View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Elsevier - Publisher Connector

ELSEVIER
Biochimica et Biophysica Acta 1500 (2000) 335–341
BIOCHIMICA ET BIOPHYSICA ACTA
BBA
www.elsevier.com/locate/bba

Succinylpurinemic autism: increased sensitivity of defective adenylosuccinate lyase towards 4-hydroxy-2-nonenal

Costantino Salerno [a,*], Werner G. Siems [b], Carlo Crifò [b]

[a] Department of Biochemical Sciences and Clinical Biochemistry Laboratory, University of Roma La Sapienza, I-00185 Rome, Italy
[b] Herzog-Julius-Hospital of Rheumatology and Orthopedics, D-38655 Bad Harzburg, Germany

Received 11 June 1999; received in revised form 18 November 1999; accepted 22 December 1999

Abstract

We studied the effect of *trans*-4-hydroxy-2-nonenal on the wild-type human adenylosuccinate lyase and on the enzyme from a patient compound-heterozygous for two missense mutations (P75A/D397Y; McKusick 103050.0003/103050.0004). Both the enzymes were inhibited by 10–50 μM *trans*-4-hydroxy-2-nonenal in a concentration-dependent manner by means of a mixed-type co-operative mechanism. A significantly stronger inhibition was noticed in the presence of the defective enzyme. Nonanal and *trans*-2,3-nonenal inhibited the enzymes to a less extent and at about 10-times higher concentrations. Hydroxylamine reversed the inhibition by *trans*-4-hydroxy-2-nonenal, *trans*-2,3-nonenal or nonanal in the case of the wild-type enzyme, but it was ineffective to reverse the inhibition by *trans*-4-hydroxy-2-nonenal on the defective enzyme. Dithiothreitol slightly decreased the inhibition exerted by *trans*-4-hydroxy-2-nonenal on both the wild-type and the defective adenylosuccinate lyase, while it did not produce practically any change in the presence of *trans*-2,3-nonenal or nonanal. © 2000 Published by Elsevier Science B.V. All rights reserved.

Keywords: Adenylosuccinate lyase; 4-Hydroxy-2-nonenal; Psychomotor retardation; Lipid peroxidation; Inherited diseases

1. Introduction

Adenylosuccinate lyase (adenylosuccinase; EC 4.3.2.2) is a tetrameric enzyme that catalyzes two distinct reactions in the purine synthesis, both involving the removal of fumarate: the conversion of succinylaminoimidazole carboxamide (SAICA) ribotide into aminoimidazole carboxamide ribotide along the de novo pathway, and the formation of AMP from adenylosuccinate (S-AMP) in the conversion of IMP into adenine nucleotides [1,2]. The catalysis follows order uni–bi kinetics with fumarate leaving the enzyme before the nucleotides. The reactions are thought to involve a β-elimination mechanism, in which deprotonation of C^{β} and cleavage of N–C^{α} in the substrate occur in a concerted manner. Little information is available on the role of enzymic amino acids in the catalysis. Studies on the enzymes from rabbit muscle and *Bacillus subtilis* suggest that Arg-60, His-61, and Arg-112 (the numbering is based on the human enzyme sequence reported in reference [3], which is shorter than that recently published on EMBL data base, accession number X65867) are located within the substrate-binding site [4,5], while His-134 acts as a general base accepting the proton from C^{β} [6]. The protection of the enzyme by dithiothreitol and EDTA suggests that thiol groups on the protein are essential for activity [1,2].

* Corresponding author. Fax: +39-6-444-0062;
E-mail: ccrifo@axcasp.caspur.it

0925-4439/00/$ – see front matter © 2000 Published by Elsevier Science B.V. All rights reserved.
PII: S0925-4439(99)00117-9

