process can be repeated several times. Preliminary experiments show that these fibers not only shorten but develop tension comparable to that obtainable with ATP.

In the above experiments, bundles of 5–10 single fibers (35–40 mm in length) separated from glycerol-washed psoas muscle were used. After the length of these fibers had been measured they were transferred to reagent on a microscope slide, and the shortening followed by measuring the length at various time intervals on a millimeter rule lying under the microscope slide. The ratio of the lost length to the initial length expressed in percent is called the shortening (Figs. 1, 2).

Since these muscle fibers contain connective tissue, the possibility exists that the above reagents act on the collagenous rather than on the myosin component of the fibers. These reagents, however, also cause threads made from myosin B to contract (Fig. 3). In order to demonstrate this shortening effect, threads prepared from purified myosin B first were immersed in a dilute reagent. This altered the threads so that it was possible to extend them several times their original length. Then, the reagent was washed out by soaking the threads successively in versenate solution and distilled H_2O, a process which "set" the threads at an extended length. When such threads are immersed in a more concentrated reagent, they contract rapidly. The contraction of a "set" fiber may be repeated several times (Fig. 3, b and c) and the shortening appears proportional to the length at which the fiber was "set". Therefore, the behavior of these threads is analogous to the behavior of fibers and these reagents act on the myosin B component of the muscle fibers.

It is too early to speculate on the mode of action of these reagents but it might be pointed out that both KSCN and KI are known to depolymerize actin[6,7] and eliminate flow birefringence of myosin solutions[8].

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1 A. SZENT-GYÖRGYI, *Chemistry of Muscular Contraction*, Academic Press, New York (1951).
2 W. J. BOWEN AND S. S. SPICER, *Federation Proc.*, 9 (1950) 16.
3 M. PRYOR, *Progress in Biophysics*, Vol. I, p. 512. Academic Press, London (1950).
4 M. PRYOR, *Nature*, 171 (1953) 213.
5 I. BANGA, J. BALO AND B. SZABO, *Nature*, 174 (1954) 788.
6 F. B. STRAUB, *Studies Inst. Med. Chem. Univ. Szeged*, 3 (1943) 23.
7 M. BARANY, N. A. BIRO AND J. MOLMAR, *Acta Physiol. Acad. Sci. Hung.*, 5 (1954) 63.
8 A. L. VON MURALT AND J. T. EDSALL, *J. Biol. Chem.*, 89 (1930) 351.

Received December 15th, 1954

The action of carboxypeptidase on ribonuclease*

A question which arises in attempts to determine the mechanism of action of enzymes, is whether the whole protein is necessary for catalytic activity. Crystalline ribonuclease (purchased from Worthington and Armour Laboratories) was selected as a suitable starting material since it is fairly stable, has a relatively simple structure, requires no co-enzyme or metal ion, is easily obtainable in electrophoretically homogeneous form, is assayed for fairly readily and is inexpensive.

Of the variety of tools available for degrading ribonuclease (RNase) the enzyme carboxypeptidase was the method of choice, since it is now used as a general method for determining the C-terminal end groups of a number of different proteins[1,2,3]. RNase was assayed using a modification of the method published by ANFINSEN *et al.*[4]. The amino acids liberated were separated by descending paper chromatography and quantitatively determined by direct photometry of the ninhydrin spots, according to the method of ROLAND AND GROSS[5].

Experiments were carried out by dissolving the substrate (RNase) in a small volume of phosphate buffer at pH 7.7 and adding carboxypeptidase to bring the total volume of the solution to 0.2 or 0.3 ml. As can be seen from Table I, the first amino acid which appears in any quantity is valine followed by leucine and phenylalanine. In addition, faint spots were discernible which corresponded to alanine and tyrosine (Table I, Experiment 1). The sixth amino acid to appear was methionine (Experiment 2). These results are exactly in accord with those obtained by ANFINSEN *et al.*[4]. Further increase of carboxypeptidase in these experiments gave no appreciable increase in digestion. Since 0.1 *M* phosphate buffer very strongly inhibited the action of carboxypeptidase[6], veronal buffer of the same pH was substituted for the phosphate. Adequate digestion took place, but under these conditions the veronal interfered with the chromatographic migration of the amino acids. However, in 0.01 *M* phosphate buffer, pH 7.8 or in alkaline water of the same pH more appreciable digestion took place (Table I, Experiments 1 and 2). Here again, valine, leucine, phenylalanine, alanine,

* This investigation was supported by funds made available from the Institutional Cancer Grant to the State University of Iowa College of Medicine.

