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COMPARATIVE STUDIES ON D-GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE

VII. STUDIES ON THE DIGESTIBILITY OF THE ENZYME ISOLATED FROM VARIOUS MAMMALS

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SUMMARY

The action of trypsin on native, PCMB-inactivated and urea-denatured PGADs isolated from different mammals has been studied. It has been found that the native enzymes in the presence of excess coenzyme are fully resistant to the action of trypsin. The native enzymes, which contain only the usual amount of bound coenzyme, are digested at a different rate, the order of decreasing digestibility being: dog > rabbit > swine > beef muscle PGAD. The phenomenon is independent of the species from which the trypsins are isolated. All these PGAD preparations, however, are digested at an *identical* high rate when the dehydrogenases are inactivated with PCMB and again at an identical and still higher rate when denatured with urea.

INTRODUCTION

In a previous paper it has been reported that native, fully active PGADs isolated from beef, swine and rabbit muscle are digested by crystalline beef trypsin at a different rate, the differences disappearing after heat denaturation[1]. It has also been found that there is an inverse relation between the enzymic activity of the rabbit PGAD and its digestibility, the native enzyme in the presence of excess coenzyme (DPN) being fully resistant to the action of crystalline trypsin, whereas the PCMB-inactivated enzyme is digested at a much higher rate than the native PGAD, which contains only the usual amount of bound coenzyme[2].

The following abbreviations are used: PGAD: D-glyceraldehyde-3-phosphate dehydrogenase; PGA: D-glyceraldehyde-3-phosphate; PCMB: *p*-chloromercuribenzoate; DPN: diphosphopyridine nucleotide; TCA: trichloroacetic acid.

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The present paper reports some results obtained by digesting enzymically active, PCMB-inhibited and urea-denatured PGADs isolated from various mammals, the proteolytic enzymes being isolated also from different species.

MATERIALS AND METHODS

Crystalline beef, swine, rabbit and dog muscle PGADs were prepared according to the method of SZÖRÉNYI AND ELÖDI[3,4]. Two to five times recrystallised preparations have been used. The tests for homogeneity had been made as described previously[1]. 1 to 2 times recrystallised beef trypsin and highly purified but not crystalline swine trypsin preparations isolated according to NORTHROP's method[5] have been used. Dog pancreatic juice was supplied by the Institute of Physiology, Medical University, Budapest*. It was diluted and stored under toluene in the refrigerator.

Proteolytic digestion: the specific activity of the proteolytic enzymes and the rate of digestion of the PGAD were determined as described previously[2].

Urea-denaturation: the PGADs treated with urea were maintained for 1 h at 37°.

The enzymic activity of the PGADs was measured by Warburg's optical method, the determinations being made in samples taken at the onset of digestion. The inhibition of the dehydrogenase, the measurements and the calculations were carried out as described previously[2].

A spectrophotometer of the Hilger Uvispek type was used.

RESULTS

1. *Digestibility of native PGADs*

The comparative experiments were carried out with beef, swine and rabbit

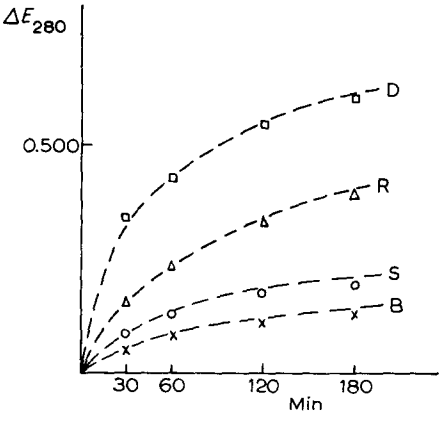

Fig. 1. Digestion of native beef, swine, rabbit and dog muscle PGAD with crystalline beef trypsin. The PGADs were dissolved in a 0.1 *M* glycine buffer containing 10^{-3} *M* KCN, pH 8.4. PGAD concn. 5 mg/ml, trypsin concn. $20.75 \cdot 10^{-4}$/TU$^{Hb}_{ml}$. The ordinate represents the increases in the optical density of the deproteinized digestion mixtures. TCA: end concn. 6.6%; B: beef; S: swine; R: rabbit; D: dog muscle PGAD.