Decreased levels of adenylosuccinase activity have been reported in approximately 40 patients showing psychomotor retardation [7–13]. A remarkable feature of the disease is the high incidence of autistic symptoms. The prognosis for survival of profoundly retarded patient is poor. The defect is transmitted as an autosomic recessive trait and characterized by the accumulation in body fluids of two normally undetectable compounds, SAICA riboside and succinyladenosine, that are the dephosphorylation products of the two substrates of the enzyme. About one-third of the patients has been analyzed at the gene level [13]. Most of the reported data refer to the defective enzymes from a Moroccan family (McKusick 103050.0001) and an Italian family (McKusick 103050.0003/103050.0004). In the former case, a single T-to-C transition in the adenylosuccinase gene leading to a Ser-413 to Pro substitution segregates with mental retardation [3]. In the case of the Italian family, the patient is compound-heterozygous for two missense mutations on different alleles [14]: a C-to-G transversion corresponding to a Pro-75 to Ala substitution and a G-to-T transversion corresponding to an Asp-397 to Tyr substitution (P75A/D397Y adenylosuccinase). In both kindred, the defective enzymes show a great heat lability and an increased sensitivity to guanadinium HCl and urea, without changing in the kinetic parameters [3,9].

It has been reported [7,15] that adenylosuccinase deficiency in a minority of patients is more severe in liver, kidney, and lymphocytes than in skeletal muscle and erythrocytes. Since the available data seem to exclude the presence of tissue-specific isozymes [3,14], this may imply that yet undefined regulatory mechanisms play a role in controlling adenylosuccinase level within certain cell types. The tissue identity of the enzyme also opens the possibility to get generalizable results on enzyme properties with enzyme preparations from different tissues. We report here that adenylosuccinase is specifically inhibited by micromolar concentrations of *trans*-4-hydroxy-2-nonenal (HNE), a major product of membrane peroxidation, which is believed to cause some of the tissue damage that occurs in vivo under conditions of oxidative stress [16] and plays an important role in diseases of central and peripheral nervous tissue [17–19].

2. Materials and methods

Nonanal, *trans*-2,3-nonenal, hydroxylamine, and dithiothreitol were supplied by Aldrich. HNE was prepared by the acid treatment (1 mM HCl) of *trans*-4-hydroxy-2-nonenal diethylacetal that was generously provided by Dr. H. Zollner (University of Graz). The concentration of HNE was determined spectrophotometrically at 224 nm by assuming a molar absorptivity of 13 750 M^{-1} [16]. Other reagents were high purity commercial samples from Sigma.

Crude preparations of wild-type (1.6 IU/g protein) and P75A/D397Y (1.2 IU/g protein) erythrocyte adenylosuccinase were obtained from healthy subjects and from patient with adenylosuccinase deficiency by step-wise precipitation in ammonium sulfate [9]. Purified preparations of erythrocyte adenylosuccinase (700 IU/g protein) were obtained from healthy volunteers by using the procedure described in reference [1] with minor modifications. Prior to exposure of the enzyme to aldehydes, dithiothreitol was removed by exhaustive dialysis. Adenylosuccinase activity was determined by following the conversion of S-AMP into AMP spectrophotometrically at 282 nm. Enzyme activity was linear under all conditions tested over the time assayed.

All experiments were performed in 10 mM Tris-HCl, 10 mM KCl, 2 mM EDTA, pH 7.4 at 37°C, unless otherwise stated. Mathematical models were fitted to the experimental data by a non-linear regression program [20]. Measurements were performed in triplicate. The standard deviation of control values was estimated from independent series of measurements performed with enzyme preparations from three different healthy subjects.

3. Results

To test the effect of HNE on the enzyme activity, adenylosuccinase (final activities between 0.5 and 4 IU/l) was incubated with various concentrations of HNE (0–500 μM) for 1–120 min at 37°C. The activity was measured by adding 0.01 volumes of 10 mM S-AMP to samples withdrawn from the incubation mixture. As shown in Fig. 1, the enzyme was inhibited by micromolar [HNE] in a concentration-dependent manner. The inhibition was achieved

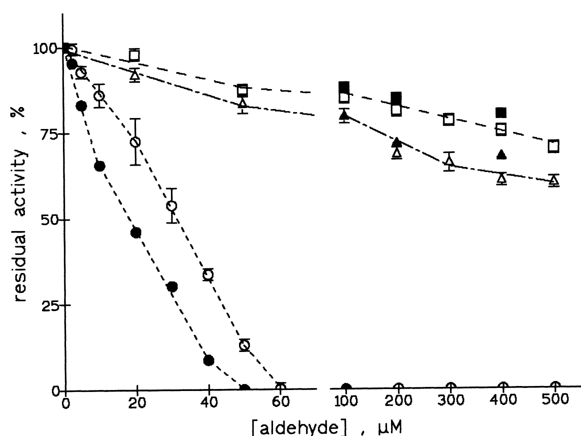

Fig. 1. Inhibition of adenylosuccinase activity by aldehydes (○●, HNE; △▲, *trans*-2,3-nonenal; □■, nonanal). Enzyme activities in the absence of aldehydes were scaled to 100%. The concentration of S-AMP was 100 μM. Open symbols refer to wild-type adenylosuccinase, while closed symbols refer to P75A/D397Y adenylosuccinase. All tests were performed in triplicate and expressed as mean values. Error bars indicate one standard deviation from the mean of independent tests performed with enzyme preparations from three different healthy subjects. All other conditions were as described in the text.

