AMINOHYDROLASES ACTING ON ADENINE, ADENOSINE AND THEIR DERIVATIVES

Hana Pospisilova, Ivo Frebort

Department of Biochemistry, Faculty of Science, Palacky University, Slechtitelu 11, 783 71 Olomouc, Czech Republic
e-mail: h.pospisilova@upol.cz

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Background: Adenine and adenosine-acting aminohydrolases are important groups of enzymes responsible for the metabolic salvage of purine compounds. Several subclasses of these enzymes have been described and given current knowledge of the full genome sequences of many organisms, it is possible to identify genes encoding these enzymes and group them according to their primary structure.

Methods and Results: This article is a short overview of the enzymes classified as adenine and adenosine deaminase. It summarises knowledge of their occurrence, genetic basis and their catalytic and structural properties.

Conclusions: These enzymes are constitutive components of purine metabolism and their impairment may cause serious medical disorders. In humans, adenosine deaminase deficiency is linked to severe combined immunodeficiency and as such the enzyme has been approved for the first gene therapy trial. The role of these enzymes in plants is unclear, since the activity has not been detected in extracts and putative genes have not been yet cloned and analyzed. A literature search and amino acid identity comparison show that Ascomycetes contain only adenine deaminase, but not adenosine deaminase, despite the fact that corresponding genes are annotated in databases as the adenosine cleaving enzymes because they share the same conserved domain.

INTRODUCTION

Out of 44 enzymes systematically labelled as aminohydrolases in the Enzyme Commission, 25 are classified as deaminases. These enzymes control important pathways in the metabolism of the components of nucleic acids¹, Fig. 1A. Genetic deficiency in these pathways in humans results in serious diseases, such as severe combined immunodeficiency caused by diminished activity of adenosine deaminase. Six of the known deaminases are involved in the salvage of adenine and its derivatives and nitrogen catabolism. This review focuses on the enzymes adenine deaminase and adenosine deaminase and covers recent progress in understanding their genetic origin and biochemical properties.

1. ADENINE DEAMINASE

Adenine deaminase (alternatively also adenine aminohydrolase or adenase, ADE, EC 3.5.4.2) catalyzes the hydrolytic cleavage of adenine to produce hypoxanthine and ammonia (Fig. 1B). ADE genes have been found in prokaryotes and lower eukaryotes such as yeasts or protozoa. Until recently, genes coding eukaryotic adenine deaminase were unknown, but genetic and physiological evidence indicated that these enzymes are present in Ascomycetes. Surprisingly, when some putative fungal adenosine deaminases (ADA, EC 3.5.4.4) have been cloned and expressed in *E. coli*, they exhibited adenine deaminase activity². At the amino acid sequence level, bacterial adenine deaminases are more closely related to

allantoinases, ureases and dihydroorotases than to fungal enzymes². Of this enzyme class, a structure of bacterial ADE from *Enterococcus faecalis* has been the first and so far the only one to become available³ (in October 2006), Fig. 2A.

1.1. Substrates, activating compounds and inhibitors of adenine deaminase

Adenine deaminases have been detected in different microorganisms, such as *Pseudomonas synxantha*⁴, *Bacillus subtilis*⁵, *Leishmania donovani*⁶, *E. coli*⁷ and *Azotobacter vinelandii*⁸. Some of the enzymes were partially purified and characterized in terms of specific activity and K_m value. The enzymatic activity showed a maximum between pH 6 (*C. fasciculata*⁹) and pH 9 (*P. synxantha*¹⁰). Optimal temperature was found to be diverse, i.e. 40-45 °C for ADE from *P. synxantha*, but 60 °C (ref.⁷) for the *E. coli* enzyme. Genes coding for ADE from *E. coli*⁷, *S. cerevisiae*¹¹ and *B. subtilis*^{5, 12} have been cloned and overexpressed in modified *E. coli*.

