Biochimica et Biophysica Acta, 662 (1981) 86–93
Elsevier/North-Holland Biomedical Press

BBA 69391

PURIFICATION, CHARACTERIZATION AND POSSIBLE FUNCTION OF α-*N*-ACYLAMINO ACID HYDROLASE FROM BOVINE LIVER

WAYNE GADE * and JERRY L. BROWN

Department of Biochemistry/Biophysics/Genetics, University of Colorado Health Sciences Center, 4200 East Ninth Avenue, Denver, CO 80262 (U.S.A.)

(Received April 21st, 1981)

Key words: α-N-Acylamino acid hydrolase; N-Acetylated protein; (Bovine liver)

α-*N*-Acylamino acid hydrolase from bovine liver has been purified approx. 1700-fold with a yield of 28%. Based on the results of studies using polyacrylamide gel electrophoresis the purified enzyme is homogeneous. This enzyme catalyzes the hydrolysis of α-*N*-acylated amino acids to yield the acyl group and the corresponding amino acid. α-Acetylmethionine was the most rapidly hydrolyzed substrate tested for this enzyme. Most of the other α-*N*-acylated amino acids used as substrates were hydrolyzed. Some, however, were cleaved at less than 1% of the rate observed for *N*-acetylmethionine. α-*N*-Acylamino acid hydrolase did not catalyze the hydrolysis of *N*-acetyl-D-alanine, *N*-acetyl-β-alanine, ε-*N*-acetyllysine, *N*-acetylglycinamide or α-*N*-acetylated peptides. When incubated with this enzyme the chloroacetyl derivatives of leucine and valine were hydrolyzed approx. 8- and 3-times, respectively, more rapidly than the corresponding *N*-acetyl derivatives. On the other hand, *N*-formylmethionine was hydrolyzed at only 60% the rate of *N*-acetylmethionine. Other studies on α-*N*-acylamino acid hydrolase have shown that it is a dimer composed of two 40 000 molecular weight monomers, it is active over a broad pH range with maximal activity at approx. pH 8.5, and requires Zn^{2+} for enzymatic activity. The substrate specificity and other properties of bovine liver α-*N*-acylamino acid hydrolase are very similar to those reported for acylase I isolated from porcine kidney [7]. We suggest that this enzyme functions in the catabolism of α-*N*-acetylamino acids resulting from the turnover of α-*N*-acetylated proteins.

Introduction

Acetylation of the NH_2-terminal residue of eukaryotic proteins is a frequent type of post-translational modification. For example, approx. 90%, 80% and greater than 50% of the intracellular proteins from mouse L-cells [1], Ehrlich ascites cells and *Neurospora crasa* [2], respectively, are α-*N*-acetylated. Our preliminary results indicate that this type of post-translational modification is a general feature of eukaryotic proteins (Brown, J.L., unpublished data). Even though it is apparent that eukaryotic proteins are frequently α-*N*-acetylated, little is known about the role of this type of modification in cell function, or the mechanisms of acetate addition to and removal from the α-NH_2-residue of proteins and peptides.

We are investigating enzymes which may function in the catabolism of α-*N*-acetylated proteins and peptides. We believe that two enzymes catalyze the ultimate release of acetate and the NH_2-terminal amino acids from acetylated NH_2-terminal peptides generated during intracellular protein turnover. These reactions are:

* Present address: University of Minnesota, Department of Biochemistry, 140 Gortner Laboratory, 1479 Gortner Avenue, St. Paul, MN 55108, U.S.A.

Abbreviations: EGTA, ethyleneglycol bis(β-aminoethyl ether)-*N,N'*-tetraacetic acid; SDS, sodium dodecyl sulfate.

I. α-*N*-acetylpeptide$_{n+1}$ + H_2O →
Peptide$_n$ + α-*N*-acetylamino acid
II. α-*N*-acetylamino acid + H_2O →
acetate + amino acid.

We have described [3] the isolation and partial characterization of bovine liver α-*N*-acetylpeptide hydrolase, which catalyzes reaction I. In this report we present a scheme for the isolation of the bovine liver enzyme, α-*N*-acylamino acid hydrolase, which catalyzes reaction II, and some of the characteristics of this enzyme. Other investigators have described enzymes which catalyze the hydrolysis of α-*N*-acetylated amino acids [4–8]. In most cases, however, these studies have not utilized purified enzymes and have not associated the enzymatic activity with protein turnover. Of these enzymes, acylase I from porcine kidney has been the most extensively studied and is the only other enzyme of this class to be purified to homogeneity [7].

