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PROPERTIES OF FRUCTOSE-1,6-BISPHOSPHATE ALDOLASE INACTIVATING ENZYMES IN RAT LIVER LYSOSOMES

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Summary

The intralysosomal localization of the enzymes that catalyse inactivation of rat liver fructose-bisphosphate aldolase (D-fructose-1,6-bisphosphate D-glyceraldehyde-3-phosphate-lyase, EC 4.1.2.13) to a form with antigenic activity was demonstrated. The inactivating enzymes like all other lysosomal markers tested except acid phosphatase, were readily solubilized by hypotonic shock. The inactivating enzyme activity was inhibited by PMSF, TPCK, TLCK and leupeptin, but not by pepstatin. On partial purification of the inactivating activity from the lysosomal fraction by DEAE-Sephadex (A-50) and Sephadex G-100 column chromatographies, it was copurified with lysosomal carboxypeptidase A and cathepsin B (EC 3.4.22.1). Studies on its substrate specificity and sensitivity to inhibitors indicated that cathepsin B and carboxypeptidase A are responsible for almost all the aldolase-inactivating activity in the lysosomal fraction.

Introduction

Proteolytic modification is known to be one mechanism of regulation of enzyme activity [1]. A typical example of this is seen in the regulation of rabbit liver fructose bisphosphatase [2]: increase of specific activity of fructose

Abbreviations: PMSF, phenylmethylsulfonyl fluoride; TPCK, *p*-tosylamide-2-phenylethylchloromethyl ketone; TLCK, *N*-α-tosyl-L-lysyl chloromethyl ketone; PCMB, *p*-chloromercuribenzoic acid; Bz-Arg-β-naphthylamide,α-*N*-benzoyl-DL-arginine-β-napthylamide; Leu-β-naphthylamide, L-leucine-β-napthylamide; Bz-Arg-*p*-nitroanilide, α-*N*-benzoyl-DL-arginine 4-nitroanilide; Z-Glu-Phe, carbobenzoxy L-glutamyl-L-phenylalanine.

bisphosphatase associated with alteration of molecular structure of the enzyme was observed in gluconeogenetic states such as fasting [3], diabetes [4] and administration of glucocorticoid [4], and these changes in the molecular properties of fructose bisphosphatase were shown to involve lysosomal proteases [2].

Previously [5], we showed that proteolytic modification of liver aldolase occurs in rats in vivo on administration of leupeptin, a thiol protease inhibitor of microbial origin, and suggested lysosomes contain proteolytic activity that catalyses inactivation of aldolase [5].

In this work we have examined the subcellular and intralysosomal distribution of aldolase-inactivating activity and characterized this enzyme activity. Results on the substrate specificities and sensitivity to protease inhibitors of partially purified enzymes indicated that most of the aldolase-inactivating activity in the lysosomal fraction of rat liver is due to cathepsin B and carboxypeptidase A. Carboxypeptidase A in this paper means lysosomal carboxypeptidase A. The term, lysosomal carboxypeptidase A instead of cathepsin A was used here following the suggestion of Barrett [6].

Materials and Methods

Materials

N-Benzoyl-DL-arginine-*p*-nitroanilide, azocasein, AMP, ATP, glucose 6-phosphate, cytochrome *c*, *N*-α-*p*-tosyl-L-lysyl chloromethylketone HCl, *N*-α-*p*-tosyl-L-phenylalanyl chloromethylketone and phenylmethanesulfonyl fluoride were purchased from Sigma Chemical Co. Z-L-Glutamyl-L-phenylalanine, Z-L-glutamyl-L-tyrosine, Z-L-glycyl-L-phenylalanine. glycyl-L-phenylalanine-NH_2, Z-L-glycyl-L-alanine-NH_2, Z-L-glycyl-L-prolyl-L-leucine, Z-L-tyrosyl-L-glutamic acid, Z-L-phenylalanine-L-tyrosine, Z-L-alanyl-L-leucine-NH_2, pepstatin and leupeptin were from the Peptide Center (Institute for Protein Research). Triose phoshate isomerase, glycerol-3-phosphate dehydrogenase and NADH (sodium salt) were from Boehringer Mannheim, F.R.G. Hemoglobin and *p*-phenylphosphate were from Wako Pure Chemical Industry Co., Kyoto. Triton WR-1339 was from Nakarai Chemicals Co. DEAE-Sephadex (A-50) and Sephadex G-100 were from Pharmacia Fine Chemicals. All other reagents were commercial analytical grade products. The lysosomal fraction was isolated from livers of rats as described previously [5]. The final pellet from 3 g liver was suspended in 3 ml of 0.25 M sucrose/1 mM EDTA, pH 7.0. After two cycles of freeze-thawing, the supernatant solution was obtained by centrifugation at 105 000 × *g*, 30 min, this was used as the source of the aldolase-inactivating enzyme.