TABLE I

SOME PRODUCTS OF THE DIGESTION OF RIBONUCLEASE WITH CARBOXYPEPTIDASE

Experiment number	*1*		*2*		*3*		*4*		
Product	*Amino acids liberated μM*	*RNase digested (%)†*	*Amino acids liberated μM*	*RNase digested (%)*	*Amino acids liberated μM*	*RNase digested (%)*	*Amino acids liberated μM*	*RNase digested (%) (a)*	*(b)*
Valine	0.18	6	0.21	11	0.39	11	0.66	22	30
Leucine	0.15	5	0.20	10	0.35	10	0.56	19	30
Phenylalanine	0.13	4	0.22	11	0.32	9	0.57	19	*
Alanine	Trace		0.20	10	0.75	22	1.2	40	**
Tyrosine	Trace		0.26	13	0.38	11	0.53	18	28
Methionine			0.15	8	0.33	10	0.30	10	16

tyrosine and methionine were identified and in addition, at least four other spots appeared. There was no decrease in RNase activity after the digestion with carboxypeptidase. In Experiment 3, the appearance of large amounts of alanine is undoubtedly due to the liberation of an additional alanine residue from the substrate.

† μMoles of amino acid: μM of RNase × 100.

* Spot too diffuse to measure accurately.

** Spot too dense to measure accurately.

Experiment 1: 45 mg (3 μM) of crystalline RNase + 0.06 mg (0.0018 μM) of crystalline carboxypeptidase, in a total volume of 0.3 ml of 0.1 M phosphate buffer pH 7.7; digestion time, 4 hours; temperature 25° C in all experiments.

Experiment 2: 30 mg (2 μM) of RNase + 0.04 mg (0.0012 μM) in a total volume of 0.2 ml phosphate buffer, 0.01 M, pH 7.8; or alkaline water, at same pH. Digestion time, 22 hours.

Experiment 3: 51.4 mg (3.44 μM) of RNase + 0.06 mg (0.0018 μM) of carboxypeptidase in a total volume of 0.3 ml of 0.01 M phosphate buffer, pH 7.8; digestion time, 24 hours.

Experiment 4: 45 mg (3 μM) of RNase + 4.7 mg (0.14 μM) in a total volume of 0.3 ml of 0.005 M phosphate buffer, pH 7.8. The carboxypeptidase did not all go into solution; suspension stirred; digestion time, part (a) 6 hours 5 minutes; part (b) 11.5 hours. Values not corrected for 4.8% moisture content of the RNase.

Further decreasing the ratio of substrate to carboxypeptidase to approximately 10:1 on a weight basis and 21:1 on a molar basis resulted in the digestion of over 20% of the RNase molecules present after six hours (Experiment 4a), assuming one mole of the C-terminal end group, valine had arisen from one molecule of RNase. Enzymic assay of the digested RNase even after 7.5 hours of incubation with the carboxypeptidase (Experiment 4b), showed no decrease in activity even though approximately 30% of the RNase molecules appear to have been divested of their C-terminal valine end group. In addition to the 6 amino acids already mentioned isoleucine was identified, plus spots corresponding to arginine, lysine, glycine and/or serine, and aspartic and/or glutamic acids. In this experiment, the phenylalanine spot was too diffuse and the alanine spot much too dense to be read accurately.

Paper electrophoresis of the enzyme at four different pH's (pH 4.6, 5.9, 6.8, and 8.5) showed only one protein spot.

A possible explanation for these results which have been observed, is that the whole, intact protein of the crystalline enzyme RNase is not necessary for the activity of this enzyme, as assayed by the method of ANFINSEN *et al.*[4]. Further work is in progress to separate the digested molecules from the remaining, intact molecules, determine the enzymic activity of each and ascertain how far digestion can proceed before enzymic activity is impaired.

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1 J. LENS, *Biochim. Biophys. Acta*, 3 (1949) 367.
2 J. I. HARRIS, *J. Am. Chem. Soc.*, 74 (1952) 2944.
3 J. I. HARRIS, C. H. LI, P. G. CONDLIFFE AND N. G. PON, *J. Biol. Chem.*, 209 (1954) 133.
4 C. B. ANFINSEN, R. R. REDFIELD, W. L. CHOATE, J. PAGE AND W. R. CARROLL, *J. Biol. Chem.*, 207 (1954) 201.
5 J. F. RONALD AND A. M. GROSS, *Anal. Chem.*, 26 (1954) 502.
6 E. L. SMITH AND H. T. HANSON, *J. Biol. Chem.*, 179 (1949) 802.

Received December 6th, 1954