rapidly (within 1-min incubation). No further changes in enzyme activity were observed over the time interval pertinent to the experiments. The fractional decrease in adenylosuccinase activity was independent of enzyme concentration and purity under the employed experimental conditions. We did not observe significant changes in the shape of the inhibition titration curve by using enzyme preparations from different healthy subjects. In contrast, P75A/D397Y adenylosuccinase showed greater sensitivity to HNE inhibition than wild-type enzyme. The inhibition was reversed by diluting the enzyme–HNE mixture. We also attempted to remove the inhibitor by dialysis. However, because of the slow and limited diffusion of HNE through the membrane (data not shown), the enzyme was only partially reactivated by this way.

We explored the effect, on the enzymatic catalysis, of aldehydes that are different from HNE by lack of the hydroxy group (*trans*-2,3-nonenal) and by lack of both the hydroxy group and the alpha,beta-double bond (nonanal). As shown in Fig. 1, *trans*-2,3-nonenal and nonanal inhibited enzyme activity to a less extent and at about ten-times higher concentrations. Nonanal exerted the smallest inhibitory effect. Addition of 250 μM hydroxylamine, which is thought to form non-dissociable oxime derivatives with the aldehydes [21], reversed at least half of the inhibition exerted by HNE or *trans*-2,3-nonenal and completely the inhibition by nonanal on the wild-type enzyme (Table 1). Hydroxylamine (250 μM) reversed the inhibition of the P75A/D397Y adenylosuccinase by *trans*-2,3-nonenal or nonanal in the same extent as in the experiments with the wild-type enzyme, but it was ineffective against HNE. Dithiothreitol (500 μM) reduced the inhibition by 20 μM HNE on both wild-type and P75A/D397Y adenylosuccinase, while it did not produce any change in the presence of 200 μM *trans*-2,3-nonenal or 200 μM nonanal. Higher concentrations of hydroxylamine and/or dithiothreitol were inhibitory (data not shown).

The effect of substrate addition to the enzyme–in-

Table 1
Effect of hydroxylamine (HA) and dithiothreitol (DTT) on the inhibition exerted by HNE, *trans*-2,3-nonenal, and nonanal

	Aldehydes			
	None	HNE (20 μM)	*trans*-2,3-Nonenal (200 μM)	Nonanal (200 μM)
None	**100.0**	**72.4 ± 2.2**	**75.3 ± 1.4**	**84.0 ± 2.1**
	100.0	*49.1*	*71.5*	*84.6*
0.25 mM HA	**99.7 ± 0.7**	**90.1 ± 1.9**	**87.5 ± 8.7**	**99.3 ± 1.0**
	96.5	*48.7*	*81.6*	*93.4*
0.50 mM DTT	**99.2 ± 1.4**	**83.5 ± 6.0**	**74.9 ± 1.6**	**84.0 ± 2.1**
	98.7	*54.3*	*71.7*	*84.2*
0.25 mM HA+0.50 mM DTT	**101.5 ± 0.5**	**93.5 ± 2.8**	**88.3 ± 8.5**	**98.2 ± 0.8**
	100.9	*52.2*	*82.9*	*93.6*

Adenylosuccinase activity is expressed as percentage of the initial activity. Bold numbers are means ± S.D. of independent tests performed with enzyme preparations from three different healthy subjects. Italic numbers refer to P75A/D397Y enzyme. all other conditions were as described in Fig. 1.

