Partial Purification and Characterization of a Pyruvate Dehydrogenase-Complex-Inactivating Enzyme from Rat Liver

By ANNEMARIE LYNEN, ERIKA SEDLACZEK and OTTO H. WIELAND

Forschergruppe Diabetes und Klinisch-chemisches Institut, Städtisches Krankenhaus München-Schwabing, Kölner Platz 1, 8000 München 40, West Germany

(Received 23 June 1977)

An enzyme inactivating the pyruvate dehydrogenase complex (inactivase) was purified about 8000-fold from rat liver by differential centrifugation, acid extraction of a lysosome-rich 25000g pellet, acetone fractionation, and adsorption on calcium phosphate gel. By exclusion chromatography on Sephadex G-100 a molecular weight of 21000 was estimated. The purified enzyme was most stable at pH 5.8 in potassium phosphate buffer, and at pH 4.5 in McIlvaine buffer. At high dilutions the enzyme was very labile and was remarkably stabilized by high salt concentrations. Enzyme activity is inhibited by native rat blood serum, iodoacetamide and leupeptin, but not by phenylmethanesulphonyl fluoride, suggesting that it belongs to the class of thiol proteinases. Among various enzymes tested, only 2-oxoglutarate dehydrogenase was attacked by the inactivase to a similar extent to the pyruvate dehydrogenase complex. Studies on the inactivation mechanism indicate that although the overall reaction is completely lost after treatment with inactivase, each individual step of the multienzyme complex retains full catalytic activity. As judged from sodium dodecyl sulphate/polyacrylamide-gel electrophoresis, the transacetylase subunit appears to be degraded into several smaller fractions.

During our studies on pyruvate dehydrogenase interconversion, rapid inactivation of the enzyme, not attributable to phosphorylation, was observed in extracts prepared from isolated rat liver mitochondria (Wieland, 1975). No inactivation occurred in the presence of small amounts of native rat blood serum or in extracts from mitochondria previously freed from lysosomes. This suggested that proteolytic degradation of the enzyme complex may be responsible for the inactivation and the protective effect of blood serum may be due to the proteinase inhibitor(s) known to be present in it. In continuation of that work we have purified a pyruvate dehydrogenase-inactivating enzyme ('inactivase') from a lysosome-enriched rat liver preparation. Below we describe the isolation procedure and some of the properties of this enzyme. Furthermore, data are presented that provide more information on the mechanism of the proteolytic attack on the PDH complex by the inactivating enzyme.

Materials and Methods

Materials

The PDH complex (EC 1.2.4.1) was purified from pig heart muscle as described by Siess & Wieland (1972). The specific activity was 2.5–3.5 units/mg of protein.

Abbreviation used: PDH complex, pyruvate dehydrogenase complex.

2-Oxoglutarate dehydrogenase (EC 1.2.4.2) was obtained as a by-product of the preparation of the PDH complex with a specific activity of 1.19 units/mg of protein.

Calcium phosphate gel was prepared as described by Keilin & Hartree (1938). α -N-Benzoyl-DL-arginine 2-naphthylamide hydrochloride was purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. N-Ethylmaleimide was from Schuchardt, Munich, Germany. Labtrol was from Merz und Dade G.m.b.H., Munich, Germany. Leupeptin (propionyl-L-leucyl-L-leucyl-L-arginal) and pepstatin (isovaleryl-L-alanyl-4-amino-3-hydroxy-6-methylheptanoyl-L-alanyl-4-amino-3-hydroxy-6-methylheptanoic acid) was kindly supplied by Professor H. Umezawa, Institute of Microbial Chemistry, Tokyo, Japan.

Acetyl-CoA acetyltransferase (EC 2.3.1.9) (enzymes A and B from ox liver) was generously given by Dr. W. Huth, Institute of Physiological Chemistry, University of Göttingen, Göttingen, Germany. Sephadex G-100 and Blue Dextran were products of Pharmacia, Uppsala, Sweden.

Enzymes and coenzymes were purchased from Boehringer, Mannheim, Germany. All other reagents were of analytical grade, obtained from Serva, Heidelberg, Germany, or E. Merck, Darmstadt, Germany.

Buffer solutions were: buffer A: 50mM-sodium acetate, pH 5.0, containing 5mM-EDTA and 2mM-dithiothreitol; buffer B: 50mM-potassium phosphate

buffer, pH 6.5, containing 5 mM-EDTA and 2 mM-dithiothreitol.