Preparation of Triton WR-1339-filled lysosomes ('Tritosomes')

Male Wistar rats weighing 300—350 g were used. Rats were injected intraperitoneally with Triton WR-1339 at a dose of 85 mg/100 g body weight, 84 h before experiments as described by Leighton et al. [17]. They were starved for 24 h before experiments and killed by decapitation. 'Tritosomes' were prepared from rat liver by the procedure of Yamamoto et al. [18].

Subfractionation of 'tritosomes' into membranes and contents

'Tritosomes' were suspended in 0.025 M sucrose (pH 7.0), left to stand for

several hours at 0°C, and then centrifuged at 105 000 × *g* for 60 min to obtain a pellet and supernatant ('tritosomal' contents). The pellet was resuspended in 1 M NaCl solution, and centrifuged at 105 000 × *g* for 60 min to obtain a supernatant (1 M NaCl washings) and a pellet ('tritosomal' membranes).

Enzyme assays

Acid phosphatase (EC 3.1.3.2) was assayed with *p*-nitrophenyl phosphate as substrate [9]. Cathepsin B was measured by the method of Otto and Bhakdi [10] with benzoyl-DL-arginine *p*-nitroanilide as substrate. Cathepsin L and cathepsin D were determined by the methods of Kirschke et al. [11] and Matsuda and Misaka [12], respectively, as modified by Kominami et al. [5]. Lysosomal carboxypeptidase A activity was assayed with Z-Glu-Phe as substrate [5]. Assays with β-napthylamide substrates were quantitated by coupling the resulting products with Fast Garnet GBC in the reaction mixture as described by Barrett [13].

Cytochrome oxidase (EC 1.9.3.1) activity was measured spectrometrically by following oxidation of reduced cytochrome *c* at 550 nm, at 25°C, as described by Wharton and Tzagoloff [14]. Glucose-6-phosphatase (EC 3.1.3.9) activity was determined by the method of Swanson [15]. The phosphate liberated was estimated by the method of Fiske and SubbaRow [16]. Catalase (EC 1.11.1.6) activity was measured by the procedure of Cohen et al. [17]. 5-Nucleotidase (EC 3.1.3.5) activity was measured by determining the release of inorganic phosphate from 5′-AMP [18]. Aldolase-inactivating activity was estimated by following the decrease in aldolase activity measured at pH 6.0; the assay mixture contained 20 μg aldolase and lysosomal fractions as indicated in a total volume of 0.1 ml of 50 mM potassium phosphate buffer, pH 6.0. The activity of aldolase was measured before addition of the lysosomal fraction and after incubation at 25°C for 1, 1.5, 3.0 and 4.5 min. 1 unit of inactivating activity was defined as $\ln[E_o] - \ln[E_t]/t$ where [E] is aldolase activity and $[E_t]$ is the activity after (t) min of incubation with inactivating enzyme.

Results

Localization of aldolase-inactivating activity in subcellular fractions of rat liver

Previously, by administration of leupeptin in vivo we demonstrated that mitochondrial lysosomal fractions prepared from rat liver contained activity to form an inactive, or less active, aldolase of unchanged subunit size [5]. In this work we have examined whether the aldolase-inactivating activity is localized in the lysosomal fraction. To obtain lysosomes little contaminated with other organelles, we used Triton WR-1339-treated rats. As shown in Table I, the 'tritosome' preparation was essentially free of mitochondria, peroxisomes and endoplasmic reticulum, as judged from the absence of the marker enzyme cytochrome oxidase, catalase and glucose-6-phosphatase, respectively. High specific activity of 5′-nucleotidase, as plasma membrane marker, was found in the 'tritosome' preparation. The relative specific activity of cathepsin L, and more especially that of cathepsin B were higher than those of other lysosomal markers. In the step of Ly-1 (lysosomal mitochondrial fraction) preparation in which the cytosol fraction was removed the specific and total activities of