Materials and Methods

Materials. Bovine liver purchased from a local butcher shop was satisfactory starting material for the isolation of α-*N*-acylamino acid hydrolase. The amino acids and *N*-acetylated amino acids were purchased from Bachem, Inc., Sigma or Schwarz/Mann Research Corp. DEAE-cellulose (DE-52 preswollen), hydroxyapatite and Sephadex were purchased from Whatman, Bio-Rad and Pharmacia, respectively. All fluorescence measurements were made using a Farrand Mark I Spectrofluorimeter.

Buffers. The following buffers were used routinely in these studies. Buffer A: 50 mM potassium phosphate (pH 7.5)/1 μM $ZnCl_2$; Buffer B: 10 mM Tris-HCl (pH 7.8)/1 μM $ZnCl_2$. The pH values were determined at 25°C.

Acylamino acid hydrolase assay. Acylamino acid hydrolase was assayed by measuring the rate of hydrolysis of *N*-acetyl-L-methionine during incubations with protein containing fractions from the various steps in the isolation procedure. The amount of methionine released was measured using a modification of the *o*-phthaldialdehyde fluorescence assay for amino acids described by Roth [9]. The incubation mixture contained 2 mM acetylmethionine in Buffer A and aliquots of the enzyme from the stages of the preparation containing between 950 and 0.5 μg protein in a total volume of 250 μl. The reaction mixture, without enzyme, was pre-warmed to 30°C and hydrolysis was started by addition of enzyme. At 1 min intervals after initiation of the reaction 25 μl aliquots were removed from the reaction mixture and placed into 10 μl of 60% perchloric acid to stop hydrolysis. Upon completion of the time course 3 ml of the *o*-phthaldialdehyde reagent (see below) was mixed with each sample. After standing for at least 5 min at room temperature the fluorescence was measured (excitation 340 nm, emission 455 nm). Standards containing concentrations of methionine ranging from 0 to 2 mM were treated as just described. Quantitation was accomplished by comparison of the fluorescence yields of the unknowns with those of the standards. Under these conditions a unit of enzyme activity is equivalent to the appearance of 1 μmol methionine/min. To screen large numbers of samples for acylamino hydrolase 5 μl of of the various fractions or appropriate dilutions of these fractions were added to 20 μl of 2 mM acetylmethionine in Buffer A and incubated at 30°C for 5 min. Hydrolysis was stopped by addition of 10 μl of 60% perchloric acid. The methionine released was then measured as described above.

o-Phthaldialdehyde reagent. The *o*-phthaldialdehyde reagent was prepared by adding 25 μl of a mixture of 2-mercaptoethanol/ethanol (15 μl/ml) to 60 ml 0.5 M sodium borate buffer (pH 9.5). Then, 200 μl of an *o*-phthaldialdehyde solution (20 mg/ml ethanol) was added.

Other assays. Protein determinations were done by a modification of the method of Lowry et al. as described by Hartree [10]. The fluorescamine assay for peptides was described in an earlier report [3].

Purification of acylamino acid hydrolase. The purification described below utilized 500 g bovine liver. All steps of the purification scheme were done at 0–4°C.

Step 1. The bovine liver was diced, suspended in 3 vol. Buffer A and homogenized with two 30 s bursts from a Willems Polytron (type 45TE, Bronwill Scientific). The cell debris were removed by centrifugation at 13 000 × *g* for 30 min. The supernatant fractions were combined to yield the crude extract.

Step 2. Protamine sulfate fractionation. The crude

extract was adjusted to 0.2% protamine sulfate by addition of a 1% protamine sulfate solution in buffer A [11]. After mixing for 60 min, the suspension was centrifuged at 13 000 × *g* for 30 min to remove the precipitated nucleic acids. Essentially all of the enzyme activity remained in the supernatant fraction.