Assay of cathepsin B₁ (EC 3.4.22.1)

Cathepsin B₁ activity was determined and defined as described by Barrett (1972), except that the assay volume was scaled down to one-tenth, and that the absorbance was measured at 546 nm. According to Barrett (1972), one unit of enzyme activity hydrolyses 1 nmol of substrate/min. The standard tube contained 10 nmol of naphthylamine. The pH of the reaction mixture was 6.0.

Assay of the PDH-complex-inactivating enzyme (inactivase)

The reaction mixture for the determination of the inactivase activity contained, in a total volume of 0.25 ml: 25 μ mol of potassium phosphate buffer, pH 6.5, 0.25 μ mol of EDTA, 0.5 μ mol of cysteine, 20 munits (8 μ g) of substrate enzyme (PDH complex), and a suitable amount of inactivase sufficient to inactivate about 50–70% of the PDH complex within 30 min. After incubation at 25°C the reaction was terminated by transferring 50 μ l of the incubation mixture to 50 μ l of an ice-cold dilution (1:100, v/v) of rat blood serum with 20 mM-potassium phosphate buffer, pH 6.5. The PDH-complex activity was assayed at 0 and 30 min of incubation, either in the combined optical test with *p*-nitroaniline and arylamine acetyltransferase (Patzelt *et al.*, 1973), or by direct measurement of NADH formation (Wieland *et al.* 1969). To correct for any unspecific inactivation of the PDH complex during incubation, controls containing in addition 0.5% (v/v) rat blood serum to block inactivase activity completely were run in parallel. The extent of inactivation of the PDH complex in the blanks was small and varied between zero and 10% of that in the serum-free incubations.

For the determination of the substrate specificity of the inactivase the assay procedure was the same, except that the enzymes listed in Table 5 replaced the PDH complex as substrate for the inactivase, and that 50 μ l samples were diluted immediately into the reaction mixture used for the determination of the respective enzyme activity. Likewise, addition of rat serum was omitted for the controls.

Definition of inactivase activity. Under the conditions described above, the inactivation of the PDH complex obeys first-order kinetics. Therefore the activity of inactivase was defined as:

$$\text{units} = \frac{\log [E_0] - \log [E_{30}]}{30}$$

where $[E_0]$ is the initial activity of the PDH complex and $[E_{30}]$ is the activity after 30 min of incubation. The specific activity is given in units/mg of protein.

Determination of the component enzymes of the PDH complex

These were assayed as described by Hayakawa *et al.* (1966), except for the following modification. The pyruvate dehydrogenase activity was determined by incubating a sample of the enzyme in a reaction mixture containing 15 μ mol of potassium phosphate buffer, pH 7.2, 25 μ mol of potassium ferricyanide, 0.1 μ mol of thiamin pyrophosphate, 1.0 μ mol of CaCl₂ and 1.0 μ mol of [¹⁴C]pyruvate (approx. 350 000 d.p.m.), in a total volume of 1.32 ml. After 15 min at 37°C the reaction was stopped by adding H₂SO₄ (final concn. 1 M), and the ¹⁴CO₂ liberated was measured by a modification of the procedure described by Schwartz & Reed (1970). ¹⁴CO₂ was adsorbed on filter paper wetted with phenethylamine and counted for radioactivity in a Packard scintillation counter, with Bray's (1960) solution as scintillant.

In the lipoate transacetylase (EC 2.3.1.12) assay the buffer concentration was 15 mM instead of 111 mM and 0.8 μ mol of dihydro-lipoamide was present instead of 10 μ mol.

In the lipoamide dehydrogenase (EC 1.6.4.3) assay the amounts of NADH and lipoamide were doubled.