TABLE I

DISTRIBUTION OF ENZYMES AND PROTEINS IN 'TRITOSOMES' AND OTHER CELLULAR SUBFRACTIONS

R.S.A., relative specific activity (specific activity found in the fraction/specific activity of the homogenate). Ly-1, lysosomal mitochondrial fraction; Ly-2, repeatedly washed lysosomal mitochondrial fraction; Ly-3, 'tritosomes'.

Enzyme	Cytoplasmic extract		Ly-1		Ly-2		Ly-3	
	Yield (% homogenate)	R.S.A.	Yield (% homogenate)	R.S.A.	Yield (% homogenate)	R.S.A.	Yield (% homogenate)	R.S.A.
Protein	52.8		12.9		5.4		0.36	
Cathepsin B	67	1.25	115	8.75	103	18.9	79	216
Cathepsin L	58.1	1.09	43.7	3.33	28.3	5.21	18.2	50.0
Cathepsin D	49.1	0.91	26.2	1.99	21.1	3.83	9.7	26.6
Acid phosphatase	64.0	1.93	22.2	2.75	11.7	3.46	4.2	19.5
Aldolase-inactivating activity	67.0	1.27	23.0	1.64	16.1	4.38	7.6	20.8
Cytochrome oxidase	35.2	0.66	18.7	1.43	7.1	1.29	0.02	0.06
Catalase	46.7	0.87	23.4	1.78	11.0	2.0	0.003	0.008
Glucose-6-phosphatase	58.1	1.06	16.5	1.54	2.2	0.40	0.26	0.77
5'-Nucleotidase	40.9	0.75	5.2	0.49	1.3	0.24	0.46	1.36

cathepsin B and L increased. Recent work of Lenney et al. [19] has indicated the presence of a heat-stable inhibitor(s) of cathepsin B and H in rat liver and we have also obtained evidence for an inhibitor(s) of cathepsin B and cathepsin L in the cytosol fraction (unpublished data). Cathepsin B and L were assayed with fractions solubilized with 0.1% Triton X-100, however, change in the concentration of Triton X-100 did not affect the activity of the cathepsins in each fraction, indicating that the higher specific activity of cathepsin B and L over that of other lysosomal markers in 'tritosomes' were not due to unsuitable assay conditions. The relative specific activity of aldolase-inactivating activity was similar to those of lysosomal markers other than cathepsins B and L, indicating that the aldolase-inactivating activity is located in the lysosomal fraction.

The distribution of aldolase-inactivating activity and lysosomal enzyme activity in the tritosomal subfractions is shown in Table II. The data show that more than 85% of the total activity of all lysosomal marker enzymes, including aldolase-inactivating enzyme, other than acid phosphatase were solubilized by hypotonic shock, while 60% of the total acid phosphatase activity was still associated with the membranes after hypotonic shock and washing with 1 M NaCl. The behavior of these lysosomal markers on various treatments is consistent with the data of Yamamoto et al. [8].

Properties of aldolase-inactivating activity

As previously reported, the inactivation of aldolase in the lysosomal fraction with time, at pH 6.0, was linear up to 50%, and did not exceed 80% [5]. The reaction was also proportional to the amount of the lysosomal fraction added (data not shown), indicating that the inactivation reaction is enzymatic. The modification catalysed by the inactivating enzyme showed an optimum pH below 4, but since the activity of aldolase as substrate decreased rapidly below pH 4, the inactivating activity below pH 4 was not examined.