Step 3. $(NH_4)_2SO_4$ precipitation and pH 3.5 extraction. The supernatant fraction (1860 ml) from Step 2 was brought to approx. 50% saturation by addition of 550 g $(NH_4)_2SO_4$ [12]. After standing for 16 h the precipitated material was isolated by centrifugation at 13 000 × *g* for 15 min. The pellets were suspended in a total of 1 l of 50 mM potassium citrate buffer (pH 3.5)/1 mM $ZnCl_2$. The suspension was stirred for 30 min and then centrifuged at 13 000 × *g* for 15 min. The resulting pellets were suspended in 500 ml Buffer A and stirred for 4 h. The insoluble material was then removed by centrifugation as described above. The resulting supernatant fraction contained the acylamino acid hydrolase. Additional extractions of the insoluble material did not significantly increase the yield of the enzyme.

Step 4. DEAE-cellulose chromatography. The supernatant fraction from the preceding step was dialyzed against Buffer B containing 0.1 M KCl (2 changes, 10 l each). This dialyzed solution was fractionated by chromatography on a column of DEAE-cellulose as described in Fig. 1.

Step 5. Hydroxyapatite chromatography. The acylamino acid hydrolase containing material

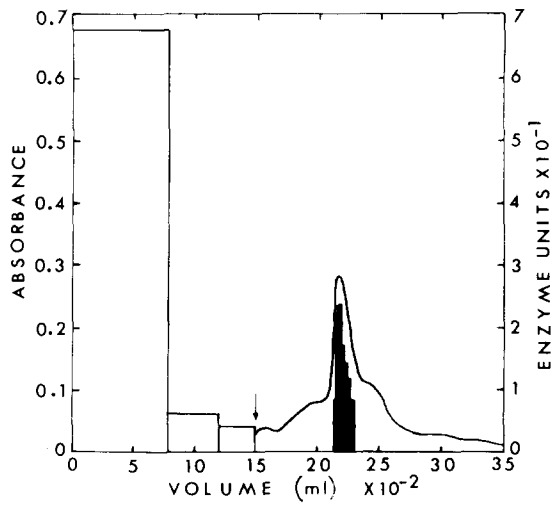

Fig. 1. Elution of acylamino acid hydrolase from DEAE-cellulose using a linear gradient of KCl. The dialyzed enzyme solution from Step 3 (see text) was applied to a column (2.4 × 40 cm) of DEAE-cellulose which had been equilibrated with Buffer B/0.1 M KCl. The column was then washed with this buffer until the absorbance (280 nm) of the eluant fell to essentially zero. At this point (indicated by the arrow) elution with a 2 l linear gradient of KCl (0.1–0.3 M) in Buffer B was initiated. The open columns indicate the absorbance (280 nm) of the material which was not adsorbed to the column under the initial conditions and of the material removed by washing with Buffer B/0.1 M KCl. The solid line represents the absorbance (280 nm) of the fractions (15 ml) collected during the elution with the KCl gradient. The fractions were assayed for acylamino acid hydrolase as indicated in Materials and Methods. Enzyme activity is indicated by the solid bars. The peak of activity was eluted at approx. 0.165 M KCl.

TABLE I

PURIFICATION OF ACYLAMINO ACID HYDROLASE

Assay conditions are as described. Units are defined as μmol acetylmethionine hydrolized/min.

Step	Protein (mg)	Enzyme activity (units)	Specific activity	Recovery (%)	Purification (-fold)
1 Crude extract	66 500	5 900	0.09	100	–
2 Protamine sulfate precipitation	31 200	5 580	0.18	94	2
3 $(NH_4)_2SO_4$ precipitation and pH 3.5 extraction	980	2 875	2.93	49	33
4 DEAE-cellulose	43	1 825	42.4	31	471
5 Hydroxyapatite	17	1 750	103	30	1 144
6 Sephadex G-150	11	1 660	151	28	1 678