Other enzymes

2-Oxoglutarate dehydrogenase (EC 1.2.4.2) was assayed as described for PDH complex (Wieland *et al.*, 1969) except that pyruvate was replaced by 2-oxoglutarate. Alcohol dehydrogenase (EC 1.1.1.1), aldolase (EC 4.1.2.13), carnitine *O*-acetyltransferase (EC 2.3.1.7), glucose 6-phosphate dehydrogenase (EC 1.1.1.49), glutamate-oxaloacetate transaminase (EC 2.6.1.1), glutamate-pyruvate transaminase (EC 2.6.1.2), lactate dehydrogenase (EC 1.1.1.27) and malate dehydrogenase (EC 1.1.1.37) were determined by standard methods as described by Bergmeyer *et al.* (1970). Citrate synthase (EC 4.1.3.7) was determined as described by Ochoa (1955). Acid phosphatase (EC 3.1.3.2) and glutamate dehydrogenase (EC 1.4.1.3) were determined by using the Boehringer test combination. 3-Hydroxyacyl-CoA dehydrogenase (EC 1.1.1.35) was assayed as described by Lynen & Wieland (1955). Acetyl-CoA acetyltransferase (EC 2.3.1.9) was determined in a coupled assay with 3-hydroxyacyl-CoA dehydrogenase as described by Lynen *et al.* (1952).

Electrophoresis

Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis was performed by the method of Weber & Osborn (1969) at a constant current of 10 mA per gel. The gels were stained for 30 min with 1% Amido Black in 7% (v/v) acetic acid and 30% (v/v) methanol. Destaining was done in 10% (v/v) acetic acid.

Protein

Protein was determined by the biuret method after removal of lipids as described by Weiss (1975), or by the method of Lowry *et al.* (1951), with Labtrol as a standard.

Results and Discussion

Studies on the mechanism of action of inactivase

The time course of inactivation of heart muscle PDH complex on incubation with a more crude rat liver lysosomal preparation and the protective effect of rat serum is illustrated in Fig. 1. Interestingly the activities of the component enzymes, in contrast with the overall activity of the complex, were not affected by the inactivase preparation. This finding, recently confirmed (G. B. Kresze, personal communication) with a highly purified inactivase preparation from our laboratory, suggests that the proteolytic attack leads to a disarrangement of the molecular organization rather than affecting the catalytic centres of the multienzyme complex. This was supported by preliminary ultracentrifugal and electron-microscopic studies (results not shown), which revealed that the PDH complex fell apart during incubation with inactivase, leaving the structure of transacetylase apparently intact. However, after sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of the inactivated samples the typical pattern of the enzyme complex had changed (Fig. 2). Band 1, representing

the lipoate transacetylase, had disappeared, and two faster-moving bands (5 and 6) became apparent. This result closely resembles the observation by Linn (1971) on the enzymic inactivation of bovine kidney 2-oxoglutarate dehydrogenase. It appears also that band 2, representing lipoamide dehydrogenase, was diminished in the inactivase-treated sample.

Isolation of inactivase from rat liver

For the purification of the inactivase, an assay with PDH complex from pig heart muscle as substrate was used. As shown in Fig. 3, the rate of inactivation of the complex depended linearly on the amount of inactivase over a 10-fold range of the latter.

(a) *Preparation of a lysosome-enriched 25000g pellet.* As starting material for the isolation of inactivase a 25000g pellet from a liver homogenate was prepared by differential centrifugation by the method of Sawant *et al.* (1964). For each preparation 25–30 normal fed female Sprague–Dawley rats (W. Gassner, Sulzfeld, Germany) of 170–200g were used. The animals were stunned by a blow on the head. Livers were removed immediately, placed in cold 0.25M-sucrose/1mM-EDTA solution, pH7 (13ml/g of liver), and homogenized in a Waring blender (Braun Starmix) driven at full speed for 2 × 30s with a 60s interval. The homogenate was adjusted to pH7.2 with 5M-KOH and fractionated in a WKF G-50-K centrifuge (WKF Forschungsgeräte, Brandau, Germany) essentially by the scheme of Sawant *et al.*

Fig. 1. *Effect of the inactivase on the overall activity of the PDH complex and the component enzymes* PDH complex (1.7 mg of protein) was incubated together with 2.6 mg of inactivase (sp. activity 0.5 units/mg of protein) in 7.5mM-potassium phosphate buffer, pH6.5, in a total volume of 0.8ml at 25°C. To terminate the inactivation reaction, samples (100 μ l each) were pipetted into 200 μ l of ice-cold rat blood serum diluted (1:100, v/v) with 20mM-potassium phosphate buffer, pH6.5, and the activities determined as described in the Materials and Methods section. ■, Lipoamide dehydrogenase; ●, lipoate transacetylase; ▲, pyruvate dehydrogenase; ○, overall reaction of PDH complex. Broken lines represent the activity in the corresponding control assays, which contained rat serum from the beginning of the experiment.