The only natural substrate of ADE is adenine¹³, but the enzyme can also hydrolyze 6-halogenpurines^{4, 14} such as 6-chloropurine. Metal ions in optimum concentration are essential for the enzymatic activity of some enzymes, i.e. Mn^{2+} for the ADEs from *Bacillus subtilis*⁵ and *Escherichia coli*⁷. In contrast, many ions act as partial or full inhibitors of ADEs^{4, 7, 8} (Ca^{2+} , Cd^{2+} , Co^{2+} , Cu^{2+} , Fe^{2+} , Hg^{2+} , Ni^{2+} , Zn^{2+} and Mn^{2+}). Metal chelators, such as 1,10-phenanthroline¹⁰, *p*-chloromercuribenzoate⁴, mercuric acetate, mercuric chloride, iodoacetic acid, NaCN, NaF, and NaN_3 ⁴

Table 1. Comparison of amino acid identity of bacterial and fungal ADEs with some prokaryotic and eukaryotic ADAs. Data were obtained using ClustalW multiple sequence alignment followed by sequence identity matrix calculation using the BioEdit software 7.0.5.3⁶⁰.

Gene identification: **1.** cryptic ADE *Escherichia coli* K12 (yicP), GeneID: 948210, **2.** ADE *Methanobacterium thermoautotrophicum* strain 29, GeneID: 1474199, **3.** ADE *Schizosaccharomyces pombe* 727h, GeneID: 2540066, **4.** putative ADE *Candida albicans* SC5314, GeneID: 3643529, **5.** ADE *Saccharomyces cerevisiae*, GeneID: 855581, **6.** ADA *Homo sapiens*, GeneID: 100, **7.** ADA *Schizosaccharomyces pombe* 727h, GeneID: 2540165, **8.** ADA *Leishmania major* strain Friedlin, GeneID: 3684336, **9.** ADA *Aspergillus fumigatus* Af293, GeneID: 3513729, **10.** ADA *Mesorhizobium loti* MAFF303099, GeneID: 1226989, **11.** ADA *Escherichia coli* K12, GeneID: 945851.

	1. ADE, <i>E. coli</i>	2. ADE, <i>M. acetivorans</i>	3. ADE, <i>S. pombe</i>	4. ADE, <i>C. albicans</i>	5. ADE, <i>S. cerevisiae</i>	6. ADA, <i>H.sapiens</i>	7. ADA, <i>S. pombe</i>	8. ADA, <i>L. major</i>	9. ADA, <i>A. fumigatus</i>	10. ADA, <i>M. loli</i>	11. ADA, <i>E. coli</i>
1. ADE, <i>E. coli</i>	ID										
2. ADE, <i>M. acetivorans</i>	0,312	ID									
3. ADE, <i>S. pombe</i>	0,061	0,064	ID								
4. ADE, <i>C. albicans</i>	0,057	0,062	0,463	ID							
5. ADE, <i>S. cerevisiae</i>	0,049	0,05	0,459	0,588	ID						
6. ADA, <i>H.sapiens</i>	0,049	0,062	0,212	0,205	0,21	ID					
7. ADA, <i>S. pombe</i>	0,047	0,042	0,345	0,322	0,358	0,162	ID				
8. ADA, <i>L. major</i>	0,061	0,051	0,347	0,379	0,367	0,2	0,338	ID			
9. ADA, <i>A. fumigatus</i>	0,061	0,055	0,533	0,458	0,463	0,208	0,326	0,364	ID		
10. ADA, <i>M. loli</i>	0,04	0,05	0,24	0,221	0,235	0,218	0,226	0,236	0,251	ID	
11. ADA, <i>E. coli</i>	0,045	0,053	0,227	0,227	0,227	0,298	0,214	0,235	0,246	0,245	ID
	identity	0,81-0,99	0,60-0,80	0,45-0,59	0,31-0,44	0,20-0,30	0,00-0,19				

Table 2: Comparison of amino acid identity among prokaryotic and eukaryotic ADAs. Data were obtained using ClustalW multiple sequence alignment followed by sequence identity matrix calculation using the BioEdit software 7.0.5.3⁶⁰.

Gene identification: **1.** ADA *Homo sapiens*, GeneID: 100, **2.** ADA *Pan troglodytes*, GeneID: 741760, **3.** ADA *Bos taurus*, GeneID: 280712, **4.** ADA *Canis familiaris* isoform 1, GeneID: 477236, **5.** ADA *Mus musculus*, GeneID: 11486, **6.** ADA *Rattus norvegicus*, GeneID: 24165, **7.** ADA *Gallus gallus*, GeneID: 419194, **8.** ADA *Xenopus tropicalis*, GeneID: 496434, **9.** ADA *Danio rerio*, GeneID: 436919, **10.** ADA *Schizosaccharomyces pombe* 972h-, GeneID: 2540165, **11.** ADA *Leishmania major* strain Friedlin, GeneID: 3684336, **12.** ADA *Plasmodium berghei* strain ANKA, GeneID: 3425563, **13.** ADA *Aspergillus fumigatus* Af293, GeneID: 3513729, **14.** ADA *Escherichia coli* K12, GeneID: 945851, **15.** ADA *Vibrio cholerae* O1 biovar eltor str. N16961, GeneID: 2614914.