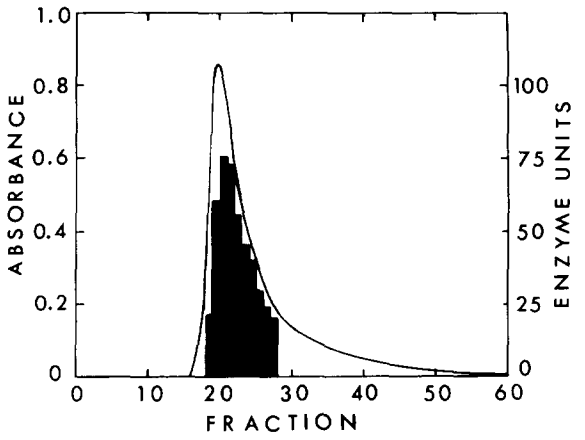

Fig. 2. Chromatography of acylamino acid hydrolase on hydroxyapatite. The pool of fractions (150 ml) from the DEAE-cellulose column containing acylamino acid hydrolase was applied directly to a column (1.5 × 15 cm) of hydroxyapatite equilibrated with Buffer B/100 mM KCl. The column was then washed with an additional 100 ml of the equilibration buffer. No material absorbing at 280 nm was present in the eluant collected during sample application and the subsequent wash. Elution of the enzyme was accomplished by use of a 250 ml linear gradient of 100 mM KCl/1 μM $ZnCl_2$ to 50 mM potassium phosphate (pH 7.4)/20 mM KCl/1 μM $ZnCl_2$. This gradient enabled elution to be conducted at approx. constant ionic strength. The solid line represents the absorbance at 280 nm of the fractions (4 ml) collected during elution. The enzymatic activity (represented by the solid bars) was measured as described in Materials and Methods.

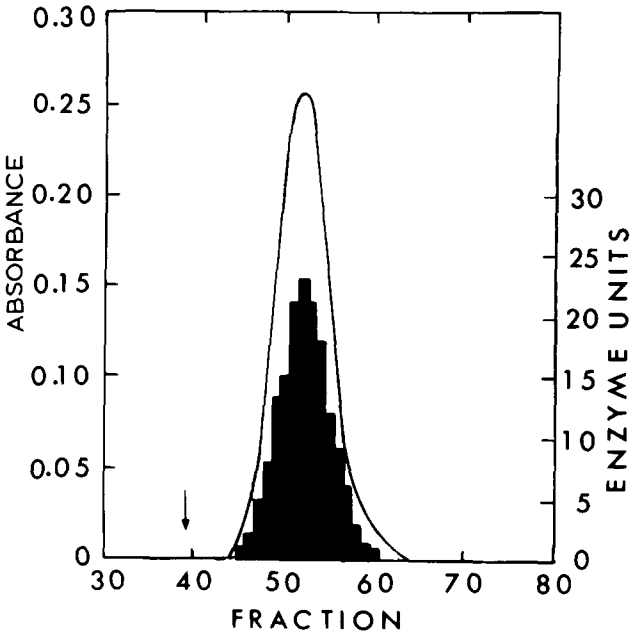

Fig. 3. Gel filtration of acylamino acid hydrolase. The concentrated sample of enzyme from Step 5 of the purification scheme was pumped onto the bottom of a column (2.5 × 85 cm) of Sephadex G-150 equilibrated with Buffer A/100 mM KCl. The column was eluted with the same buffer using upward flow at the rate of 20 ml/h. The void volume of the column is indicated by the arrow. The solid line represents the absorbance at 280 nm of each fraction (4 ml). Acylamino acid hydrolase activity (represented by the solid bars) was measured as described in Materials and Methods..

obtained in the previous step was applied directly to a column of hydroxyapatite. The details of this step are described in Fig. 2. The fractions containing significant levels of enzyme were combined and concentrated to approx. 1 ml by vacuum dialysis against Buffer A/100 mM KCl.

Step 6. Sephadex G-150 gel filtration. The concentrated sample of acylamino acid hydrolase was applied to a column of Sephadex G-150 and eluted as described in Fig. 3. The fractions containing enzyme activity were pooled and the resulting material concentrated by vacuum dialysis against Buffer A.

A summary of the purification scheme is presented in Table I.

Results

Evaluation of the purity of acylamino acid hydrolase

The results of electrophoresis of aliquots of the material obtained from Step 6 of the purification scheme in two different polyacrylamide gel systems are shown in Fig. 4. In other experiments using polyacrylamide gel electrophoresis, non-denaturing gels [13] were stained for acylamino acid hydrolase activity using the method described by Sugiura et al. [15] for the detection of peptidase activity. Aliquots from Steps 1 and 6 of the purification scheme containing 0.3 units of enzyme activity were electrophoresed as described in Fig. 4. The gels were then incubated at 30°C for 30 min in 50 ml of 50 mM Tris-HCl (pH 8.0) plus 1 mM $ZnCl_2$ containing 25 mg 3-(*p*-iodophenyl)-2-(*p*-nitrophenyl)-5-phenyl-2H-tetrazolium chloride, 5 mg phenazine methosulfate, 10 units L-amino acid oxidase and 25 mg acetylmethionine as substrate. Control samples were treated as described above except acetylmethionine was not included in the incubation mixture. Enzymatic