The aldolase-inactivating activity was almost completely inhibited by treat-

TABLE II

DISTRIBUTION OF ALDOLASE-INACTIVATING ACTIVITIES IN TRITOSOMAL SUBFRACTIONS

Enzyme	'Tritosomes' S.A.	Tritosomal subfractions					
		Content		1 M NaCl washings		Membranes	
		S.A.	yield (%)	S.A.	yield (%)	S.A.	yield (%)
Protein			56.8		15.9		27.3
Cathepsin B **	19.4	28.2	91	7.12	7.7	0.72	1.3
Cathepsin L **	57.5	67.9	72.8	66.9	21.3	11.3	5.9
Cathepsin D *	0.604	0.80	82.7	0.41	12.6	0.094	4.7
Acid phosphatase **	0.38	0.125	20.7	1.26	59.7	0.25	19.7
Carboxypeptidase A *	0.54	0.83	96.1	0.32	2.7	0.054	1.2
Aldolase-inactivating activity	0.66	0.89	84.3	0.53	14.5	0.025	1.2

* The specific activities (S.A.) of cathepsin D and carboxypeptidase A are shown in μmol leucine formed/min per mg protein.

** The specific activities of acid phosphatase, cathepsin B and cathepsin L are shown as nmol *p*-nitrophenol, μmol *p*-nitroanilide and A_{366}/min per mg protein, respectively.

TABLE III

EFFECTS OF PROTEASE INHIBITORS ON ALDOLASE-INACTIVATING ACTIVITY OF RAT LIVER LYSOSOMES

The lysosomal fraction from rat liver was suspended in 0.25 M sucrose/1 mM EDTA pH 7.0 and subjected to two freeze-thawing cycles, and then centrifuged at 105 000 × *g*, 30 min. The supernatant (2.1 mg/ml) was incubated at 25°C with various effectors for 10 min in 0.1 M potassium phosphate buffer, pH 6.0 and then the reaction was initiated by the addition of 20 μg purified aldolase. The reaction volume was 0.1 ml.

Inhibitor	Concentration	Aldolase-inactivating activity (% inhibition)
Leupeptin	10 μg/ml	78
E-64	10 μg/ml	80
Chymostatin	10 μg/ml	58
Pepstatin	10 μg/ml	2
PMSF	1 mM	79
TPCK	1 mM	88
TLCK	1 mM	73
EDTA	1 mM	1
Iodoacetate	0.1 mM	68
PCMB	0.05 mM	80

ment with TPCK, TLCK, PMSF, leupeptin or thiol inhibitors such as PCMB and iodacetate (Table III). Chymostatin was also slightly inhibitory, but pepstatin, a strong inhibitor of cathepsin D, had no effect. The results obtained with protease inhibitors suggest that PMSF-sensitive protease as well as thiol proteases such as cathepsin B and/or L is involved in inactivation of aldolase.

Partial purification of aldolase-inactivating enzyme from rat liver lysosomes

For identification of the aldolase-inactivating enzyme(s), the enzyme was partially purified. The lysosomal fraction of rat liver solubilized by one freeze-thawing cycle was fractionationed with 40—70% $(NH_4)_2SO_4$. The resulting precipitate was passed through a column of Sephadex G-25 equilibrated with 20 mM potassium phosphate buffer, pH 6.0, and active fractions were applied to a column of DEAE-Sephadex (A-50) equilibrated with 20 mM potassium phosphate buffer, pH 6.0. The column was washed with the same buffer, and then the enzyme was eluted with a linear gradient of 0—0.3 M NaCl/20 mM potassium phosphate buffer, pH 6.0. As shown in Fig. 1, most of the aldolase-inactivating activity was eluted as a broad peak with 0.15—0.25 M NaCl. On increasing the salt concentration, carboxypeptidase A activity against Z-Glu-Phe was eluted following cathepsin B activity Bz-Arg-β-naphthylamide hydrolysing activity without Leu-β-naphthylamide hydrolysing activity. The fractions containing aldolase-inactivating activity seemed to include both cathepsin B and carboxypeptidase A activities. The unadsorbed fraction contained Bz-Arg-β-naphthylamide and Leu-β-naphthylamide hydrolysing activities, but no aldolase-inactivating activity. These activities are possibly due to cathepsin H described by Kirschke et al. [20]. Consistent with these results, highly purified cathepsin H showed no aldolase-inactivating activity [21]. Cathepsin L is usually eluted with a lower salt concentration than cathepsin B [22], but no aldolase-inactivating activity due to cathepsin L was detected under the present experimental conditions.