	1. ADA, <i>H. sapiens</i>	2. ADA, <i>P. troglodytes</i>	3. ADA, <i>B. taurus</i>	4. ADA, <i>C. familiaris</i>	5. ADA, <i>M. musculus</i>	6. ADA, <i>R. norvegicus</i>	7. ADA, <i>G. gallus</i>	8. ADA, <i>X. tropicalis</i>	9. ADA, <i>D. rerio</i>	10. ADA, <i>S. pombe</i>	11. ADA, <i>L. major</i>	12. ADA, <i>P. berghei</i>	13. ADA, <i>A. fumigatus</i>	14. ADA, <i>E. coli</i>	15. ADA, <i>V. cholerae</i>
1. ADA, <i>H. sapiens</i>	ID														
2. ADA, <i>P. troglodytes</i>	0.983	ID													
3. ADA, <i>B. taurus</i>	0.887	0.881	ID												
4. ADA, <i>C. familiaris</i>	0.856	0.856	0.856	ID											
5. ADA, <i>M. musculus</i>	0.807	0.807	0.826	0.79	ID										
6. ADA, <i>R. norvegicus</i>	0.829	0.823	0.837	0.79	0.94	ID									
7. ADA, <i>G. gallus</i>	0.646	0.646	0.66	0.649	0.636	0.652	ID								
8. ADA, <i>X. tropicalis</i>	0.695	0.692	0.703	0.675	0.708	0.708	0.719	ID							
9. ADA, <i>D. rerio</i>	0.634	0.629	0.629	0.626	0.63	0.622	0.64	0.726	ID						
10. ADA, <i>S. pombe</i>	0.167	0.167	0.189	0.177	0.174	0.177	0.196	0.132	0.161	ID					
11. ADA, <i>L. major</i>	0.2	0.197	0.192	0.192	0.206	0.209	0.202	0.204	0.201	0.349	ID				
12. ADA, <i>P. berghei</i>	0.217	0.22	0.223	0.215	0.226	0.234	0.235	0.227	0.217	0.174	0.156	ID			
13. ADA, <i>A. fumigatus</i>	0.207	0.209	0.223	0.202	0.228	0.234	0.24	0.225	0.201	0.329	0.356	0.219	ID		
14. ADA, <i>E. coli</i>	0.29	0.287	0.284	0.276	0.302	0.293	0.295	0.293	0.288	0.223	0.237	0.208	0.241	ID	
15. ADA, <i>V. cholerae</i>	0.276	0.276	0.276	0.26	0.29	0.279	0.3	0.301	0.271	0.205	0.239	0.213	0.232	0.698	ID
	identity	0.81-0.99	0.60-0.80	0.45-0.59	0.31-0.44	0.20-0.30	0.00-0.19								

Table 3. Comparison of amino acid identity between putative plant adenosine/AMP deaminases and the enzymes from other organisms containing the same conserved domain cd00443. Data were obtained using ClustalW multiple sequence alignment followed by sequence identity matrix calculation using the BioEdit software 7.0.5.⁶⁰

Gene identification: **1.** ADA/AMPD *Arabidopsis thaliana*, GeneID: 825826, **2.** ADA/AMPD *Oryza sativa* (japonica cultivar-group), GeneID: 4344179, **3.** ADA/AMPD *Homo sapiens* GeneID: 161823, **4.** ADA/AMPD *Bos taurus*, GeneID: 512667, **5.** ADA/AMPD *Danio rerio* GeneID: 619263, **6.** ADA/AMPD *Anopheles gambiae* str. PEST, GeneID: 1274840, **7.** ADA/AMPD *Ustilago maydis* 52l, GeneID: 3628643, **8.** ADA/AMPD *Gibberella zeae* PH-1 GeneID: 2784988, **9.** ADA/AMPD *Aspergillus nidulans* FGSC A4, GeneID: 2876437, **10.** ADA/AMPD *Aspergillus terreus* NIH2624, GeneID: 4355279, **11.** ADA/AMPD *Synechococcus elongatus* PCC 6301. GeneID: 3198705.