Fig. 2. Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of PDH complex from pig heart (a) before and (b) after treatment with inactivase

PDH complex (4.7 mg of protein) was incubated together with 219 μ g of inactivase in a total volume of 1 ml under standard conditions. The inactivation reaction was stopped after 30 min by addition of leupeptin to give a final concentration of 0.15 μ M. Samples (100 μ l each) were incubated for 2 h at 37°C in 1.3% sodium dodecyl sulphate and dialysed against 10 mM-sodium phosphate buffer, pH 7.0, containing 0.1% sodium dodecyl sulphate and 0.1 mM-2-mercaptoethanol. Samples containing 50 μ g of PDH complex were applied to the gel. Control experiments with inactivase and leupeptin gave no visible protein bands.

(1964), except that the 3300g supernatant was centrifuged for 30 min at 25000g in a Sorvall SS 34 rotor. The 25000g pellet was resuspended in 50 mM-potassium phosphate buffer, pH 6.5, containing 1 mM-

Fig. 3. Inactivation of the PDH complex as a function of the amount of inactivase

The standard assay contained 24.6 munits of PDH complex and increasing amounts of inactivase (sp. activity 5.13 units/mg of protein) as indicated. For further details see the Materials and Methods section.

EDTA, and freeze-dried for 24 h. The product could be stored at 4°C in a desiccator for up to 1 month without significant loss of activity. Table 1 summarizes the steps for preparation of the lysosome-rich fraction. The increase in total activity (about 4-fold) is probably due to the removal of inhibitor(s). Acid phosphatase tested as lysosomal marker enzyme was purified 2-fold compared with the homogenate.

(b) *Purification of inactivase.* 1. Extraction of the freeze-dried pellet. The freeze-dried material was extracted by stirring for 3 days at 4°C with a solution of 500 mM-(NH₄)₂SO₄ in 15 mM-HCl and the final pH was adjusted to 3.5 with 1 M-HCl; 10 ml of solution/g of freeze-dried material was used. After centrifugation for 15 min at 43 500g in a Sorvall SS 34 rotor an opalescent supernatant containing almost all of the inactivase was obtained.

2. Acetone fractionation. Before the acetone fractionation the (NH₄)₂SO₄ concentration of the extract was adjusted to 250 mM by dilution with deionized water. The mixture was then cooled to +2°C, and acetone (-15°C) was slowly added within 10 min to give 38% saturation. During this operation the temperature fell to -2°C. After 5 min centrifugation at 14000g the precipitate was discarded. To the supernatant, acetone (-15°C) was added to give 65% saturation. After centrifugation as above the precipitate was dissolved in buffer A containing 200 mM-NaCl to a protein concentration of about 1.5 mg/ml. This solution was dialysed against 10 vol. of saturated (NH₄)₂SO₄ in buffer A for 2 h at 4°C. The inactivase was contained in the precipitate, which was collected by centrifugation at 43 500g for 10 min and dissolved in the smallest possible volume of buffer A.

Table 1. *Preparation of a lysosome-rich fraction from rat liver*

Mean values $\pm$ S.E.M. for six different preparations are given. The units of inactivase activity are defined in the Materials and Methods section.

Fraction	Protein (mg/g of liver)	Inactivase		Purification factor	Yield (%)	Acid phosphatase		Purification factor	Yield (%)
		Activity (units/g of liver)	Sp. activity (units/mg of protein)			Activity (units/g of liver)	Sp. activity (units/mg of protein)		
Homogenate	197 $\pm$ 5	4.1 $\pm$ 0.7	0.02 $\pm$ 0.003	1	100	6.54 $\pm$ 0.20	0.03 $\pm$ 0.001	1	100
750g supernatant	171 $\pm$ 8	3.2 $\pm$ 0.9	0.02 $\pm$ 0.005	1	78	5.72 $\pm$ 0.50	0.03 $\pm$ 0.002	1	87
330g supernatant	135 $\pm$ 4.1	5.3 $\pm$ 1.5	0.04 $\pm$ 0.01	2	129	4.80 $\pm$ 0.20	0.03 $\pm$ 0.002	1	73
25000g pellet	34 $\pm$ 3.2	15.5 $\pm$ 2.5	0.45 $\pm$ 0.07	22	378	2.60 $\pm$ 0.32	0.08 $\pm$ 0.009	2.7	40

Table 2. *Purification of rat liver inactivase from the freeze-dried lysosome-rich 25000g pellet*

Starting material was 36g of freeze-dried pellet. The units of inactivase activity and experimental details are described in the text.