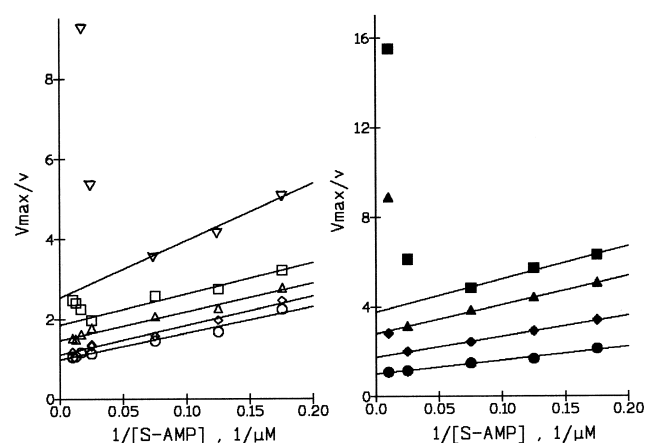

Fig. 2. Double reciprocal plot of specific velocity (v/V_{max}) against [S-AMP] at different fixed concentrations of HNE. The concentrations of HNE were: ○●, none; ◇◆, 25 μM; △▲, 35 μM; □■, 40 μM; ▽, 50 μM. Open symbols refer to wild-type adenylosuccinase, while closed symbols refer to P75A/D397Y adenylosuccinase. All other conditions were as described in Fig. 1.

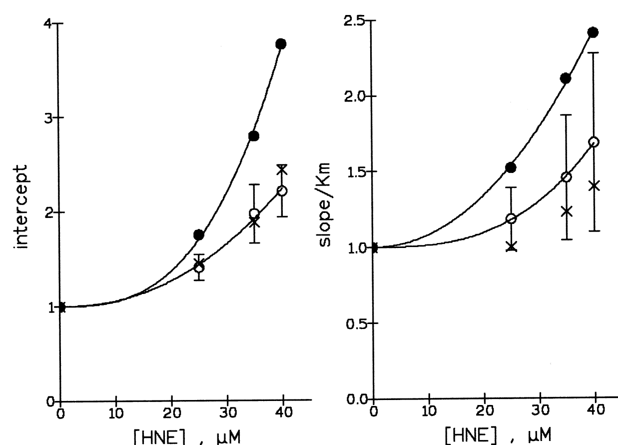

Fig. 3. Ordinate-intercept and slope/K_m replots. Each curve represents data from a family of reciprocal plots obtained as shown in Fig. 2. The experiments were performed by using highly purified wild-type adenylosuccinase (×) as well as crude enzyme preparations (○, wild-type; ●, P75A/D397Y). Error bars indicate one standard deviation from the mean of independent tests performed with enzyme preparations from three different healthy subjects.

hibitor mixture was tested by varying S-AMP concentration at different fixed concentrations of HNE. Both wild-type and P75A/D397Y adenylosuccinase showed Michaelian relations between steady-state velocity and S-AMP concentration in the absence of HNE. Substrate inhibition was observed at relatively high [S-AMP] only in presence of HNE, as shown in the double reciprocal plots reported in Fig. 2. Replots of slope/K_m and ordinate intercept of the extrapolated linear part of the curves versus [HNE] revealed a co-operative mixed-type inhibition by the hydroxyalkenal (Fig. 3). Table 2 reports the kinetic constants of the enzymatic reaction calculated by using Hill's algorithm under rapid equilibrium assumption [22]. The kinetic analysis is consistent with the view that HNE reacts with both the free enzyme and the enzyme–substrate complex (though with different affinity), leading to kinetically ineffective enzyme species. The affinity of HNE for P75A/D397Y adenylosuccinase is significantly higher than that for the wild-type enzyme, in good agreement with the results obtained in the inhibition titration experiments (Fig. 1).

4. Discussion

Lipid peroxidation is always combined with the formation of reactive aldehydes [23,24], HNE being a major aldehyde produced in vivo during the peroxidation of polyunsaturated fatty acids [16]. This

Table 2
Kinetic constants of the enzymatic reaction catalyzed by adenylosuccinase