	<div> <div>1. ADA/AMPD, A. thaliana</div> <div>2. ADA/AMPD, O. sativa</div> <div>3. ADA/AMPD, H. sapiens</div> <div>4. ADA/AMPD, B. taurus</div> <div>5. ADA/AMPD, D. rerio</div> <div>6. ADA/AMPD, A. gambiae</div> <div>7. ADA/AMPD, U. maydis</div> <div>8. ADA/AMPD, G. zeae</div> <div>9. ADA/AMPD, A. nidulans</div> <div>10. ADA/AMPD, A. terreus</div> <div>11. ADA/AMPD, S. elongatus</div> </div>										
	ID	ID	ID	ID	ID	ID	ID	ID	ID	ID	ID
1. ADA/AMPD, A. thaliana	0.603										
2. ADA/AMPD, O. sativa	0.247	0.242									
3. ADA/AMPD, H. sapiens	0.327	0.328	0.622								
4. ADA/AMPD, B. taurus	0.344	0.335	0.457	0.58							
5. ADA/AMPD, D. rerio	0.365	0.328	0.279	0.397	0.385						
6. ADA/AMPD, A. gambiae	0.351	0.32	0.215	0.315	0.3	0.277					
7. ADA/AMPD, U. maydis	0.163	0.174	0.091	0.197	0.184	0.158	0.171				
8. ADA/AMPD, G. zeae	0.191	0.182	0.204	0.207	0.209	0.206	0.162	0.192			
9. ADA/AMPD, A. nidulans	0.261	0.257	0.189	0.274	0.288	0.291	0.261	0.267	0.429		
10. ADA/AMPD, A. terreus	0.185	0.169	0.113	0.178	0.172	0.162	0.142	0.113	0.11	0.162	
11. ADA/AMPD, S. elongatus	identity	0.81-0.99	0.60-0.80	0.45-0.59	0.31-0.44	0.20-0.30	0.00-0.19				

also inhibit adenine deaminases. Other inhibitors of these enzymes are mostly substrate analogs, such as purine⁹, coformycin and its derivatives⁶, 6-methyl- and dimethylaminopurine⁴, hypoxanthine⁹, and 4-aminopyrazolo [3,4-d]pyrimidine⁴.

1.2. Genetic relationship of adenine deaminases

Protein BLAST results of prokaryotic and eukaryotic ADEs clearly show that fungal enzymes are only weakly similar to bacterial and archaeal ones (Tab. 1). Differences between archaeal and bacterial ADEs themselves are moderate, suggesting that they have close genetic origin as shown by phylogenetic analysis (Fig. 3A). These prokaryotic proteins form a separate group that is clearly distinct from any other ADE or ADA enzymes as indicated by low degree of amino acid sequence identity. Known eukaryotic ADE enzymes share a very high degree of similarity with putative fungal ADAs and they also contain conserved amino acid domains that associate them with bacterial and mammalian ADAs as well.

1.3. Protein structure of adenine deaminase

The enzyme AAH1 from *Saccharomyces cerevisiae* is a typical representative of ADEs from lower eukaryotes. The gene coding for this protein (GeneID: 855581) is located on chromosome XIV (NC_001146) at the position from 359598 to 360641 (1043 bp) and has no introns. According to amino acid domain search, the enzyme belongs to the metal-dependent hydrolase superfamily, adenosine deaminase (ADA) family and more specifically to the sub-family of α/β barrel enzymes, adenine deaminase family 2. The main structural feature of this protein is the conserved domain cd01320 that is characteristic for a monomeric zinc-dependent protein, which catalyzes the irreversible hydrolytic deamination of both adenosine, as well as 2'-deoxyadenosine, to ammonia and inosine or deoxyinosine, respectively. The same domain is present in the majority of adenosine deaminases.

A typical representative of bacterial ADEs is a *yicP* cryptic adenine deaminase from *E. coli* K12. The encoding gene (GeneID: 945851) is localized on the genome at the position from 3841987 to 3843753 (1766 bp). The enzyme belongs to the same superfamily, adenine deaminase family 1² and contains the conserved domain cd01295 (AdeC: adenine deaminase directly deaminating adenine to form hypoxanthine), which is different from the cd01320 domain of AAH1. Similar domain architecture is found for the enzyme from *Enterococcus faecalis*, the structure of which has been solved recently (Fig. 2A).

