ACTIVE RELAXATION OF UNSTRIATED MUSCLE PRODUCED BY PROTEOLYTIC ENZYMES AND THE MODE OF ACTION OF THESE ENZYMES

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Unstriated muscle which has been killed by heat is a suitable preparation for the study of certain effects of substances on the contractile mechanism (Singh, 1954 *a, b, c*; 1955 *a, b*). The circular muscle of the stomach of the frog has the interesting property, that its contractile mechanism is actively relaxed by substances that denature proteins. According to modern views, denaturation consists of an alteration of the specific internal structure of the protein wherein the closely folded peptide chains unfold (Haurowitz, 1950). Similarly, the contraction of muscle is supposed to be due to folding of the contractile protein; so relaxation would be due to unfolding of the muscle proteins. The process of relaxation would be similar to denaturation of proteins.

In the mechanism of enzymatic hydrolysis, it is presumed that the first phase of the enzymatic action is the combination of the enzyme with its substrate, the second phase of the enzymatic hydrolysis is the hydrolytic cleavage of the substrate while it is bound to the enzyme forming a complex. The formation of this complex presumably loosens some bonds of the substrate so that the enzyme is then able to act.

The polypeptide chains might be at first unfolded by the enzymes and then undergo hydrolytic cleavage. The unfolding action of the proteolytic enzymes can be tested on heat killed frog's stomach muscle. It has been found that the active relaxation of heated unstriated muscle is caused either by the enzyme itself or by the medium in which it acts.

Experimental

Experiments were performed on unloaded strips of the circular muscle of the stomach of the frog, *Rana tigrina*. Both dying muscle and heat killed muscles were used. The muscles were killed by heating at 50° C. for 10 minutes. They were then treated with various concentrations of enzymes, trypsin, pepsin and papain. The enzymes were dissolved in frog saline and pH was adjusted to the requisite value by adding sodium bicarbonate, phosphate or hydrochloric acid. The control muscles were immersed in boiled enzymes; trypsin and pepsin were boiled for 10 minutes and papain for 15 minutes. Papain is rather resistant to boiling. In the present paper the strength of the enzymes is represented by the quantity of powdered material dissolved in saline; these were trypsin (Merck), papain (B.D.H.), pepsin B.P. (Burgoyne). The enzymes were allowed to act for 24 hours at 37° C.; for allowing milder action some experiments were performed at 30° C. By active relaxation of dead muscle is implied that it lengthened without the application of any external force.

RESULTS

Action of trypsin.—Trypsin powder was used in concentration of 0·5 to 1 per cent. at pH 8. In 21 heat killed muscles, it caused active relaxation in 18 muscles by 3 to 70 per cent., contraction in 1 muscle and had no effect on 2 muscles (Fig. 1, Table I). The concentration of the enzyme has to be

TABLE I

Frog's stomach muscle, killed by heating to 50° C. for 10 minutes

Active relaxation reduced by trypsin

No. of Experiment	Active relaxation per cent.		No. of Experiment	Active relaxation per cent.	
	Effect of trypsin	Control		Effect of trypsin	Control
1	66	30	12	41	0
2	10	4	13	−12	4
3	70	10	14	13	0
4	10	10	15	20	0
5	50	10	16	28	6
6	30	12	17	20	−14
7	30	30	18	28	8
8	44	10	19	28	12
9	44	12	20	29	3
10	46	0	21	17	14
11	20	4			

suitably adjusted, so as to be mild, as contraction appears to occur at the hydrolytic stage, and active relaxation as a preliminary effect.

On dying muscle, trypsin did not produce any significant effect, but the control muscles were contracted, so that the effect of trypsin was to produce active relaxation (6 experiments).

Action of papain.—The action of papain was studied in solutions of pH 1 (6 experiments), pH 2 (6 experiments), pH 3 (6 experiments), pH 4 (6 experiments), pH 5 (6 experiments), pH 6 (12 experiments), pH 7 (6 experiments), pH 8 (6 experiments). It produced active relaxation only at pH 6–7 or 1; in other solutions it did not produce any significant effect (Table II).

