hypersensitivity to noise and lights. Many patients show severe mental retardation, language delay, slurred pronunciation and limited vocabulary.

Four variants of adenylosuccinate lyase deficiency have been distinguished

with purely neurologic clinical picture characterised by severe psychomotor retardation, epilepsy, axial hypotonia with normal tendon reflexes, and impressive autistic features including absent or poor eye contact, stereotypies, tantrums, agitation, and auto-aggressivity. The further evolution is characterised by absent or minimal psychomotor development and persistence of the autistic behaviour, if present. Patients with type II adenylosuccinate lyase deficiency suffer only from slight to moderate psychomotor retardation, show a transient auditory and visual contact disturbance, but do not manifest epilepsy. Type III is similar to type I from the neurologic point of view. It is remarkable for severe growth failure, with muscle wasting starting between 1 and 2 years of age. Type IV is characterised by a clinical symptomatology between those of type I and type II: an intermediate degree of psychomotor development, minor epilepsy and stereotypies, aggressive behaviour, and normal eye contact.

Diagnosis is based on the presence in urine and cerebrospinal fluid of SAICA riboside and succinyladenosine [23], both normally undetectable with routine techniques, although succinyladenosine concentrations around 1 μM have been reported in control cerebrospinal fluids [95]. SAICA riboside can be detected by a modified Bratton-Marshall test [96], performed on fresh urine stored at -20°C, owing to the instability of the analyte at room temperature and alkaline pH. False positive results are recorded in patients who receive antibiotics, particularly sulfonamides, and/or antiepileptic drugs. Other methods for detecting succinylpurines require two-dimensional thin-layer chromatography with staining of imidazole compounds [97], silica thin-layer chromatography with liquid nitrogen-induced phosphorescence [98], determination of UV absorbance at 270 nm and 250 nm [99], and capillary electrophoresis [100]. High pressure chromatography with ultraviolet detection remains the method of choice to confirm diagnosis [23]. In profoundly retarded type I and type III patients, cerebrospinal fluid concentrations of both succinylpurines are 100 to 200 μM, and the succinyladenosine / SAICA riboside ratio is between 1 and 2 (Fig. 7). In mildly-retarded type II patients, the cerebrospinal fluid concentration of SAICA riboside is around 100 μM, but strikingly that of succinyladenosine is four- to fivefold higher. In patients with intermediate clinical symptomatology (type IV), concentrations of both succinyladenosine and SAICA riboside tend to be elevated, but the ratio of the compounds is between 1 and 2 [92].

Several methods have been described for the assay of adenylosuccinate lyase in human tissues. The activity of the enzyme with S-AMP can be readily measured by a dual wavelength spectrophotometric method based on the decrease in absorbance at 282 nm minus that at 320 nm [72]. A similar assay with SAICAR is an order of magnitude less sensitive and cannot be utilised with patients' biopsies. Radiochemical assay of adenylosuccinate lyase requires synthesis of ^{14}C-labelled fumarate and separation of the reaction products by thin-layer chromatography [101]. A rapid and accurate microassay of adenylosuccinate lyase by capillary electrophoresis has been reported [24]. Studies in patients with adenylosuccinate lyase deficiency show that the activity of the enzyme is lost to a different extent in various tissues, and is normal in others [23, 64, 102]. Enzymatic activity was markedly reduced in liver, kidney, and peripheral

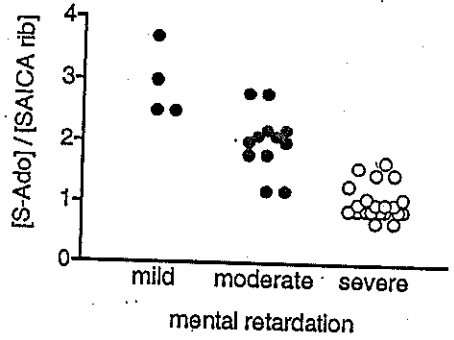

Figure 7. Adenylosuccinate lyase deficiency. Succinyladenosine (S-Ado) / SAICA riboside (SAICA rib) ratio as a function of patients' mental status.

The functional consequences of decreased adenylosuccinate lyase activity on the purine synthesis *de novo* and the conversion of IMP into AMP were assessed in fibroblast cultures by using labelled precursors [21, 103]. Paradoxically, incorporation of [^{14}C]formate into the purine nucleotides was found enhanced in adenylosuccinate lyase deficient cells as compared to control fibroblasts, indicating an increase of *de novo* synthesis. In fibroblats of type I patients, stimulation of *de novo* synthesis did not result in significant accumulation of labelled intermediates. When fibroblasts of type I patients were incubated with [^{14}C]hypoxanthine, the flux into adenine nucleotides and the steady-state level of labelled S-AMP were similar to those measured in control cells. By the contrast, in the case of type II patients' fibroblasts, small amounts of labelled SAICAR, SAICA riboside, S-AMP, and succinyladenosine accumulated upon addition of [^{14}C]formate, while a marked increase of labelled S-AMP and succinyladenosine was observed upon addition of [^{14}C]hypoxanthine. Flux of labelled hypoxanthine into adenine nuceotides was reduced by about 75%, but still present in fibroblasts of type II patients, indicating that the metabolic block was overcome at least in part by the increased steady-state level of S-AMP. Experiments with engineered adenylosuccinate lyase [29, 36, 63] indicate that most defective enzymes from type I patients are kinetically indistinguishable from the wild-type enzyme, but show great heat lability and increased sensitivity to denaturating agents. Enzymes of type II patients are thermostable proteins, but elicit impaired catalytic functions.