2. ADENOSINE DEAMINASE

Adenosine deaminase (alternatively also adenosine aminohydrolase, ADA, EC 3.5.4.4) catalyzes irreversible deamination of adenosine and 2'-deoxyadenosine to inosine and deoxyinosine. This kind of enzyme is present in prokaryotic and eukaryotic organisms in different forms and belongs to the family of α/β barrel enzymes² similarly to the adenine deaminases. Given their widespread occurrence and importance in the medical field, these enzymes have been investigated more profoundly than adenine

deaminases. ADA is found in a variety of prokaryotes and eukaryotes (insects, lower vertebrates, fish, mammals and possibly also in plants).

In prokaryotes and eukaryotes, proteins that exhibit adenosine deaminase activity include not only the classical ADA (EC 3.5.4.4), but also ADA regulatory proteins (only in prokaryotes) with different conserved domains (EC 2.1.1.63) and tRNA-specific adenosine deaminases (ADATs), which are structurally very similar to ADAs. ADAT was the first prokaryotic RNA editing enzyme to be identified in *E. coli*¹⁵. As described above, fungal ADEs are very similar at the amino acid sequence level to microbial ADAs and for this reason if classified solely on sequence homology could be easily mistaken.

2.1. Mammalian adenosine deaminase

Two different isoenzymes¹⁶ of ADA, ADA1 and ADA2, found in higher eukaryotes are encoded by different genes¹⁷. In humans, almost all ADA activity is attributed to a single-chain Zn-binding protein ADA1, whereas ADA2 is found in negligible quantities in serum and may be produced by monocytes/macrophages¹⁸. ADA1 is expressed in all human tissues, its activity levels being relatively high in thymus and duodenum (about 10 nkat/mg), whereas more than 500-fold lower activity was reported in liver¹⁹. The molecular basis for tissue-specific expression of ADA has been studied by analyzing ADA1 gene promoter and interactions of nuclear proteins with enhancer elements²⁰. Specific regulatory regions of the gene coding for ADA1 in humans were investigated in transgenic experiments using ADA1 gene and promoter fragments linked to a bacterial chloramphenicol acetyl transferase (CAT) reporter gene²⁰. A 232-bp region (-211 to +11) from the ADA1 gene promoter confers high basal reporter gene (CAT) expression in transient transfection assays in mammalian cells^{21, 22}.

Mutations in the ADA1 gene that block its expression cause severe combined immunodeficiency, whereas mutations leading to overexpression cause hemolytic anemia. Lack of ADA1 activity leads to an accumulation of dATP that causes inhibition of the ribonucleotide diphosphate reductase activity, the enzyme that synthesizes DNA and RNA which are required in a large amount during lymphocyte proliferation. There is also some evidence that the other different allele (ADA2) may lead to autism.

Recently, ADA2 has been identified as a member of a new class of ADGFs (ADA-related growth factors), which is present in almost all organisms¹⁸.

Two additional types of adenosine deaminase are found in higher eukaryotes, ADATs and ADARs. Adenosine deaminase acting on RNA (ADAR) has the ability to deaminate adenosines in any long double-stranded RNA and converting them to inosines. ADARs are commonly found in animals, but not known in other organisms. ADARs are presumed to evolve from the adenosine deaminases acting on tRNAs (ADATs), by steps including fusion of two or more double-stranded-RNA binding domains to a common type of zinc-containing adenosine-deaminase domain²³.

Fig. 1. Adenine deaminase (ADE, EC 3.5.4.2): A. Role of ADE in salvage pathways of adenine, hypoxanthine and their nucleosides (*Saccharomyces cerevisiae*), B. Catalytic reaction of ADE.