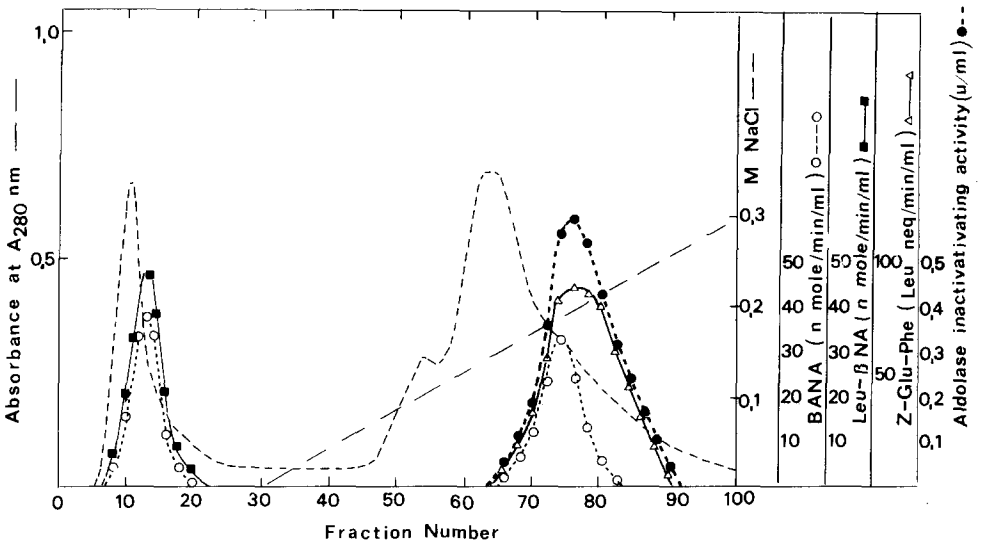

Fig. 1. DEAE-Sephadex (A-50) column chromatography of aldolase-inactivating enzyme(s) from rat liver lysosomes. DEAE-Sephadex column (2.6 X 15 cm) was equilibrated at 4°C with 10 mM potassium phosphate buffer, pH 6.0/5 mM 2-mercaptoethanol. The enzymes were eluted with a linear gradient formed with 200 ml vol. of buffer and buffer with 0.3 M NaCl. Fractions of 4.5 ml were collected at a flow rate of 50 ml/h.

The fractions containing aldolase-inactivating activity were pooled and concentrated by ultracentrifugation (Amicon PM-10) and then applied on a column of Sephadex G-100 equilibrated with 20 mM acetate buffer, pH 5.0/ 0.15 M NaCl. Two peaks of carboxypeptidase A were eluted, one in the void volume and a higher peak in later fractions (Fig. 2). Cathepsin B appeared as a symmetrical peak well separated from carboxypeptidase A. Aldolase-inactivating activity was recovered in the fractions containing carboxypeptidase A and in those containing cathepsin B. The apparent molecular weights of the major peaks of carboxypeptidase A and cathepsin B were calculated to be 91 000 and 27 000, respectively, on the basis of the positions of elution of molecular weight markers (data not shown). These values for the molecular weight are consistent with previously reported data [23,24]. Multiple forms of carboxypeptidase A from rat liver lysosomes were reported by Matsuda and Misaka [12], who separated three peaks of activity by Sephadex G-200 column chromatography. Since we used a Sephadex G-100 column, the high molecular weight form of carboxypeptidase A may not be separated into two fractions on our column. Thus, aldolase-inactivating enzyme was copurified with two enzymes, carboxypeptidase A and cathepsin B.

Therefore, we next examined the effects of various protease inhibitors on partially purified carboxypeptidase A and cathepsin B. The experiment was carried out with carboxypeptidase A preparation purified by Sephadex G-100 column chromatography. The enzyme activity was assayed with both Z-Glu-Phe and aldolase as substrates. Same inhibitors shown in Table III were used. Z-Glu-Phe hydrolysing and aldolase-inactivating activities were inhibited to similar extents by PMSF, and neither was inhibited by the other inhibitors