The potential mechanisms whereby adenylosuccinate lyase deficiency may provoke mental retardation include deficiency of purine nucleotides, toxic effects by derivatives of the enzyme substrates, and impairment of energy metabolism. Mutations leading to a total loss of adenylosuccinate lyase activity in all tissues are probably incompatible with life, because they would suppress *de novo* purine synthesis and render cells completely dependent on the purine salvage pathway. Current knowledge of the relative importance of *de novo* purine synthesis during development is very limited, and the relative importance of maternally supplied purines in embryogenesis is not well understood.

From the dual function of adenylosuccinate lyase, one would expect its deficiency to lead to decreased concentrations of purines, particularly adenine nucleotides. However, *in vivo* studies by means of ^{31}P magnetic

small decrease in the adenylate pool in patient's muscle at the end of recovery from ischemic work, whereas measurements of the concentration of adenine and guanine nucleotides in freeze-clamped liver, kidney, and muscle did not reveal significant changes from normal values [105].

Toxic effects by intracellular and/or extracellular derivatives of the substrates of the defective enzyme have been hypothesised, since S-AMP metabolism was impaired to a greater extent than SAICAR metabolism in patients with much less mental retardation and autistic behaviour [25]. This finding was interpreted by assuming that SAICA riboside is the offending compound and that succinyladenosine can protect from the toxic effects. Owing to the resemblance of both succinylpurines to adenosine and their accumulation in the cerebrospinal fluids of the patients, the possibility was explored that they might interfere with cerebral adenosine receptors and thereby with the numerous physiologic functions of adenosine. However, current data indicate that neither succinyladenosine nor SAICA riboside are able to interfere with binding and uptake of adenosine by crude membrane fractions of rat cerebral cortex [106], to modify the effect of adenosine on the transmission at glutamatergic synapses between the Schaffer collateral and commissural axons and the CA1 pyramidal neurons in rat brain, or to modify the response of single hippocampal neurons to application of glutamate, *N*-methyl-D-aspartate, or kainate by microionophoresis [107]. Significant degeneration of hippocampal pyramidal neurons has been observed only after reverse microdialysis with SAICA riboside over a period of 5 to 10 hours, while no sign of neuronal damage was noticed by injecting SAICA riboside in rat brain [108]. Dizocilpine, a noncompetitive blocker of the ion channels associated with the *N*-methyl-D-aspartate receptors, did not protect against SAICA riboside perfusion by microdialysis, suggesting that the damage was not due to the activation of these receptors as it was observed in a wide variety of neurotoxic insults. Succinyladenosine did not produce damage and did not protect against SAICA riboside damage.

Several *in vivo* experiments suggest that adenylosuccinate lyase deficiency may be responsible for the deterioration of cell bioenergetics, at least under conditions of metabolic stress. ^{31}P-Magnetic resonance spectroscopy of patient's calf muscle indicated that, during the rest, intracellular ATP and phosphocreatine were markedly reduced, whereas inorganic phosphate content was higher than in controls [104]. After ischemic exercise, phosphocreatine recovery behaved as a monoexponential process with a time constant significantly higher than those obtained with healthy volunteers. Moreover, at the end of recovery, ATP content in the patient was lower than at rest, whereas controls did not show any ATP change. Further evidence for an impaired response of the patient's metabolism was obtained by means of an intraveneous fructose load that was expected to induce metabolic stress by reducing intracellular ATP content and by increasing AMP deaminase activity, which in turn promotes purine catabolism [64]. Indeed, the increase in plasma Mg^{2+} upon fructose injection was significantly above control levels and could be taken as indirect evidence of intracellular ATP loss. ATP depletion could be also suggested by the increase in plasma K^+ and by the slow decrease of blood glucose concentration. Whereas the rise in plasma potassium was probably due to a transient impairment of ATP-dependent pumps, the fructose-induced hypoglycemia, exceptionally observed in healthy subjects, could be attributed to defective gluconeogenesis, that

As far as the brain metabolism is concerned, preliminary *in vivo* studies by ^{31}P magnetic resonance spectroscopy did not reveal significant changes in nucleotide phosphate content [110], while measurements by positron emission tomography indicated a markedly reduced uptake of 2-fluoro-2-deoxyglucose in the cortical areas of three patients with adenylosuccinate lyase deficiency, which suggested an impairment of glucose metabolism in brain [111]. The lack of the signal showed a regional distribution that differ from that of adenosine A_1 receptors and adenosine uptake sites. Direct inhibitory effect by SAICA riboside and succinyladenosine on the glycolysis was excluded by *in vitro* experiments showing that the succinylnucleosides did not alter glucose metabolism in isolated hepatocyte model [103].