2.2. Substrates, activating compounds and inhibitors of adenosine deaminase

Substrates of different adenosine deaminase enzymes are adenosine²⁴ and adenine²⁵ and their derivatives, such as 2',3'-deoxyadenosine²⁶, 2'-deoxyadenosine²⁷, 3'-deoxyadenosine²⁵, isopropylideneadenosine²⁸, adenine arabinoside²⁹, 2'-dAMP³⁰, 5'-AMP³¹, adenosine-3'-monophosphate³², ADP and ATP³¹, cAMP³², double-stranded RNA³³, tRNA³⁴, and other purine compounds as 2-amino-6-chloropurine riboside²⁸, 6-chloropurine riboside and 6-methoxypurinriboside³⁵, 6-methylaminopurine ribonucleoside²⁵, and formycin A²⁷.

Metals ions in specific concentrations are in some cases essential for the activity. MgCl_2 and CoSO_4 had a remarkable activating effect on ADA from *Aspergillus terricola*³⁶. Fe^{3+} or Sn^{2+} are promoting the enzymatic reaction of *Nocardioide* sp. J-326TK³¹ and *Streptomyces* sp.³² adenosine deaminases. For *Bacillus cereus* enzyme, the activity is stabilized by NH_4^+ or K^+ , while it is irreversibly lost in the absence of these or a few other monovalent cations³⁷. Activating compounds were also described for the enzymes from *Argopecten irradians concentricus* (NH_4Cl , $(\text{NH}_4)_2\text{SO}_4$)³⁸, *Candida albicans* enzyme (8-aza-adenine, adenine, AMP, ATP, IMP,

inosine, *N*-acetyl-D-glucosamine)³⁹ and *Mus musculus* (dibutyryl-cAMP)⁴⁰.

The value of pH optimum significantly varies among enzymes from different sources, but usually confines to the range between pH 5 and 8. The pH range to observe enzymatic activity was shown to be pH 5-9 for *Bos taurus* ADA⁴¹, pH 4-8 for *Candida albicans*³⁹ and pH 3.5-5.5 for *Streptomyces* sp.³² enzyme. Temperature optimum ranges from 25 °C for ADA from camel (*Camelus dromedaries*)⁴² to 55 °C for the one from *Streptomyces* sp.³².

Wide spectrum of inhibitors has been described for adenosine deaminases. *Aspergillus terricola* enzyme is inhibited by the 2-mercaptoethanol, iodoacetic acid, Mn^{2+} , reduced glutathione and by EDTA, which indicates that this ADA is a metalloenzyme. As mentioned previously, MgCl_2 and CoSO_4 have a remarkable activating effect, whereas MnCl_2 shows a slight inhibitory effect on enzyme activity³⁶. *Candida albicans* ADA is inhibited by 1-methyl adenosine, AMP, *p*-chloromercuribenzoate, *p*-hydroxymercuribenzoic acid and theophylline³⁹. ADA from *Argopecten irradians concentricus* has been found sensitive to these inhibitors: 2'-deoxyinosine, 2-amino-4-hydroxypteridine, folic acid, hypoxanthine, inosine, *p*-chloromercuribenzoate and purine riboside³⁸. The enzymatic reaction of *Nocardioide*

sp. ADA is potently inhibited by Hg^{2+} , Ag^{2+} , Cd^{2+} , Mg^{2+} , Mn^{2+} , Zn^{2+} , *o*-phenanthroline and pentachlorophenol, and noticeably inhibited by 8-bromoadenosine, theobromine and theophylline³¹. *Klebsiella* sp. ADA is also inhibited by metal ions (Fe^{2+} , Hg^{2+} , Ni^{2+} , Zn^{2+})²⁹. Other microbial ADAs are inhibited by Hg^{2+} , iodoacetamide, iodoacetic acid, *N*-ethylmaleimide, *p*-chloromercuribenzoate, Pb^{2+} , Zn^{2+} (*Micrococcus sodonensis*²⁸), Ag^+ , Hg^{2+} , *o*-phenanthroline (*Streptomyces* sp.³²) and *p*-chloromercuribenzoate (*E. coli*²⁵). *Plasmodium falciparum* ADA activity is blocked by 2,6-diaminopurine, 6-methylamino riboside, 6-methylmercaptapurine riboside, adenine and cordycepin⁴³.

In case of *Homo sapiens* adenosine deaminase, binding of 1,6-dihydro-6-(hydroxymethyl)purine riboside and coformycin causes changes in the protein conformation. Both human ADA1 and chicken (*Gallus gallus*) ADA1 show similar substrate specificity and sensitivity to the inhibitors 2'-deoxycoformycin and erythro-9-(2-hydroxy-3-nonyl)-adenine hydrochloride²⁶. On the other hand, ADA2 from chicken is not inhibited by erythro-9-(2-hydroxy-3-nonyl)-adenine hydrochloride⁴⁴.