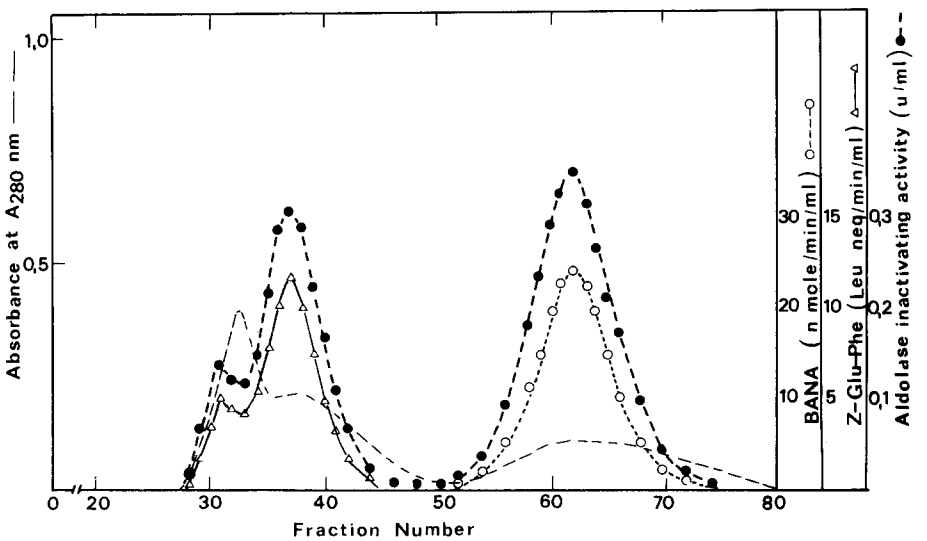

Fig. 2. Fractionation of aldolase-inactivating activity and cathepsin B and carboxypeptidase A. Concentrated eluate (2.5 ml, 9.8 mg) from a DEAE-Sephadex column (Fig. 1) was applied to a Sephadex G-100 column (1.6 × 90 cm) equilibrated at 4°C with 20 mM acetate buffer, pH 5.0/0.15 M NaCl/5 mM 2-mercaptoethanol. Material was eluted with the equilibrating buffer at a rate of 12 ml/h and fractions of 2.2 ml were collected.

tested. The optimal pH values for inactivation of aldolase and hydrolysis of Z-Glu-Phe were 4.0 and 5.3, respectively (data not shown). This fraction had high activity on Z-Glu-Phe, Z-Glu-Tyr and Z-Gly-Phe, which are often used for assay of carboxypeptidase A [25], but caused no appreciable hydrolysis of Z-L-Gly-Arg, or Z-L-Gly-Phe-NH_2, a substrate for endopeptidase, or L-Gly-Phe-NH_2, a substrate for aminopeptidase substrate. Esterolytic activity of the

TABLE IV

EFFECTS OF PROTEASE INHIBITORS ON CATHEPSIN B

The experiment was carried out with pooled fractions No. 55—69 from the column shown in Fig. 2. These fractions were concentrated by ultrafiltration (Amicon PM-10), a protein concentration of this solution was preincubated at 25°C with various effectors for 10 min in 0.1 M potassium phosphate buffer, pH 6.0, and aliquots were removed for assay of aldolase-inactivating activity (1 μg) and cathepsin B (15 μg).

Inhibitor	Concentration (mM)		Aldolase-inactivating activity (% inhibition)	Cathepsin B (% inhibition)
Leupeptin	10	μg/ml	88	82
E-64	10	μg/ml	89	83
Chymostatin	10	μg/ml	65	60
PMSF	1	mM	2	5
TPCK	1	mM	92	88
TLCK	1	mM	93	90
EDTA	1	mM	0	0
PCMB	0.05	mM	90	92
Iodoacetate	0.1	mM	85	80

enzyme against Ac-Tyr-OEt, Ac-Phe-OEt, Ac-Try-OEt or Bz-Arg-OEt was detectable. The preparation also had no effect on protein substrates, e.g., acid-denatured hemoglobin and azocasein. These results are consistent with those obtained in studies on the substrate specificity of carboxypeptidase A from pig kidney [25], indicating that carboxypeptidase A is one of the aldolase-inactivating enzymes in lysosomes.

The effect of protease inhibitors on partially purified cathepsin B are shown in Table IV. Leupeptin, TPCK and TLCK, which strongly inhibit the activity of cathepsin B [24], inhibited aldolase-inactivating activity. PMSF had no effect on either the Bz-Arg-*p*-nitroanilide cleaving or aldolase-inactivating activity. Thus, cathepsin B is also responsible for the aldolase-inactivating activity in the lysosomes. The optimum pH values for aldolase-inactivating activity and Bz-Arg-*p*-nitroanilide hydrolysing activity were 4.0 and 6.2, respectively (data not shown).