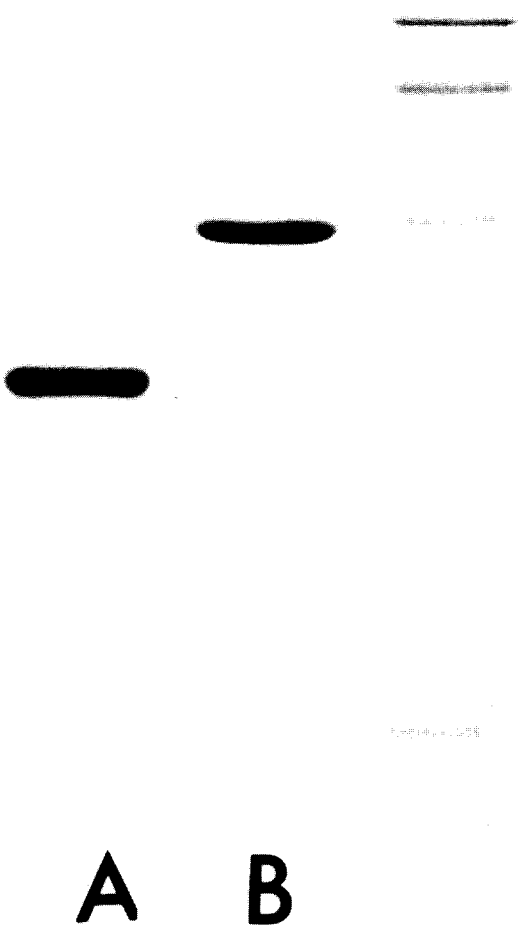

Fig. 4. Evidence for purity of acylamino acid hydrolase. Samples of the material from Step 6 of the purification scheme were examined by polyacrylamide gel electrophoresis in the systems described by Davis [13] and by Laemmli and Jonathan [14]. In each case 20 μg protein were applied to the gels. The gels were stained with Coomassie Blue. The figure is a composit of 2 gels. Tracts A and B show the results obtained in the Davis and the Laemmli and Jonathan systems, respectively. The tract adjacent to B contains a series of standards. In order of distance migrated from the top of the gel the identity and monomer weights of these standards are: Phosphorylase *B* (92 500), bovine serum albumin (67 000), ovalbumin (43 000), carbonic anhydrase (31 000), soybean trypsin inhibitor (21 000) and lysozyme (14 000).

activity was indicated by a red band of the reduced tetrazolium salt. A single band of enzymatic activity was observed in both the crude and purified enzyme preparations. This activity co-migrated with Coomassie Blue staining material observed after electrophoresis of the purified enzyme. These results suggest that acylamino acid hydrolase is the major and probably the only enzyme in liver which catalyzes the hydrolysis of acetylmethionine.

Substrate specificity of acylamino acid hydrolase

The relative rates of hydrolysis of various *N*-acylamino acids in the presence of α-*N*-acylamino acid hydrolase are shown in Table II. Of the acetylamino acids tested as substrates, certain of those with nonpolar side chains were the most rapidly hydrolyzed, i.e., acetylmethionine, acetylleucine and acetylalanine. Acetylvaline and acetylglycine, however, were cleaved at a substantially lower rate than the other compounds of this class. The chloroacetyl derivatives of valine and leucine were hydrolyzed 8-

TABLE II

RELATIVE RATES OF HYDROLYSIS OF ACYLAMINO ACIDS IN PRESENCE OF α-*N*-ACYLAMINO ACID HYDROLASE

All relative rates of hydrolysis were determined using the standard *o*-phthaladialdehyde assay conditions as described. All rates are expressed relative to the rate of hydrolysis of acetylmethionine (160 μmol/min per mg protein).