Other inhibitors of human ADA include 2-aminopurine, adenine, guanosine, inosine, Cu^{2+} , Hg^{2+} , and *p*-chloromercuriophenylsulfonate⁴⁵, 2,6-diaminopurine, 6-methylamino riboside, 6-methylmercaptapurine riboside, cordycepin, deoxycoformycin, erythro-9-(2-hydroxy-3-nonyl)adenine⁴³, 2,6-diaminopurine sulfate, 4-amino-5-imidazole carboxamide hydrochloride, 4-amino-5-imidazole carboxamide ribonucleoside, 6-chloropurine, and iodopurine⁴⁶.

Purine riboside was a competitive inhibitor of *Mus musculus* ADA, whereas 2'-3'-*o*-isopropylidene adenosine acted as an uncompetitive inhibitor. The enzyme activity was also inhibited by theophylline (40 % loss of the original activity), caffeine (30 %), L-cysteine (50 %) and strongly by Hg^{2+} (98 %). In addition, purine analogs such as inosine, purine, alpha-adenosine and adenine showed variable inhibition effects. ADA activity towards 3'-deoxyadenosine and 6-chloropurine riboside decreased to 70 % and 60 % of the original value, respectively. However, the activity with 2'-*o*-methyl adenosine increased by 30 % comparing to the activity obtained with adenosine⁴⁰.

ADA from bovine spleen (*Bos taurus*) is inhibited by erythro-9-(2-hydroxy-3-nonyl)adenine, erythro-9-(2-hydroxy-3-nonyl)-3-deazaadenine⁴⁷, 9-(*p*-aminobenzyl)adenine, *p*-chloromercuribenzoate and purine riboside⁴¹.

Another known mammalian ADA from *Camelus dromedaries* is inhibited by Mg^{2+} , Mn^{2+} and purine riboside⁴².

As a part of a program to explore structure-activity relationships for extremely tight binding of coformycin inhibitors to adenosine deaminase, a series of analogues containing the imidazo[4,5-*e*][1,2,4]triazepine ring system was synthesized and showed to display inhibitory properties towards mammalian ADA in *in vitro* tests⁴⁸.

2.3. Genetic relationship and structures of adenosine deaminases

Human ADA1 gene is located on the chromosome 20 (20q12-q13.11). The gene is 32,213 bp long and contains

A.

B.

Fig. 2. Structures of adenine and adenosine deaminases: A. Adenine deaminase from *Enterococcus faecalis* (PDB entry 2ICS) obtained as recombinant protein from *E. coli*³, B. Adenosine deaminases – 1) whole structure of ADAT from *Escherichia coli* (PDB entry 1Z3A), 2) Z-β domain of ADAR1 from *Homo sapiens* (PDB entry 1XMK), 3) whole structure of ADA from *Bos taurus* (PDB entry 1VFL). The structural coordinates were visualized with Open-Source PyMOL 0.99rc2 (DeLano Scientific LLC, Palo Alto, CA, USA).

11 introns, the coding sequences being only 4,53 % of the whole gene. The encoded protein belongs to the metallo-dependent hydrolase superfamily, adenosine deaminase (ADA) family and contains adenosine deaminase (ADA) conserved domain cd01320.

Typical prokaryotic gene representative, ADA gene from *Escherichia coli* K12, is located in the position from 1700257 to 1701258 of the genome (1001 bp). The encoded enzyme contains the same conserved domain as a human ADA.

Protein structures have been solved for prokaryotic and eukaryotic ADAs, a variety of domains and chains of ADAR, and for four chains of bacterial ADAT. No structure of a plant enzyme of this class is known. Comparison of protein structures of three typical representatives of these enzymes is shown in Fig. 2B.

Fig. 3. Fitch phylogenetic unrooted tree (Phylogram) of adenine or adenosine deaminases. The analysis was done with the software BioEdit software 7.0.5.3⁶⁰ and TreeView 1.6.6 (<http://taxonomy.zoology.gla.ac.uk/rod/rod.html>): A. Bacterial, archaeal and fungal ADEs, B. Prokaryotic and eukaryotic adenosine deaminases.