Fraction	Volume (ml)	Protein (mg)	Specific activity (units/mg of protein)	Activity yield (%)	Purification factor
Acid (NH ₄) ₂ SO ₄ extract	350	745.5	3.8	100	1
Acetone precipitate (38–65% satd.)	58	78.8	26.4	72.5	7
(NH ₄) ₂ SO ₄ precipitate after dialysis	8.0	54.2	34.4	65.0	9
First calcium phosphate-gel elution	7.7	8.0	117.8	32.8	31
Second calcium phosphate-gel elution	5.3	0.9	190.5	5.8	50

3. Calcium phosphate-gel adsorption. The solution from step 2 was dialysed for 2h against 20 vol. of buffer B. Although inactivase loses some activity at pH 6.5, this procedure was essential for complete adsorption on the gel. To the dialysed solution a suspension of calcium phosphate gel was added (protein/gel dry weight = 3.5:22, w/w) and stirred for 10 min. The inactivase was eluted from the gel by two washings with 500 mM-(NH₄)₂SO₄ in 15 mM-HCl, pH 2.9, each being $\frac{1}{3}$ of the volume before adsorption. The eluates were combined and concentrated without loss of activity by dialysis for 2h against 1 litre of saturated (NH₄)₂SO₄ in buffer A. The precipitate was dissolved in buffer A to give about 4mg of protein/ml. The purification steps are summarized in Table 2.

Properties of the purified inactivase

Molecular weight. The molecular weight of inactivase determined by chromatography on a Sephadex G-100 column (2cm $\times$ 95cm) calibrated with ovalbumin (mol.wt. 45000), chymotrypsinogen A (mol.wt. 25000) and cytochrome *c* (mol.wt. 12400) in buffer A containing 500 mM-NaCl was about 21000.

This value was obtained at various stages of enzyme purification.

Stability. The stability of purified inactivase largely depends on protein concentration. Below 0.5 mg of protein/ml in buffer A some 80% of activity was lost overnight at 4°C, but no loss of activity occurred at 3.5 mg of protein/ml in buffer A when stored at 4°C for 2 weeks or at -14°C for 1 year. The stability of highly diluted solutions (less than 0.5 mg of protein/ml) of inactivase was greatly improved when buffer A was supplemented with 500 mM-NaCl or 500 mM-(NH₄)₂SO₄.

The stability of the enzyme in different buffers is shown in Fig. 4. In phosphate buffer the enzyme was most stable at pH 5.8, whereas in McIlvaine buffer pH 4.5 was optimal. The pH optimum for inactivation of the PDH complex by highly purified inactivase was about 5.0 (Fig. 5) whereas with crude preparations it was pH 6.5 (results not shown). For this reason and taking into account the stability of the substrate (PDH complex), pH 6.5 was chosen for the standard assay.

Effects of various reagents on inactivase. In Table 3 evidence is presented that inactivase belongs to the family of thiol proteinases. Cysteine (in combination

with EDTA) markedly stimulated the activity of the inactivase, whereas iodoacetamide caused an almost complete loss of enzyme activity. *N*-Ethylmaleimide had only a weak inhibiting effect. Consistent with this view is the observation that leupeptin, a known inhibitor of thiol proteinases (Barrett, 1974), markedly inhibited the inactivase, whereas pepstatin, an inhibitor of carboxyl proteinases (Barrett & Dingle, 1972), was ineffectual. The effect of rat serum was probably attributable to α_2 -macroglobulin, which is known to be a potent proteinase inhibitor (Barrett & Starkey, 1973).

Substrate specificity. Inactivase resembles the lysosomal proteinase cathepsin B₁ in molecular weight, thiol requirement and pH optimum. Inactivase, however, could be separated from cathepsin B₁ by calcium phosphate-gel adsorption, as shown in

Fig. 4. *Stability of the inactivase in different buffers* Portions (10 μ l) of inactivase corresponding to 2.75 μ g of protein were added to 1.0ml of 100mM-potassium phosphate (○) or 110mM-McIlvaine (1921) buffer (●) and incubated at the pH values indicated at 25°C for 30min, before use in the standard assay.