TABLE II

Frog's stomach muscle, killed by heating to 50° *C. for* 10 *minutes*

Active relaxation produced by papain

No. of Experiment	Active relaxation per cent. at pH 6–7		No. of Experiment	Active relaxation per cent. at pH 1	
	Effect of papain	Control		Effect of papain	Control
1	38	10	1	0	−8
2	32	17	2	32	0
3	25	18	3	32	14
4	21	8	4	31	0
5	0	−18	5	−3	10
6	14	3	6	22	20

Action of pepsin.—The action of pepsin is very powerful. In concentrations of 0·2 to 1 per cent. of pepsin powder at pH 1 to 3, the muscles disintegrated. In 0·1 per cent., pepsin produced only contraction (6 experiments). The effect was less marked in 0·05 per cent. (6 experiments), and not significant in lower concentrations (Table III). Though pepsin by itself did not produce any active relaxation, the acid medium of pH 1 itself caused active relaxation of some muscles (Singh and Singh, 1954 *b*). In many muscles, there was no such effect, and in these muscles also pepsin only produced contraction. Pepsin, therefore, has no unfolding action. The preliminary unfolding is produced by the acid medium, and in these experiments, also by the preliminary heating.

TABLE III

Frog's stomach muscle, killed by heating to 50° C. for 10 minutes

Contraction produced by pepsin at pH 1

No. of Experiment	Contractions by 0·1 per cent. pepsin powder; per cent. of initial length		No. of Experiments	Contractions by 0·05 per cent. pepsin powder; per cent. of initial length	
	Effect of pepsin	Control		Effect of pepsin	Control
1	60	100	1	95	106
2	67	105	2	91	103
3	43	102	3	100	104
4	70	86	4	65	100
5	45	100	5	73	100
6	60	105	6	95	107

The action of pepsin thus differs from that of trypsin or papain. Experiments were also performed at 30° C. to produce a milder action which is necessary for unfolding of the polypeptide chains, as stronger action produces hydrolysis. The concentrations used were 1 per cent. (6 experiments), 0·5 per cent. (6 experiments), 0·2 per cent. (6 experiments) and 0·1 per cent. (6 experiments). No active relaxation was observed.

DISCUSSION

These experiments show that before the proteolytic enzymes can produce hydrolytic cleavage, the polypeptide chains have to be unfolded; this may be produced by the enzymes themselves or the medium. The action of pepsin differs from that of trypsin and papain. It appears to attack those bonds in the protein molecule, the dissolution of which does not result in active relaxation.

Active relaxation of unstriated muscle can thus be used to test some important reactions of proteins. Active relaxation of the contractile mechanism of unstriated muscle is caused by (*a*) denaturing agents; (*b*) distilled water; and (*c*) some proteolytic enzymes. The first reaction indicates unfolding of polypeptide chains during denaturation. Since the water molecule is a typical dipole, one would expect the hydrogen bond between the NH and CO groups to be cleaved by water molecules and the water in

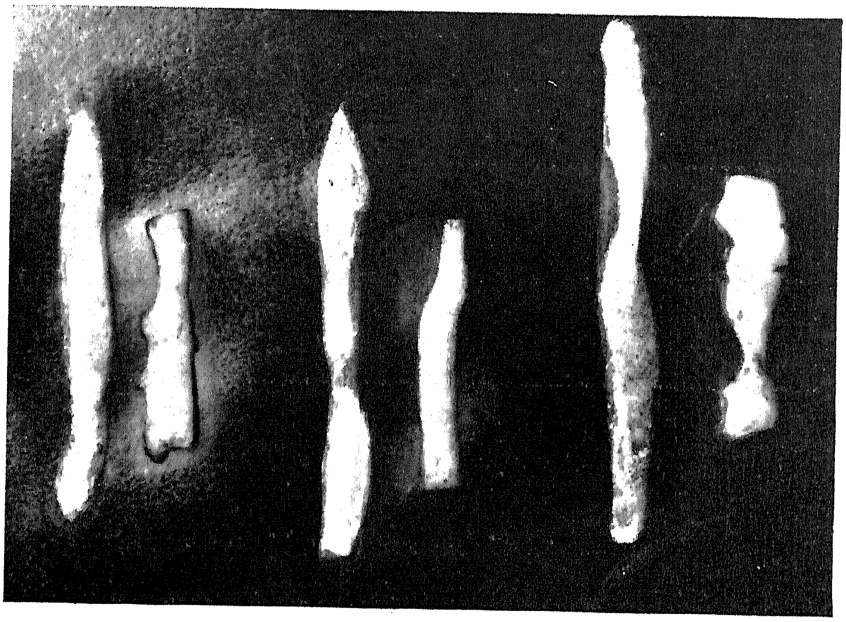

Fig. 1. Frog's stomach muscle, killed by heating to 50° C. for 10 minutes. Effect of trypsin (Elongated muscle). The shorter muscles are the controls.

this way to penetrate between the folded polypeptide chains (Bernal, 1940). This probably represents the mechanism of active relaxation by distilled water. The action of some proteolytic enzymes suggests that they also unfold the polypeptide chains as a preliminary step, or cause denaturation of proteins, thus acting as *denaturases*.

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