Reaction	Equation	Parameter	Adenylosuccinase	
			Wild-type	P75A/D397Y
$E+S \overset{K_m}{\rightleftharpoons} ES \rightarrow E+P$	$\frac{V_{max}}{v} = \frac{K_m}{[S]} + 1$	K_m (μM)	5 ± 2	6.1
$E + nI \overset{K_i}{\rightleftharpoons} EI_n$	$\frac{slope}{K_m} = 1 + \left(\frac{[I]}{K_i}\right)^n$	K_i (μM)	45 ± 7	34
		n	3 ± 1	2.1
$ES + mI \overset{aK_i}{\rightleftharpoons} ESI_m$	$intercept = 1 + \left(\frac{[I]}{aK_i}\right)^m$	aK_i (μM)	37 ± 4	28
		m	2.3 ± 0.2	2.9

compound has a high biological activity and exhibits a number of cytotoxic, mutagenic, and genotoxic effects [16]. It is considered that at least some of the damage observed in free radical pathology is mediated by HNE and other aldehydes, which may act as 'second toxic messenger' of the primary free radical event. The hypothetic sequence free radicals–lipid peroxidation–aldehyde formation–damage was proposed by Esterbauer et al. [16]. The most cell types and organs possess high capacities for removal of HNE by different metabolic pathways to prevent HNE accumulation and toxic consequences [25–27]. Nevertheless, HNE concentration is significantly increased in plasma, various organs, and cell types under certain conditions of oxidative stress [28,29]. Under those conditions, HNE concentrations up to the micromolar range were measured, e.g. 6 μM in reperfused small intestine [30,31]. Of special importance is the subcellular compartmentation of HNE and other aldehydic lipid peroxidation products. It is assumed that in hydrophobic regions of membranes much higher HNE concentrations occur. For membranes in isolated peroxidized microsomes even a HNE concentration of approximately 4.5 mM was calculated [32].

HNE binding to proteins is of great importance concerning the pathophysiological consequences of free radical-initiated reactions. The I_{50}-values for HNE inhibition have been reported for several enzymes [21,33–36]: glucose-6-phosphatase (70 μM), adenylate cyclase (2.7 μM), 5′-nucleotidase (>5 mM), DNA polymerase α (370 μM), DNA polymerase β (290 μM), and Na^+- K^+-ATPase (120 μM). The binding of HNE to glucose-6-phosphate dehydrogenase and glyceraldehyde-3-phosphate dehydrogenase results to irreversible enzyme inactivation [37,38]. The chemical mechanism of alkenal and hydroxyalkenal binding to proteins have been studied intensively by the group of Uchida and Stadtman [38–42]. The findings of these authors may have important implications for the accumulation of proteins modified by aldehydes in vivo [43], a process that is believed to be involved in age- and disease-related impairment of cellular function [44,45].

In our experiments a reversible inhibition of adenylosuccinase by HNE was observed. The mutated enzyme was more sensitive in comparison to the wild-type. The 50% inhibitions were reached with about 17 and 32 μM, respectively. The binding of HNE to adenylosuccinase was characterized by using analogs (*trans*-2,3-nonenal, nonanal) lacking in some of the functional groups as well as by adding specific reagents. We found that the lack of the hydroxy group greatly reduced the affinity of the inhibitor for the enzymes. The inhibitory effect was further reduced removing the alpha,beta-double bond. The result obtained in the presence of hydroxylamine (see Table 1) could be taken as evidence that the carbonyl group of nonanal was involved in the binding, but it did not exclude more complex mechanisms in the case of HNE and *trans*-2,3-nonenal. Indeed, it could be hypothesized that these alkenals bind to the protein through a reaction involving the alpha,beta-double bond, in which the carbonyl function is preserved, and that the reversal of inhibition by hydroxylamine was due to electrostatic and/or steric hindrance of the oxime group. This proposal was in line with the finding that, when P75A/D397Y adenylosuccinase was used instead of the wild-type enzyme, hydroxylamine reversed the inhibition by *trans*-2,3-nonenal or nonanal, but it was ineffective against HNE. Experiments with dithiothreitol gave indirect evidence for a thio–ether linkage between HNE and the enzyme. The addition of dithiothreitol reduced the enzyme inhibition by HNE, but it did not reduce the enzyme inhibition by *trans*-2,3-nonenal or nonanal.

