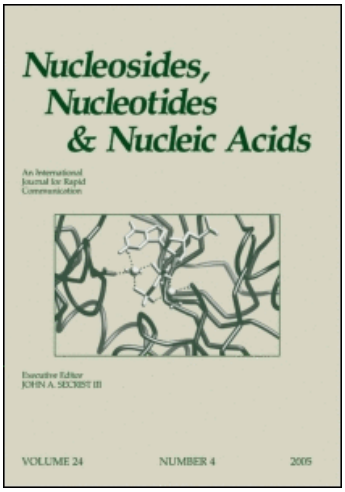


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# Biochemical and Molecular Genetic Correlation in Adenylosuccinate Lyase Deficiency

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# Biochemical and Molecular Genetic Correlation in Adenylosuccinate Lyase Deficiency

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## ABSTRACT

An homology model of human adenylosuccinate lyase structure shows that P100A substitution distorts the amino acid chain of domain I in the proximity of His-86, which behaves as general acid in the catalysis, and may expose Cys-98 and Cys-99 to oxidising agents. This model is in line with the observation that the defective protein is strongly inhibited by 4-hydroxy-2-nonenal, an hydroxyalkenal that is known to form thio-ether linkage with proteins.

*Key Words:* Adenylosuccinate lyase; Enzyme defect; Crystal structure; Protein destabilisation; Oxidative stress; 4-Hydroxy-2-nonenal.

## INTRODUCTION

Adenylosuccinate lyase (ADSL) deficiency is an autosomal recessive inborn error of purine synthesis characterised by diminished levels of enzyme activity and accumulation SAICA riboside and S-Ado. These succinylpurines are the dephosphorylation products of the two substrates of the enzyme. Affected individuals show various degrees of psychomotor retardation and behaviour abnormalities often accompanied by

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epilepsy, hypotonia, growth retardation and muscular wasting. ADSL is a homotetramer containing four active sites that are located at the four corners of the structure and formed by residues from three subunits. Molecular analysis of the affected families reveals the existence of about 30 different mutations in the ADSL gene, mostly in compound heterozygous form. It is widely accepted that impairment of enzyme activity is due either to remarkable susceptibility to thermal stress or to changes in the active site structure that leads to anomalous handling of the substrates. As a rule, mutations that decrease the stability of the overall protein structure decrease the activity of the enzyme with the two substrates in parallel. By contrast, mutations that affect the active site cause accumulation of S-Ado to a more marked extent than SAICA riboside.

The significance of protein destabilisation is unclear in the case of the P100A/D422Y compound heterozygote,[1] since the enzyme shows a Tm value that is decreased by only 8°C (to 51°C, from 59°C of control samples) and kinetic constants that are very similar to those of the wild-type protein.[2] An homology model of human ADSL, created by using the crystal structure of *T. maritima* ADSL as template,[3] shows that P100A substitution distorts the amino acid chain of domain I in the proximity of His-86, which behaves as general acid in the catalysis, while the D422Y mutation involves a cluster of helices on the outskirts of the tetrameric enzyme far away from the active site. Correct geometry of domain I may be essential to preserve enzyme function, since the structure of the hyperthermophilic archaebacterium *P. aerophilum* is reinforced in this point by a disulfide bridge that fixes Cys-87 (corresponding to residue 101 in the human sequence) to the helix 3-helix 4 pair.[4] Distortion of amino acid chain by P100A substitution may expose Cys-98 and Cys-99 to oxidising agents. Indeed, we showed[5] that P100A/D422Y enzyme is strongly inhibited by 4-hydroxy-2-nonenal (HNE), a major product of membrane peroxidation, which is believed to cause some of tissue damage that occur in vivo under conditions of oxidative stress. Kinetic analysis is consistent with the view that HNE 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 HNE is slightly reversed by dithiothreitol suggesting a thio-ether linkage between HNE and the enzyme.



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