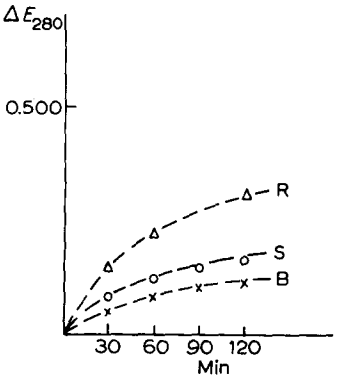

Fig. 2. Digestion of native PGADs with purified swine trypsin. The PGADs were treated as described in the legend to in Fig. 1. Trypsin concn. $17 \cdot 10^{-4}$/TU$^{Hb}_{ml}$. B: beef; S: swine; R: rabbit muscle PGAD.

* The authors are indebted to Dr. A. KOVÁCH for kindly supplying the pancreatic juice samples.

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(20 samples each) and 3 dog-muscle PGAD preparations. Five crystalline beef trypsin and 2 purified swine trypsin preparations have been used. The pancreatic juice samples were obtained from 3 different dogs.

As stated previously, the native, fully active PGADs are hydrolysed at a different rate when digested with crystalline beef trypsin. The same effect had been observed when using purified swine trypsin and dog pancreatic juice as a proteolytic agent (Figs. 1, 2 and 3).

The native beef PGAD is digested slowly and under the same experimental conditions the dog PGAD is broken down much more rapidly. The rate of digestion of the swine PGAD and that of the rabbit PGAD lie in between these two extremes. It is interesting that the beef, swine and rabbit PGADs are digested at a similar rate, irrespective of whether isolated endopeptidases or pancreatic juice—a mixture of endo- and exopeptidases—are used. The dog PGAD, however, is more susceptible to the combined action of proteolytic enzymes (Table I).

When the various PGADs were preincubated 1–2 h in a refrigerator in the presence of excess coenzyme (20–30 equivalents of DPN) before addition of trypsin they were fully resistant to proteolysis: incubation with trypsin for several hours at 37° did not result in measurable digestion.

*2. Digestibility of PCMB-inactivated and urea-denatured PGADs**

According to our previous experience, if the SH groups of the rabbit PGAD are blocked with increasing amounts of PCMB, simultaneously with the progressive de-

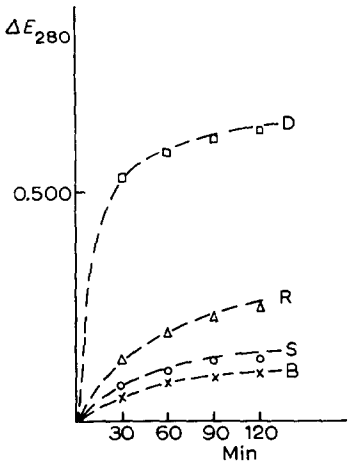

Fig. 3. Digestion of native PGADs with dog pancreatic juice. The PGADs were treated as described in the legend to in Fig. 1. The pancreatic juice was diluted with the same buffer. Proteolytic activity expressed in TU: $23 \cdot 10^{-4}/TU/\frac{Hb}{ml}$. B: beef; S: swine; R: rabbit; D: dog muscle PGAD.

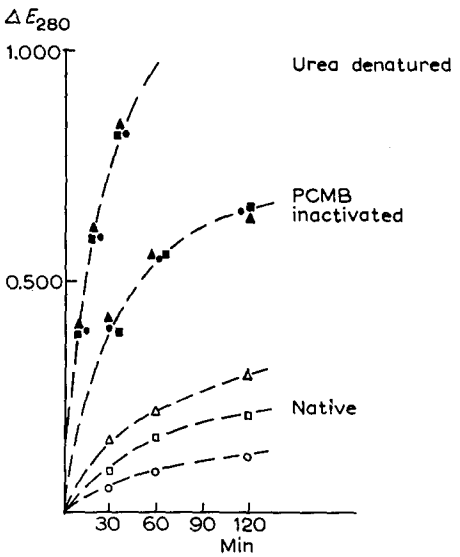

Fig. 4. Digestibility of native, PCMB-inactivated and urea-denatured PGADs isolated from various mammals. The experiments were carried out in a 0.1 *M* glycine buffer, pH 8.3. Inhibition was brought about by addition of 14 equiv. of PCMB, the dehydrogenase activity at the onset of the digestion being zero. Denaturation was carried out with 6 *M* urea at 37°. ○● beef; □● swine; ▲△ rabbit PGAD.