Substrates	Relaitve rates
Ac-Met	100
ClAc-Leu [a]	80
f-Met	60
ClAc-Val	38
Ac-Leu	25
Ac-Ala	24
Ac-Glu	20
Ac-Val	5
Ac-Gly	4
Ac-Asn	4
Ac-Phe	0.8
Ac-Tyr	0.2
Ac-His	0.6
Ac-Asp	0.6
Ac-β-Ala	0
Ac-D-Ala	0

[a] ClAc, chloroacetyl and f-Met, formylmethionine.

and 3-times, respectively, faster than the corresponding acetyl derivatives, but *N*-formylmethionine was not cleaved as rapidly as *N*-acetylmethionine. α-*N*-Acylamino acid hydrolase distinguishable between acetylalanine and the acetyl derivatives of D-alanine and β-alanine. The latter two compounds were not hydrolyzed in presence of this enzyme. *N*-Acetyllysine, acetylarginine, acetylcystiene and acetylglycinamide were incubated with acylamino acid hydrolase for as long as 2 h. No hydrolytic products could be detected by the standard assay or when aliquots of these incubation mixtures were subjected to paper electrophoresis at either pH 1.9 or 6.5, using 3000 V for 30 min followed by staining with ninhydrin. The unhydrolyzed substrates, however, could be detected by the method described by Netecki and Goodman [16]. Under the same conditions as described above, methionine and alanine could be easily identified when acetylmethionine and acetylalanine were incubated with the enzyme. The acetyl derivatives of dialanine, trialanine and tetraalanine were tested as substrates for acylamino acid hydrolase as described previously [3]. Hydrolytic products could not be detected when aliquots of these reaction mixtures were assayed by either the *o*-phthaldialdehyde or fluorescamine assays. Increasing the length of the incubation period did not alter these results. The results of the substrate specificity studies suggest that α-*N*-acylamino acid hydrolase requires substrates composed of certain L-amino acids which have acylated α-amino groups and free α-carboxyl groups. All substrates tested that met these requirements, however, were not hydrolyzed. The basis for the differences in the rates of hydrolysis of some substrates and the failure to hydrolyze others are unknown. It is clear, however, that the rates of hydrolysis are influenced by the identity of both the amino acid and the acyl group.

pH optimum

The apparent pH optimum of α-*N*-acylamino acid hydrolase was approx. 8.5. However, enzyme activity was greater than 90% of maximal in the pH range from 6.0 to 10.3. The enzyme activity decreased rapidly in acidic range and no activity could be detected at pH 3.8. Greater than 90% of the original activity was restored when the pH was readjusted to 7.8 by dialysis against Buffer B.

Effect of sulfhydryl reagents

α-*N*-Acylamino acid hydrolase was completely inactive in the presence of 1 mM levels of either dithiothreitol or β-mercaptoethanol. The activity of this enzyme was not altered by incubation with 10 mM iodoacetamide or with 1 mM *p*-chloromercuribenzoyl sulfonic acid.

Metal ion requirements

α-*N*-Acylamino hydrolase was inactivated by a 30 min incubation (30°C) with 1 mM 1,10-phenanthroline in Buffer A. Similar treatment of the enzyme with 1 mM EDTA or 1 mM EGTA, however, did not reduce its ability to catalyze the hydrolysis of acetylmethionine. After treatment with 1,10-phenanthroline and dialysis against Buffer A to remove the chelator, complete enzymatic activity could be restored by addition of 1 μM levels of Zn^{2+}. Other divalent cations including Ca^{2+}, Cd^{2+}, Co^{2+}, Cu^{2+}, Fe^{2+}, Mg^{2+} and Mn^{2+} at concentrations up to 1 mM failed to reactivate the 1,10-phenanthroline-treated enzyme. These results are consistent with the idea that acylamino acid hydrolase requires Zn^{2+} for activity.

Enzyme stability

α-*N*-Acylamino acid hydrolase is relatively stable when stored in the presence of Zn^{2+} (i.e., Buffer A or B) at 4°C. Under these conditions, acylamino hydrolase loses less than 20% of its activity per month. Enzyme activity in solutions containing less than 300 μg protein/ml deteriorated more rapidly. For example, storage of the enzyme at a protein concentration of 30 μg/ml resulted in loss of activity of approx. 35% per week.

Enzyme and subunit sizes

The molecular weight of α-*N*-acylamino hydrolase was estimated to be approx. 80 000 by gel filtration on a Sephadex G-150. The monomer weight of acylamino acid hydrolase was estimated to be approx. 40 000 by electrophoresis in polyacrylamide gels containing SDS.

