LVII. THE INFLUENCE OF AMINO-ACIDS ON HYDROLYSIS BY PANCREATIC LIPASE.

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THE effect of amino-acids on pancreatic lipase has been studied as a preliminary to a general investigation of the influence of proteins on hydrolysis by this enzyme, following results previously reported that certain proteins may influence the mode of action of lipase. It was found [Platt and Dawson, 1925] that the enzyme exerts preferentially an "esterase" or "lipase" action when associated with albumin and globulin respectively.

Amino-acids have been shown to increase the action of certain enzymes; Jacoby and Umeda [1914] found that glycine, alanine, leucine, tyrosine and glutamic acid accelerate hydrolysis by soya-bean urease. This promoter effect is apparently specific for α -amino-acids. Kato [1923], using glycine as a typical amino-acid, investigated the phenomenon in more detail and eliminated any possible effect due to the buffer value of the added amino-acid by conducting the hydrolysis in the presence of a phosphate buffer mixture. He suggests that a definite chemical combination between glycine and urease is indicated.

Sherman and his co-workers have made exhaustive investigations on the influence of amino-acids on various amylase preparations. All α -amino-acids tested were found to accelerate the hydrolysis of starch by amylase, while other compounds containing an amino-group, carboxyl group or both had no influence or even an inhibitory effect on the enzyme. Sherman and Walker [1923] advance the hypothesis that the apparent accelerating power of amino-acids is due to their retarding the destruction of the enzyme, to which they ascribe a protein structure. In support of this they have shown that the amino-acids have a relatively greater action at higher temperatures, when the destruction of the enzyme by hydrolysis will be greater.

Falk and Nelson [1912] have stated that amino-acids and products of protein hydrolysis are capable of hydrolysing esters. An analysis of their results shows that the actual amounts of hydrolysis observed are small, while sufficient attention does not appear to have been given to the control of the hydrogen ion concentration. In preliminary experiments, I carried out control estimations in which ester and amino-acid were incubated without the addition of enzyme. When the change in hydrogen ion concentration, due to the addition of amino-acid to ester and buffer or ester and water mixtures, was carefully controlled, no hydrolysis of ester due to amino-acid alone could be

demonstrated. This result has been confirmed by Bosman [1926], who repeated some of Falk's experiments and proved conclusively that any hydrolysis of ester which does occur is due, not to any specific action of the amino-acids, but to the acidity developed in the medium on the addition of these compounds.

With regard to previous work on the influence of amino-acids and related substances on lipase, Magnus [1904] was unable to activate an inactive preparation of liver lipase by the addition of glycine, alanine and tyrosine, while Terroine [1920] stated that glycine and Witte's peptone have no influence on the lipolytic action of pancreatic juice. Willstätter and Memmen [1923], however, observed a small increase in the rate of hydrolysis of tributyrin by pancreatic lipase on the addition of alanine, and a large increase on the addition of the tripeptide leucylglycylglycine. The hydrogen ion concentration was controlled by adding an ammonia-ammonium chloride buffer solution to the digestion mixtures.

EXPERIMENTAL.

The enzyme was used either in the form of a powder prepared by treatment of pig's pancreas with acetone and ether, or as a clear glycerol extract obtained by digesting the powder with 87 % glycerol and filtering through a hardened filter-paper. Ethyl butyrate and olive oil were chosen as substrates. When using the former the activating power on an amino-acid was obtained by determining the number of cc. of $N/10$ NaOH required to neutralise the acid formed from 0.5 cc. of ester in the presence and absence of amino-acid. The amino-acids were added from freshly prepared solutions adjusted to the correct p_H , the final concentration of amino-acid in the digestion mixture being 0.01 M .

The amount of hydrolysis of olive oil was estimated by the technique of Willstätter and Waldschmidt-Leitz [1923]. The titration with alcoholic potash was carried out in an alcohol-ether mixture using thymolphthalein as indicator. Amino-acids were added to the control estimations immediately before titration, in order to correct for the titration values of these substances in alcoholic solution.

Hydrolysis of ethyl butyrate.

The first experiments with the ethyl butyrate were carried out in the presence of a phosphate buffer mixture p_H 7.0. It was found that the increase in hydrolysis due to amino-acids under these conditions was small, owing to the very marked activating power of phosphates on lipase. The estimations on ethyl butyrate were, therefore, made without the addition of buffer solutions, the reaction mixtures being adjusted to p_H 7.6 initially.

Exp. 1. Mixtures were made up containing

- 10 cc. water
- 2 cc. amino-acid solution
- 2 cc. suspension containing 10 mg. of enzyme
- 0.5 cc. ethyl butyrate.

These were incubated with continuous shaking for three hours at 37°. The results are the mean of duplicate experiments corrected for the suitable controls.

Amino-acid added (0.01 <i>M</i>)	Mean titration value cc.
—	2.25
Glycine	2.60
Alanine	2.60
Leucine	2.65
Aspartic acid	3.25
Glutamic acid	3.90
Histidine	5.35

Exp. 2. Conditions as in *Exp. 1* except that 0.5 cc. of glycerol extract 1 was used as source of enzyme.

Amino-acid added	Mean titration value cc.
—	0.88
Glycine	1.37
Alanine	1.33
Leucine	1.35
Aspartic acid	1.90
Glutamic acid	2.35
Histidine	4.10
Lysine	1.88
Ornithine	2.00

The effect of the amino-acids is more marked when the glycerol extract is used. This is probably because protein activating substances have been removed by this further purification.

Hydrolysis of olive oil.