* In these experiments dog PGAD has not been investigated. Crystalline beef trypsin was used throughout.

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TABLE I

COMPARISON OF THE DIGESTIBILITY OF NATIVE PGADs

Expt. No.	*Proteolytic enzyme*	*Quantity of proteolytic enzyme* $10^{-4}/TU\,/\frac{Hb}{ml}$	*Digestion ratio* after 30 min incubation*			
			beef	*swine*	*rabbit*	*dog*
			PGAD			
17	Beef trypsin	15	100	150	235	400
19	Beef trypsin	30	100	180	250	400
25	Beef trypsin	30	100	170	230	—
20	Swine trypsin	10	100	150	230	—
40	Swine trypsin	15	100	150	250	—
21	Dog pancreatic juice	11	100	210	—	600
35	Dog pancreatic juice	30	100	200	400	1300

* The rate of digestion in this experiment was determined by measuring the amount of protein precipitable with 6.6% TCA.

crease of dehydrogenase activity there is an increase in its digestibility[2]. The rate of proteolysis reaches a maximum value when all the SH groups are blocked and the catalysis of the PGA oxidation is completely abolished. The rate of digestion is not increased by further addition of PCMB. The same effect has been found when partially and completely inhibited swine and beef PGADs were tested for digestibility. The stability of the various partially PCMB-inactivated PGADs towards proteolysis varies, however, from one PGAD to the other. Much smaller differences are observed when the rates of digestion of 50% PCMB-inhibited beef, swine and rabbit PGADs are compared than when those of the native enzymes are compared. Moreover, the enzymes isolated from these three animals are digested by trypsin at an *identical* high rate if the dehydrogenases are completely inactivated by PCMB (Fig. 4).

As noted previously[2], the urea-denatured rabbit PGAD is digested at a still higher rate than the PCMB-fully-inactivated enzyme. The same results have been obtained when urea-denatured swine and beef PGADs were hydrolysed. In comparing the rates of digestion of the 3 denatured enzymes, no difference in their digestibility was found (Fig. 4).

DISCUSSION

The PGADs isolated from various mammals (cattle, swine, rabbit, cat and dog) and crustacean species have been investigated in our Institute. The enzymes prepared from mammals could not be differentiated by any of the usual enzymological, immunological or physico-chemical methods[6,7,8]. As the data presented show, there is, however, a well-defined difference between the rate of proteolysis of the native, fully active enzymes, the order of decreasing digestibility being: dog > rabbit > swine > beef. The phenomenon is not altered by using either trypsins isolated from different species or a mixture of endo- and exopeptidases. These differences disappear when the functional groups of the beef, swine and rabbit PGADs are blocked with PCMB, or when the enzyme is denatured with urea or heat, thus suggesting that the peptide linkages susceptible to the action of trypsin are alike in all the 3 enzymes tested. Therefore, it can be assumed that the specific steric configuration of the native PGADs might be responsible for their different resistance to the hydrolytic action

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of trypsin. The above data support the assumption that the PCMB-inactivated dehydrogenase is an intermediate between the native, active protein and the urea-denatured protein, and no longer showing the steric configuration characteristic for the different type of animal.

As for the protective effect of the coenzyme, the results suggest that the enzyme–coenzyme complex formation stabilises the tertiary structure of all these PGADs to such an extent that—under the experimental conditions employed—none of them can be degraded by trypsin.

FISCHER *et al.* have found that α-amylases isolated from various mammals and moulds are not digested by trypsin when combined with their full complement of metal ions. But when their metal content is removed they become susceptible to hydrolysis, thus indicating that the role of calcium in amylases is to stabilise the secondary and tertiary structure of the protein[9].

The above results suggest that the use of proteolysis can reveal subtle differences in the secondary and tertiary structure of the proteins.

ACKNOWLEDGEMENT

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