Table 4. Further differentiation between the two enzymes is indicated by the fact that the inactivase is much more sensitive to inhibition by leupeptin than is cathepsin B₁. At a leupeptin concentration of 0.15 μ M the activity towards PDH complex is almost completely blocked, but there is still 65% of activity towards α -*N*-benzoyl-DL-arginine 2-naphthylamide. Additional evidence that the inactivase is different from cathepsin B₁ is provided by the fact that aldolase, a substrate of cathepsin B₁ (Otto, 1971), is essentially not inactivated. When tested under standard conditions with PDH complex as substrate, the inactivase activity was 57.3 units/ml, compared with 0.67 unit/ml with aldolase as substrate.

Further information on the substrate specificity of the inactivase is presented in Table 5, which shows that a variety of mitochondrial and non-mitochondrial enzymes are rather poor substrates for the inactivase. So far only 2-oxoglutarate dehydrogenase

Fig. 5. *Effect of pH on the activity of the inactivase* The activities were determined under standard conditions, except that the pH of the potassium phosphate buffer was varied.

Table 3. *Effect of different compounds on the activity of inactivase*

For reaction with iodoacetamide or *N*-ethylmaleimide, the inactivase (730 ng of protein) was preincubated in 100 μ l of 50mM-potassium phosphate buffer, pH 6.5, for 10 min at 25°C. The reaction was stopped by addition of mercapto-ethanol (final concentration 8mM) and the enzyme activity was determined under standard conditions. For reaction with pepstatin, leupeptin or rat blood serum, the substance was added directly to the standard assay mixture. The inactivase concentration was 365 ng in a total volume of 250 μ l.

Substance added	Final concentration	Relative activity (%)
EDTA	1.0mM	100
Cysteine	2.0mM	
None	—	42
Iodoacetamide	2.0mM	5
<i>N</i> -Ethylmaleimide	2.0mM	70
Leupeptin	0.15 μ M	20
Pepstatin	15 μ M	97
Rat blood serum	0.5%	0

Table 4. *Separation of inactivase and of cathepsin B₁ by calcium phosphate gel*
For further details and definition of units see the text.

Fraction	Sp. activity of the inactivase (units/mg)	Purification factor	Sp. activity of cathepsin B ₁ (units/mg)	Purification factor
Acid extract of the freeze-dried lysosomal preparation	12	1	225	1
Acetone fraction	23	1.9	442	2
(NH ₄) ₂ SO ₄ precipitate	42	3.5	683	3
Calcium phosphate-gel eluate	166	13.8	230	1
Calcium phosphate-gel supernatant	7.3	0.6	1010	4.5

Table 5. *Substrate specificity of inactivase*

Inactivase activity against PDH complex or the various enzymes listed was assayed and calculated as described in the Materials and Methods section. The amount of inactivase (specific activity 190 units/mg of protein) was 170gn/assay. The activity of the inactivase with PDH complex (0.9 pmol/assay) as substrate was set at 100%.

Enzyme used as substrate (source)	Protein		Relative activity of inactivase (%)
	(μg/assay)	(pmol/assay)	
Acetyl-CoA acetyltransferase			
Enzyme A (ox liver)	2.5	15	<1
Enzyme B (ox liver)	0.7	4.0	<1
Alcohol dehydrogenase (horse liver)	0.2	2.5	<1
Aldolase (rabbit skeletal muscle)	1.5	9.5	1.2
Carnitine <i>O</i> -acetyltransferase (pigeon breast muscle)	2.5	45.4	<1
Citrate synthase (pig heart)	0.5	5.0	<1
Glucose 6-phosphate dehydrogenase (yeast)	12.5	100	<1
	0.12	1	<1
Glutamate dehydrogenase (ox liver)	0.3	0.14	6.3
	3.0	1.4	4.8
Glutamate-oxaloacetate transaminase (pig heart)	1.0	9.1	3.1
Glutamate-pyruvate transaminase (pig heart)	0.1	0.9	<1
3-Hydroxacyl-CoA dehydrogenase (pig heart)	0.25	3.7	<1
Lactate dehydrogenase (pig heart)	0.25	1.8	7.9
Malate dehydrogenase (pig heart)	0.025	0.4	<1
	0.25	4.0	<1
2-Oxoglutarate dehydrogenase (pig heart)	12.0	4.6	89

has been found to be inactivated to a similar extent to PDH complex. In addition, our inactivase also acts on the pyruvate dehydrogenase and 2-oxoglutarate dehydrogenase complexes purified from bovine kidney (G. B. Kresze, personal communication). Whether our enzyme leads to inactivation of carbamoyl phosphate synthase, observed to occur in mitochondrial extracts (Rubio & Grisolia, 1977), remains to be established. From preliminary experiments it appears that the phosphorylated and dephosphorylated forms of the PDH complex are attacked at similar rates.