These results indicate that carboxypeptidase A and cathepsin B are major components of the inactivating activity of aldolase in lysosomes.

Discussion

In previous work [5] we found that decrease of aldolase activity by injection of leupeptin may be due to proteolytic modification by lysosomal protease(s).

Recently, it was reported that in rabbits prolonged starvation resulted in accumulation of an inactive, or less active, form of aldolase without loss of antigenic activity, as on injection of leupeptin, and the involvement of lysosomal protease(s) in decrease of liver aldolase was suggested [26].

In the present work we examined the aldolase-inactivating enzyme(s) in lysosomes. We determined the intralysosomal localization of aldolase-inactivating activity as shown in Table I. Otto and Bhakdi [10] reported that cathepsin B inactivated rabbit muscle aldolase preferentially, but later Kirschke et al. [11, 27] showed that Otto's preparation of cathepsin B contained three enzymes, B, L and H, and Towatari et al. [22] indicated that both cathepsin B and cathepsin L inactivate not only rabbit muscle aldolase but also rat liver aldolase. Bond and Barrett [21] recently showed that rabbit muscle aldolase was inactivated by cathepsin B and cathepsin L, but not by cathepsin H. Another possible enzyme involved in inactivation of aldolase is carboxypeptidase. It is well known that enzymic removal of the COOH-terminal tyrosine residue from rabbit liver aldolase results in about 40% inactivation of the enzyme [28]. Our preliminary data showed that incubation of rat liver aldolase with carboxypeptidase Y from yeast or carboxypeptidase A from bovine pancreas resulted in time-dependent enzyme inactivation in association with release of certain amino acids. Thus, cathepsin B, cathepsin L and lysosomal carboxypeptidase are possible aldolase-inactivating enzymes in lysosomes. On DEAE-Sephadex (A-50) or Sephadex G-100 column chromatography (Figs. 1 and 2), these enzymes are clearly separated. Most of the aldolase-inactivating activity was recovered in the fractions containing carboxypeptidase A or cathepsin B. Moreover, studies with protease inhibitors and on substrate specificity supported the idea that carboxypeptidase A and cathepsin B are responsible for the aldolase-inactivating activity in lysosomes (Table III and

IV). Petell and Lebherz [29] reported that inactivation of mouse liver aldolase, observed during tissue homogenization, was prevented by the addition of leupeptin and PMSF. These results are also consistent with our findings.

The mechanism of proteolysis of rabbit muscle aldolase by cathepsin B was investigated by Nakai et al. [30] and also recently by Bond and Barrett [21]. They demonstrated that cathepsin B caused release of the dipeptide Ala-Tyr from the carboxyterminal of the enzyme with loss of enzymatic activity. Petell and Lebherz [29] also reported that an inactive form of mouse liver aldolase produced during tissue homogenization did not have C-terminal tyrosine residues. Thus, not only rabbit muscle aldolase, but rat liver aldolase is inactivated by limited proteolysis around the carboxy terminal residues of the enzyme molecule. Preliminary results on this problem have been reported [31].

Previously, we reported [5] that the cytosolic activities of carboxypeptidase A and cathepsin B increased in the liver of rats treated with leupeptin, but that the increase in activity of free cathepsin B was much less than that of carboxypeptidase A. Unpublished observations showed that the free aldolase-inactivating activity in the liver of leupeptin-treated rats was completely inhibited by addition of PMSF, an inhibitor of carboxypeptidase A, but only partially inhibited by leupeptin, a specific inhibitor of cathepsin B. A possible explanation for these results is that a inhibitor of cathepsin B is present in the cytosol. The marked increase in total activity of cathepsin B on removal of the cytosol shown in Table I also supports the idea of the existence of this inhibitor, and in fact, heat-stable inhibitors of cathepsin B and H in rat liver were demonstrated [19]. The problems of the interaction of free cathepsin B activity with natural cathepsin B inhibitors and the inactivation of aldolase in vivo require further study.

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