Adenylosuccinase deficiency results in mental retardation and some further clinical symptoms, which lead in total to a severe disease not only of the central and peripheral nervous system. The pathogenesis of the symptoms is still debated, since deficiency of metabolites which are normally formed distally from the enzyme defect, as well as accumulation of intermediates proximally thereof, could have deleterious effects. [8,9]. Our experiments on inhibition of adenylosuccinase activity by HNE, which is much stronger for the point mutated enzyme, suggest that free radicals and lipid peroxidation products may be responsible of the impairment of enzyme activity and, at least in part, of the subsequent tissue damage. The importance of free radical-initiated changes for different diseases and disturbances of the central and peripheral nervous systems was demonstrated for stroke, Alzheimer's disease, traumatic spinal cord injury, cerebral circulatory insufficiency, Huntington's

disease, systemic amyloidosis and others [17–19,46]. Our hypothesis is supported by the high frequency in succinylpurinemic autism of symptoms, which are connected with increased formation of free radicals and lipid peroxidation products, such as convulsions, muscle wasting, and recurrent infections of the upper respiratory tract [8,12].

References

[1] L.B. Barnes, S.H. Bishop, Adenylosuccinate lyase from human erythrocytes, Int. J. Biochem. 6 (1975) 497–503.
[2] R.L. Stone, H. Zalkin, J.E. Dixon, Expression, purification, and kinetic characterization of recombinant human adenylosuccinate lyase, J. Biol. Chem. 268 (1993) 19710–19716.
[3] R.L. Stone, J. Aimi, B.A. Barshop, J. Jaeken, G. Van den Berghe, H. Zalkin, J.E. Dixon, A mutation in adenylosuccinate lyase associated with mental retardation and autistic features, Nat. Genet. 1 (1992) 59–63.
[4] S.U. Gite, R.F. Colman, Affinity labeling of the active site of rabbit muscle adenylosuccinate lyase by 2-[(4-bromo-2,3-dioxobutyl)thio]adenosine 5′-monophosphate, Biochemistry 35 (1996) 2658–2667.
[5] T.T. Lee, C. Worby, Z.Q. Bao, J.E. Dixon, R.F. Colman, Implication of His68 in the substrate site of Bacillus subtilis adenylosuccinate lyase by mutagenesis and affinity labeling with 2-[(4-bromo-2,3-dioxobutyl)thio]adenosine 5′-monophosphate, Biochemistry 37 (1998) 8481–8489.
[6] T.T. Lee, C. Worby, J.E. Dixon, R.F. Colman, Identification of His-141 in the active site of Bacillus subtilis adenylosuccinate lyase by affinity labeling with 6-(4-bromo-2,3-dioxobutyl)thioadenosine 5′-monophosphate, J. Biol. Chem. 272 (1997) 458–465.
[7] J. Jaeken, G. Van den Berghe, An infantile autistic syndrome characterized by the presence of succinylpurines in body fluids, Lancet 2 (1984) 1058–1061.
[8] J. Jaeken, S.K. Wadman, M. Duran, F.J. Van Sprang, F.A. Beemer, R.A. Holl, P.M. Theunissen, P. De Cock, F. Van den Bergh, M.F. Vincent, G. Van den Berghe, Adenylosuccinase deficiency an inborn error of purine nucleotide synthesis, Eur. J. Pediatr. 148 (1988) 126–131.
[9] C. Salerno, C. Crifò, O. Giardini, Adenylosuccinase deficiency: a patient with impaired erythrocyte activity and anomalous response to intravenous fructose, J. Inherit. Metab. Dis. 18 (1995) 602–608.