As shown in Table 5, glucose 6-phosphate dehydrogenase was not inactivated under standard conditions (pH 6.5). The fact that also at pH 6.0 no significant inactivation occurred (results not shown) suggests that the inactivase differs from the proteinase

described by Towatari *et al.* (1976). The inactivase under study displays some of the properties described by Kirschke *et al.* (1977) for cathepsin L. Both enzymes are thiol proteinases of nearly the same molecular weight and pH optimum. However, no information is available as to whether cathepsin L degrades enzymes and thus can be regarded as a regulatory factor in enzyme catabolism. The specificity of our inactivase, which appears directed towards the mitochondrial 2-oxo acid dehydrogenase complexes, suggests a physiological control of the turnover of these multienzyme complexes.

We thank Dr. E. A. Siess for valuable discussions and for critical reading of the manuscript. This study was supported by the Deutsche Forschungsgemeinschaft, Bad Godesberg, Germany.

References

- Barrett, A. J. (1972) *Anal. Biochem.* **47**, 280–293
- Barrett, A. J. (1974) in *Proteinase Inhibitors* (Fritz, H., Tschesche, H., Greene, L. J. & Truscheit, eds.), pp. 531–540, Springer-Verlag, Berlin
- Barrett, A. J. & Dingle, J. T. (1972) *Biochem. J.* **127**, 439–441
- Barrett, A. J. & Starkey, P. M. (1973) *Biochem. J.* **133**, 709–724
- Bergmeyer, H.-U., Gawehn, K. & Grassl, M. (1970) in *Methoden der Enzymatischen Analyse* (Bergmeyer, H.-U., ed.), pp. 388–483, Verlag Chemie, Weinheim
- Bray, G. A. (1960) *Anal. Biochem.* **1**, 279–285
- Hayakawa, T., Harashima, M., Ide, S., Hamada, M., Okabe, K. & Koike, M. (1966) *J. Biol. Chem.* **241**, 4694–4699
- Keilin, D. & Hartree, E. F. (1938) *Proc. R. Soc. London Ser. B* **124**, 397–405
- Kirschke, H., Langner, J., Wiederanders, B., Ansorge, S. & Bohley, P. (1977) *Eur. J. Biochem.* **74**, 293–301
- Linn, T. C. (1971) *Arch. Biochem. Biophys.* **161**, 505–514
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Lynen, F. & Wieland, O. (1955) *Methods Enzymol.* **1**, 566–573
- Lynen, F., Wessely, L., Wieland, O. & Rueff, L. (1952) *Angew. Chem.* **64**, 687
- McIlvaine, T. C. (1921) *J. Biol. Chem.* **49**, 183
- Ochoa, S. (1955) *Methods Enzymol.* **1**, 685–694
- Otto, K. (1971) in *Tissue Proteinases* (Barrett, A. J. & Dingle, J. T., eds.), pp. 1–18, North-Holland, Amsterdam
- Patzelt, C., Löffler, G. & Wieland, O. H. (1973) *Eur. J. Biochem.* **33**, 117–122
- Rubio, V. & Grisolia, S. (1977) *FEBS Lett.* **75**, 281–284
- Sawant, P. L., Shibko, S., Kumta, U. S. & Tappel, A. L. (1964) *Biochim. Biophys. Acta* **85**, 82–92
- Schwartz, E. R. & Reed, L. J. (1970) *J. Biol. Chem.* **245**, 183–187
- Siess, E. A. & Wieland, O. H. (1972) *Eur. J. Biochem.* **26**, 96–105
- Towatari, T., Tanaka, K., Yoshikawa, D. & Katunuma, N. (1976) *FEBS Lett.* **67**, 284–288
- Weber, K. & Osborn, M. (1969) *J. Biol. Chem.* **244**, 4406–4412
- Weiss, L. (1975) Habilitationsschrift Fachbereich Medizin, Universität München
- Wieland, O. H. (1975) *FEBS Lett.* **52**, 44–47
- Wieland, O., von Jagow-Westermann, B. & Stukowski, B. (1969) *Hoppe-Seyler's Z. Physiol. Chem.* **350**, 329–334