Discussion

The α-*N*-acylamino acid hydrolase isolated in this study catalyzed the hydrolysis of *N*-acyl groups from

most of the α-*N*-acylated amino acids tested. Acetyl, chloroacetyl and formyl groups were all removed from appropriate substrates in the presence of this enzyme. α-*N*-Acylamino acid hydrolase did not catalyze the hydrolysis of acetate from the *N*-acetylated derivatives of D-alanine or β-alanine nor from ε-*N*-acetyllysine. Neither *N*-acetylglycinamide nor *N*-acetylated peptides were substrates for this enzyme. These observations indicate that α-*N*-acylamino acid hydrolase is specific for the removal of acyl residues, probably acetate in vivo, from the α-amino group of L-amino acids.

Of the other enzymes described that hydrolyze α-*N*-acylamino acids to yield the acyl group and the free amino acids [4–8], acylase I [4–7] from porcine kidney is the most thoroughly studied. While the substrate specificity of acylase I is somewhat different from that of bovine liver α-*N*-acylamino acid hydrolase, these enzymes have many features in common. For example, both enzymes cleave acetylmethionine more rapidly than the other α-*N*-acetylated amino acids. They both catalyze the hydrolysis of most of the *N*-acetylated amino acids but in both cases the acetylated nonpolar amino acids are hydrolyzed more rapidly. These enzymes also cleave *N*-acetylglutamate relatively efficiently but not *N*-acetylasparatate. Neither enzyme releases acetate from the *N*-acetylated basic amino acids. Other common features include a similar size and subunit composition, i.e., both enzymes are dimers consisting of monomers of approx. 40 000 molecular weight. In addition, both enzymes apparently require Zn^{2+} for activity, both have a pH optimum of approx. 8.5, and they are not affected by reagents that react with sulfhydryl groups but are inactivated by reagents that reduce disulfide bonds. Thus, although there are some differences in the relative rates of hydrolysis of specific acetylated amino acids by α-*N*-acylamino acid hydrolase and acylase I, these differences appear to be minor, and it is likely that both enzymes serve similar, if not identical, functions in the liver and kidney, respectively. The specific functions of these enzymes, however, have not been established.

The impetus for isolating α-*N*-acylamino acid hydrolase from bovine liver was provided by our earlier studies on enzymes that may be involved in the catabolism of α-*N*-acetylated proteins. These studies lead to the isolation of an enzyme, α-*N*-acylpeptide hydrolase, which cleaves α-*N*-acetylated peptides to yield an α-*N*-acetylated amino acid and the corresponding peptide [3]. Based on this observation and the knowledge that turnover of α-*N*-acetyl groups is dependent upon protein turnover [1] we postulated a mechanism for the catabolism of α-*N*-acetylated proteins that requires an initial attack by endoproteases to yield an *N*-acetylated peptide [3]. α-*N*-Acylpeptide hydrolase then removes the *N*-acetylated amino acid and finally α-*N*-acylamino acid hydrolase cleaves the *N*-acetylamino acid to yield acetate and the corresponding amino acid. If this mechanism is correct, both α-*N*-acylpeptide hydrolase and α-*N*-acylamino acid hydrolase must be present in the same cells since *N*-acetylated amino acids do not appear to cross plasma membranes [17] and thus, cannot be transported to other cells for their eventual metabolism. We have now satisfied this requirement of the model by showing that both α-*N*-acylpeptide hydrolase and α-*N*-acylamino acid hydrolase are components of liver cells.

Data obtained in this and other studies also support the proposed model. For example, alanine, glycine, and serine are the major *N*-acetylated terminals identified for proteins isolated from a variety of sources [2]. Our preliminary results (Gade, W., unpublished data) show that these amino acids are also the major acetylated terminal residues in the soluble proteins from Ehrlich ascites cells, L-cells and *Neurospora crasa*. α-*N*-Acylpeptide hydrolase efficiently removes acetylalanine, acetylglycine and acetylserine from the terminals of all the small (less than six amino acids) peptides we have tested [3], and bovine liver α-*N*-acylamino acid hydrolase efficiently cleaves acetylalanine and acetylglycine. We have not yet tested *N*-acetylserine as a substrate for the bovine liver enzyme. Acylase I, however, hydrolyzes acetylserine at about the same rate as acetylalanine. Based on these observations it appears that α-*N*-acylpeptide hydrolase and α-*N*-acylamino acid hydrolase have the proper substrate specificities to function as we propose in the intracellular breakdown of α-*N*-acetylated proteins.