Exp. 3. Mixtures were made up consisting of

- 2 cc. ammonia-ammonium chloride buffer p_H 9.2
- 2 cc. solution containing 10 mg. calcium chloride
- 2 cc. amino-acid solution
- 2 cc. suspension containing 10 mg. enzyme
- 4 cc. water
- 2.5 g. olive oil.

These were shaken for three minutes and incubated at 37° for a further 57 minutes. The results are expressed as the percentage of the oil hydrolysed and are the mean of duplicates corrected for controls.

Amino-acid added	% hydrolysis
—	12.7
Glycine	13.8
Aspartic acid	18.7
Glutamic acid	19.3
Arginine	12.4
Lysine	19.6
Tryptophan	13.3
Phenylalanine	14.5

Exp. 4. Conditions as in *Exp. 3* except that 1 cc. of glycerol extract 2 was used.

Amino-acid added	% hydrolysis
—	19.8
Glycine	22.45
Alanine	22.55
Leucine	22.55
Aspartic acid	26.7
Glutamic acid	27.9
Histidine	32.5

Exp. 5. Conditions as above using 0.5 cc. glycerol extract 3.

Amino-acid added	% hydrolysis
—	13.7
Aspartic acid	21.0
Proline	15.9
Tyrosine	16.1

A consideration of the above data shows that all the amino-acids tested, with the exception of arginine, accelerate the hydrolysis of ethyl butyrate and of olive oil by pancreatic lipase. The simple monoamino-monocarboxylic acids have small effects which are equal in magnitude, while histidine has the greatest promoter action of any of the substances tested. The acceleration of hydrolysis is not due to a change in the hydrogen ion concentration on the addition of amino-acid, since the p_H of the solutions was carefully controlled by colorimetric and electrometric observations.

It was found, however, that amino-acids do not influence hydrolysis by lipase in acid solution. This was shown by a similar series of experiments to those reported above, in which acetate buffer mixtures at p_H 5.4 for ethyl butyrate and p_H 4.7 for olive oil replaced the alkaline buffer solutions. This result suggests that amino-acids accelerate hydrolysis by stabilising the enzyme in neutral or alkaline but not in acid solutions, and a certain amount of experimental evidence has been obtained which confirms this view.

In the first place it was observed that the promoter effect of the added amino-acid is greater at higher temperatures. Thus the addition of 0.01 *M* aspartic acid raises the optimum temperature for the hydrolysis of olive oil by lipase from 37° to 44°, when the estimations are carried out in the usual manner in the presence of alkaline buffer mixtures and calcium chloride.

This indicates that amino-acids retard the destruction of the enzyme by hydrolysis, since such a decomposition, which involves the splitting up of either the lipase or of associated substances upon which the stability of the enzyme depends, will normally be greater at higher temperatures. If this explanation is correct, the enzyme when heated in the absence of substrate should retain its lipolytic activity longer when the incubation is carried out in the presence of added amino-acid than when heated in aqueous or buffer suspensions alone. To test this point mixtures of enzyme and buffer solution were made up as for the estimation of hydrolysis of olive oil; aspartic acid or histidine was added to one series, and all were incubated at 37° before adding the substrate. Aspartic acid or histidine was then added to those mixtures which did not already contain amino-acid, and the amount of hydrolysis of olive oil in one hour determined. To estimate the amount of

inactivation of enzyme which had taken place, the activity of the enzyme which had not been previously heated was determined in the usual manner.

Exp. 6. The mixtures incubated previous to determining the amount of hydrolysis contained

2 cc. buffer solution p_H 9.2 or 4.7
 2 cc. solution containing 10 mg. $CaCl_2$
 2 cc. solution containing enzyme
 2 cc. amino-acid solution or water
 2 cc. water.

Enzyme	p_H	Amino-acid added*	Hours of previous incubation	% hydrolysis		
				Previously incubated		Freshly prepared
				Alone	With amino-acid present	
A. 0.5 cc. glycerol extract	4	9.2 Aspartic acid	1	13.9	18.0	19.2
B. "	9.2	"	21	8.1	11.1	19.2
C. 0.5 cc. glycerol extract	5	9.2 Histidine	4	11.4	14.0	14.7
D. "	9.2	"	24	0.5	3.3	14.7
E†. 1 cc. glycerol extract	4	9.2 Aspartic acid	4	5.3	18.6	31.4
F. 0.5 cc. glycerol extract	3	4.7 "	4	5.2	5.2	7.3

* The final concentration of amino-acid was 0.01 *M*.

† No calcium chloride was added during the initial incubation.

A consideration of the above results shows that amino-acids increase the stability of lipase in alkaline but not in acid solutions. This is in agreement with the finding that amino-acids accelerate hydrolysis by lipase in alkaline but not in acid media.

It will be observed (*Exp. 6, E*) that the destruction of lipase in alkaline solution is more rapid when calcium salts are absent. This point is being investigated further, since it is of interest in connection with the claim by Willstätter, Waldschmidt-Leitz and Memmen [1923] that lipase from any source can be completely activated by the addition of calcium chloride, albumin and an ammonia-ammonium chloride buffer mixture. It has already been shown [Platt and Dawson, 1925] that the addition of small amounts of phosphates will further increase the activity of lipase in such a medium, and during the present investigation it was found that amino-acids can replace the albumin to produce a system which has a greater activating power for lipase than that of Willstätter.

SUMMARY.

1. Amino-acids accelerate the hydrolysis, in alkaline or neutral but not in acid solutions, of ethyl butyrate and olive oil by pancreatic lipase.
2. The acceleration is probably due to the increased stability of the enzyme in alkaline solutions in the presence of amino-acids.

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