Several therapeutic trials have been performed to reduce the detrimental effects of adenylosuccinate lyase deficiency. Two attempts have been made to decrease succinylpurine levels in biological fluids [105]. Allopurinol was administered at a dose of 33 mg/kg for 3 weeks because of its known inhibitory effect on the *de novo* purine synthesis. Sodium benzoate was given at the dose of 250 mg/kg for 4 weeks since it is known to decrease the availability of glycine, one of the precursors of the *de novo* pathway. Both treatments modified neither the cerebrospinal fluid and plasma concentrations of succinylpurines nor the urinary excretion of these compounds. With the aim of replenishing the adenine nucleotide pool without increasing the level of hypothetically toxic metabolites, some patients were treated for several months with oral supplements of adenine (100-300 mg/day) combined with allopurinol (60-240 mg/day), which avoided the oxidation of adenine into its poorly soluble nephrotoxic product, 2,8-dihydroxyadenine [102]. No clinical improvement or reduction of succinylpurine synthesis was recorded, with the exception of some acceleration of body growth in type III patients. A slight improvement of patients' behaviour was obtained by giving aminoimidazole carboxamide at the dose of 10 mg/kg for 10 days and of 20 mg/kg for 20 days [105]. Aminoimidazole carboxamide administration resulted in a twofold increase in the excretion of uric acid but did not modify the excretion of succinylpurines. Administration of 1.8 g/kg D-ribose with the aim to improve purine synthesis [112] actually caused hyperuricemia and hyperuricosuria in one patient, probably because of the stimulation of the synthesis of 5-phosphoribosyl-1-pyrophosphate, a key regulatory substrate in purine metabolism. Minor changes in succinylpurine levels were observed. D-Ribose supply was accompanied by a slight improvement in the patient's behaviour and a progressive reduction of seizure frequency, which increased dramatically on two attempts to withdraw the drug. Substitution of D-ribose with an equivalent amount of D-glucose did not result in an increase of seizure frequency. D-Ribose was given to the patient for about 2 years without complaints; afterward, new worsening of the symptom was observed. A slight improvement in patient's condition was obtained [113] by substituting D-ribose with uridine at the dose of 0.4 g/kg. The patient showed decreased seizure frequency and electroencephalographic abnormalities. Uridine had been considered to be superior to D-ribose for the treatment of behavioural abnormalities in patients with nucleotidase superactivity [114].

6. Conclusions

The study of purine metabolism, and particularly of its inborn errors in humans,

medicine. Inherited deficiencies behave as "experiments in nature" that can be utilised for analysing the role of the enzymes in the pathway. The discovery of adenylosuccinate lyase deficiency led to a revaluation the enzyme and prompted to a deep analysis of protein structure and/or gene organisation. Adenylosuccinate lyase deficiency is a prototype for the elucidation of the molecular basis of genetic heterogeneity in inborn errors and for the assessment of genotype-phenotype correlation. Since the symptoms are indistinguishable from autism in some of the cases, adenylosuccinate lyase deficiency provides one of the first examples of monogenic defect that causes behavioural dysfunction.

A reliable estimation of the incidence of adenylosuccinate lyase deficiency is not possible at present since specific purine analysis is only available in few laboratories, but the small number of patients detected up to now in not evidence that this is a rare disorder. Most of the patients have been found in Central Europe and Mediterranean countries, and in population of Gypsy origin. An Italian patient was discovered by screening less than 100 subjects with diagnosis of autism. A search for adenylosuccinate lyase deficiency should be included in the screening program of metabolic investigations, in all infants with unexplained neonatal seizures, severe infantile epileptic encephalopathy, developmental delay, hypotonia, and/or autistic features.

7. Electronic data-base information

Adenylosuccinate Lyase Mutations Database Home Page, http://www.icp.ucl.ac.be/adsldb/
European Molecular Biology Laboratory (EMBL) Nucleotide Sequence Database, http://www.ebi.ac.uk/embl/index.html (for the ADSL gene [accession number X65867])
GenBank, http://www.ncbi.nlm.nih.gov/Genbank (for the ADSL coding sequence [accession number AF106656])
Humam Gene Mutation Database, http://www.uwcm.ac.uk/uwcm/mg/hgmd0.html
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/omin/ (for ADSL [MIM 103050])
RCSB Protein Data Bank, http://www.rcsb.org/ (for the coordinates of *P. aerophilum* ADSL [accession number 1dof]; for the C^{α} coordinates of *B. subtilis* ADSL [accession number 1f10])

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