[10] J. Krijt, I. Sebesta, A. Svehlakova, A. Zumrova, J. Zeman, Adenylosuccinate lyase deficiency in a Czech girl and two siblings, Adv. Exp. Med. Biol. 370 (1995) 367–370.
[11] D. Valik, P.T. Miner, J.D. Jones, First U.S. case of adenylosuccinate lyase deficiency with severe hypotonia, Pediatr. Neurol. 16 (1997) 252–255.
[12] G. Van den Berghe, M.F. Vincent, J. Jaeken, Inborn errors of the purine nucleotide cycle: adenylosuccinase deficiency, J. Inherit. Metab. Dis. 20 (1997) 193–202.
[13] S. Marie, H. Cuppens, M. Heuterspreute, M. Jaspers, E.Z. Tola, X.X. Gu, E. Legius, M.F. Vincent, J. Jaeken, J.J. Cassiman, G. Van den Berghe, Mutation analysis in adenylosuccinate lyase deficiency: eight novel mutations in the re-evaluated full ADSL coding sequence, Hum. Mutat. 13 (1999) 197–202.
[14] D. Verginelli, B. Luckow, C. Crifò, C. Salerno, M. Gross, Identification of new mutations in the adenylosuccinate lyase gene associated with impaired enzyme activity in lymphocytes and red blood cells, Biochim. Biophys. Acta 1406 (1998) 81–84.
[15] G. Van den Berghe, J. Jaeken, Adenylosuccinase deficiency, Adv. Exp. Med. Biol. 195A (1986) 27–33.
[16] H. Esterbauer, R.J. Schaur, H. Zollner, Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes, Free Radic. Biol. Med. 11 (1991) 81–128.
[17] J.E. Springer, R.D. Azbill, R.J. Mark, I.G. Begley, G. Waeg, M.P. Mattson, 4-Hydroxynonenal, a lipid peroxidation product, rapidly accumulates following traumatic spinal cord injury and inhibits glutamate uptake, J. Neurochem. 68 (1997) 2469–2476.
[18] R.J. Mark, M.A. Lovell, W.R. Markesbery, K. Uchida, M.P. Mattson, A role for 4-hydroxynonenal, an aldehyde product of lipid peroxidation, in disruption of ion homeostasis and neuronal death induced by amyloid beta-peptide, J. Neurochem. 68 (1997) 255–264.
[19] L.M. Sayre, D.A. Zelasko, P.L. Harris, G. Perry, R.G. Salomon, M.A. Smith, 4-Hydroxynonenal-derived advanced lipid peroxidation end products are increased in Alzheimer's disease, J. Neurochem. 68 (1997) 2092–2097.
[20] T. Tabata, R. Ito, Effective treatment of the interpolation factor in Marquardt's nonlinear least-squeres fit algorithm, Comput. J. 18 (1975) 250–251.
[21] W.G. Siems, S.J. Hapner, F.J.G.M. Van Kijk, 4-Hydroxynonenal inhibits Na^{+}-K^{+}-ATPase, Free Radic. Biol. Med. 20 (1996) 215–223.
[22] I.H. Segel, Enzyme Kinetics: Behavior and Analysis of Rapid Equilibrium and Steady-State Enzyme Systems, John Wiley and Sons, New York, 1975.
[23] G. Poli, M.U. Dianzani, K.H. Cheeseman, T.F. Slater, J. Lang, H. Esterbauer, Separation and characterization of the aldehydic products of lipid peroxidation stimulated by carbon tetrachloride or ADP-iron in isolated rat hepatocytes and rat liver microsomal suspensions, Biochem. J. 227 (1985) 629–638.
[24] H. Esterbauer, Aldehydic products of lipid peroxidation, in: D.C.H. McBrien, T.F. Slater (Eds.), Free Radicals, Lipid Peroxidation and Cancer, Academic Press, New York, 1982, pp. 101–128.
[25] W. Siems, H. Zollner, T. Grune, H. Esterbauer, Metabolic fate of 4-hydroxynonenal in hepatocytes: 1,4-dihydroxynonene is not the main product, J. Lipid Res. 38 (1997) 612–622.
[26] T. Grune, W.G. Siems, T. Petras, Identification of metabolic