In addition to the proper substrate specificity, the turnover numbers calculated for α-*N*-acylpeptide hydrolase and α-*N*-acylamino acid hydrolase are in reasonable agreement with the turnover number calculated for cathepsin C. The latter enzyme is a

lysosomal enzyme that appears to function in the degradation of nonacetylated peptides produced by the action of intracellular endopeptidases [18] during protein catabolism. The turnover number for α-*N*-acylpeptide hydrolase estimated from the rate of hydrolysis of *N*-acetyltrialanine is approx. 500 mol of substrate/mol enzyme per s. Based on the rate of hydrolysis of *N*-acetylmethionine the turnover number for α-*N*-acylamino acid hydrolase is approx. 200 mol substrate/mol enzyme per s. By comparison, the turnover number for cathepsin C is approx. 100 when this enzyme is assayed in vitro using glycyl-L-phenylalanylamide acetate as substrate [18]. Additionally, the K_m values (Gade, W., unpublished data) for α-*N*-acylpeptide hydrolase and α-*N*-acylamino acid hydrolase are approx. 1 mM. These values are in good agreement with the K_m measured for cathepsin C [18]. Other calculations show that the average intracellular concentrations of α-*N*-acylamino acid hydrolase and α-*N*-acylpeptide hydrolase, estimated from the enzyme units in the crude extracts of bovine liver and the specific activities of the purified enzymes, are approx. 0.2 and 0.1 mM, respectively. These levels are adequate to prevent significant accumulation of α-*N*-acetylated peptides and amino acids as a result of the intracellular degradation of α-*N*-acetylated proteins. While we do not have direct proof that α-*N*-acylpeptide hydrolase and α-*N*-acylamino acid hydrolase function as proposed in the model, the substrate specificities of these enzymes and the observations that both enzymes are present in the same cells, that their turnover numbers and K_m values are similar to another enzyme involved in the intracellular catabolism of proteins, and that the levels of these enzymes are sufficient to perform their suspected function are all compatable with the proposed roles for these enzymes in the catabolism of α-*N*-acetylated proteins.

Acknowledgements

This investigation was supported by Grant No. CA 12574, awarded by the National Cancer Institute, DHEW.

References

1 Brown, J.L. (1979) J. Biol. Chem. 254, 1447–1449
2 Brown, J.L. and Roberts, W.K. (1976) J. Biol. Chem. 251, 1009–1014
3 Gade, W. and Brown, J.L. (1978) J. Biol. Chem. 253, 5012–5018
4 Birnbaum, S.M., Levintow, L., Kingsley, R.B. and Greenstein, J.P. (1952) J. Biol. Chem. 194, 455–470
5 Baslow, M.H. and Lenney, J.F. (1967) Can. J. Biochem. 45, 337–340
6 Goldstein, F.B. (1976) J. Neurochem. 26, 45–49
7 Kordel, W. and Schneider, F. (1976) Biochim. Biophys. Acta 445, 446–475
8 Krisch, K. (1963) Biochem. Z. 337, 531–545
9 Roth, M. (1971) Anal. Chem. 43, 880–882
10 Hartree, E.E. (1972) Anal. Biochem. 48, 422–427
11 Rhodes, C., Germershausen, J. and Suskind, S.R. (1971) Methods Enzymol. 22, 80–86
12 Di Jeso, F. (1968) J. Biol. Chem. 243, 2022–2023
13 Davis, B.J. (1964) Ann. N.Y. Acad. Sci. 121, 404–427
14 Laemmli, U.K. and Jonathan, K. (1971) J. Mol. Biol. 62, 465–467
15 Sugiura, M., Ito, Y., Hirano, K. and Sawaki, S. (1977) Anal. Biochem. 81, 481–484
16 Netecki, D.E. and Goodman, J.W. (1966) Biochemistry 5, 665–672
17 Hokin, L.E. and Hokin, M.R. (1963) Annu. Rev. Biochem. 32, 553–578
18 Mycek, M.J. (1970) Methods Enzymol. 29, 285–315