Sequences of adenosine deaminase coding genes of prokaryotes and eukaryotes are more related each other than those of adenine deaminases. The sequences of the genes from higher eukaryotes, mammals particularly, are almost identical. At protein level, the degree of identity among mammalian enzymes is very high and clearly separates this group from the others (Tab. 2). The same relationship is shown in the phylogenetic tree (Fig. 3B). Noticeably, fungal enzymes form group, which is the least similar with the others. Although these enzymes are annotated as ADAs, there is actually no experimental evidence that the respective proteins show ADA-like activity. Contradictory to the annotation, the enzyme from *Schizosaccharomyces pombe* (SPBC1198.02) shows no activity with adenosine, but readily acts on adenine^{2,54}, thus being ADE not ADA. It is likely that also other closely related enzymes from Ascomycetes possess ADE, but not ADA activity and maybe adenosine deaminases are not presented in lower eukaryotes at all.

These genes from lower eukaryotes contain no introns, but introns occupy about 95 % of the DNA sequence in mammalian ADA genes. Typically, the ADA gene has 11 introns. Dog ADA gene contains an additional 40 bp intron that may have evolved by an excision from the 5'-

part of the 9th intron. Mammalian ADA genes range from 23,593 bp (mouse) to 33,391 bp (chimpanzee). The most significant intron sequence similarity is between human and chimpanzee and then between mouse and rat, where the introns 5, 7, and 11 are the most conserved.

2.4. Adenosine deaminases in plants – an open question

Despite the adenosine deaminase enzymes have been thoroughly described in prokaryotes, lower eukaryotes and mammals, there are only scarce reports on these enzymes in plants. Although completed genome databases of *Arabidopsis thaliana* and other plants show presence of putative encoding genes, the corresponding proteins have not been obtained so far nor the activity towards adenosine demonstrated. Some articles even speculate that adenosine deaminase is not present at all in plant tissue^{49, 50, 51} or that enzyme activity is too low and the main enzyme for adenosine recycling in plants is adenosine kinase (EC 2.7.1.77)^{52, 53}.

The corresponding hypothetical plant gene product contains an adenosine/AMP conserved domain cd00443 (different from cd01320 present in other ADAs) that is also present in some prokaryotic and eukaryotic enzymes annotated as ADA, adenosine/AMP deaminase or ADA-

like proteins. On amino acid sequence level, putative plant enzymes show close relationship between each other, while they differ from ADAs of other origin, especially from those of microorganisms (Tab. 3).

CONCLUSIONS

Nowadays, the study of adenine/adenosine deaminases is receiving raising attention. Several active recombinant enzymes from microorganisms were prepared so far, such as ADEs from *Escherichia coli*⁷, *Enterococcus faecalis*³, *Saccharomyces cerevisiae*⁵⁴ and *Schizosaccharomyces pombe*⁵⁴. These proteins have been produced mainly for biochemical and structural studies, but there is also a possible *in vitro* application of the recombinant proteins, i.e. in analytical chemistry. Development of a biosensor for the assay of adenine in biological fluids that is based on adenine deaminase/xanthine oxidase/peroxidase system is in progress (Petr J, Pospíšilová H, Frébort I, Nistor M, unpublished results).

Human adenosine deaminase is very intensively investigated at the medical field because of diseases induced by lack or excess of its activity. Acquired knowledge is then used in attempting to therapy the adenosine deaminases-based illness. For examples, enzyme replacement therapy, using red blood cells as a source of encapsulated human ADA, restored both T and B cell function of patients with adenosine deaminase deficient form of severe combined immunodeficiency (SCID)⁵⁵ and hematopoietic stem cell gene therapy led to the successful reconstitution of immune function in a child who showed a poor response to PEG-ADA enzyme replacement⁵⁶. ADA deficiency was selected for the first approved human gene therapy trial⁵⁷. Determination of ADA could be used as a non-specific marker of T-lymphocyte activation⁵⁸. As a model system for human therapy, mouse is often used also in the investigation of ADA-linked disorders. Hence, murine ADA was cloned and expressed in modified *E. coli* and monolayer cultures of murine CI-1D LM (thymidine kinase⁻) fibroblast cells derived from bone marrow stromal cells of a (C57BL/6J × C3H/HeJ)F₁ mouse and human embryonic kidney (HEK) 293 cells⁵⁹.

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