pathways of the lipid peroxidation product 4-hydroxynonenal in in situ perfused rat kidney, J. Lipid Res. 38 (1997) 1660–1665.
[27] W.G. Siems, A.M. Pimenov, H. Esterbauer, T. Grune, Metabolism of 4-hydroxynonenal, a cytotoxic lipid peroxidation product, in thymocytes as an effective secondary antioxidative defense mechanism, J. Biochem. 123 (1998) 534–539.
[28] H. Esterbauer, H. Zollner, R.J. Schaur, Hydroxyalkenals: cytotoxic products of lipid peroxidation, ISI Atlas of Science: Biochemistry 1 (1988) 311–317.
[29] T. Grune, W. Siems, W. Schneider, Accumulation of aldehydic lipid peroxidation products during postanoxic reoxygenation of isolated rat hepatocytes, Free Radic. Biol. Med. 15 (1993) 125–132.
[30] W.G. Siems, T. Grune, H. Esterbauer, 4-Hydroxynonenal formation during ischemia and reperfusion of rat small intestine, Life Sci. 57 (1995) 785–789.
[31] T. Grune, W.G. Siems, J. Kowalewski, H. Esterbauer, Postischemic accumulation of lipid peroxidation product 4-hydroxynonenal in rat small intestine, Life Sci. 55 (1994) 693–699.
[32] J.F. Koster, R.G. Slee, A. Montfoort, J. Lang, H. Esterbauer, Comparison of the inactivation of microsomal glucose-6-phosphatase by in situ lipid peroxidation-derived 4-hydroxynonenal and exogenous 4-hydroxynonenal, Free Radic. Res. Commun. 1 (1986) 273–287.
[33] G. Poli, F. Chiarpotto, F. Biasi, R. Pavia, E. Albano, M.U. Dianzani, Enzymatic impairment induced by biological aldehydes in intact rat liver cells, Res. Commun. Chem. Pathol. Pharmacol. 38 (1982) 71–76.
[34] A. Benedetti, R. Fulcieri, M. Comporti, Inhibition of calcium sequestration activity of liver microsomes by 4-hydroxyalkenals originating from the peroxidation of liver microsomal lipids, Biochim. Biophys. Acta 793 (1984) 489–493.
[35] L. Paradisi, C. Panagini, M. Parola, G. Barrera, M.U. Dianzani, Effects of 4-hydroxynonenal on adenylate cyclase and 5′-nucleotidase activities in rat liver plasma membranes, Chem. Biol. Interact. 53 (1985) 209–217.
[36] E. Wawra, H. Zollner, R.J. Schauer, H.M. Tillian, E. Schauenstein, The inhibitory effect of 4-hydroxy-nonenal on DNA-polymerases alpha and beta from rat liver and rapidly dividing Yoshida ascites hepatoma, Cell Biochem. Funct. 4 (1986) 31–36.
[37] L.I. Szweda, K. Uchida, L. Tsai, E.R. Stadtman, Inactivation of glucose-6-phosphate dehydrogenase by 4-hydroxy-2-nonenal. Selective modification of an active-site lysine, J. Biol. Chem. 268 (1993) 3342–3347.
[38] K. Uchida, E.R. Stadtman, Covalent attachment of 4-hydroxynonenal to glyceraldehyde-3-phosphate dehydrogenase. A possible involvement of intra- and intermolecular cross-linking reactions, J. Biol. Chem. 268 (1993) 6388–6393.
[39] K. Uchida, S. Toyokuni, K. Nishikawa, S. Kawakishi, H. Oda, H. Hiai, E.R. Stadtman, Michael addition-type 4-hydroxy-2-nonenal adducts in modified low-density lipoproteins markers for atheroslerosis, Biochemistry 33 (1994) 12487–12494.
[40] B. Friguet, E.R. Stadtman, L.I. Sweda, Modification of glucose-6-phosphate dehydrogenase by 4-hydroxynonenal. Formation of cross-linked protein that inhibits the multicatalytic protease, J. Biol. Chem. 269 (1994) 2139–21643.
[41] K. Uchida, E.R. Stadtman, Selective cleavage of thioether linkage in proteins modified with 4-hydroxynonenal, Proc. Natl. Acad. Sci. USA 89 (1992) 5611–5615.
[42] K. Uchida, L.K. Szweda, H.-Z. Chae, E.R. Stadtman, Immunochemical detection of 4-hydroxynonenal protein adducts in oxidized hepatocytes, Proc. Natl. Acad. Sci. USA 90 (1993) 8742–8746.
[43] B. Friguet, L.I. Szweda, E.R. Stadtman, Susceptibility of glucose-6-phosphate dehydrogenase modified by 4-hydroxy-2-nonenal and metal-catalyzed oxidation to proteolysis in the multicatalytic protease, Arch. Biochem. Biophys. 311 (1994) 168–173.
[44] E.R. Stadtman, Protein oxidation and aging, Science 257 (1992) 1220–1224.
[45] E. Chiarpotto, F. Biasi, A. Scavazza, S. Camandola, M.U. Dianzani, G. Poli, Metabolism of 4-hydroxy-2-nonenal and aging, Biochem. Biophys. Res. Commun. 207 (1995) 477–484.
[46] Y. Ando, N. Nyhlin, O. Suhr, G. Holmgren, K. Uchida, M. El Sahly, T. Yamashita, H. Teraraki, M. Nakamura, M. Uchino, M. Ando, Oxidative stress found in amyloid deposits in systemic amyloidosis, Biochem. Biophys. Res. Commun. 17 (1997) 497–502.