Enzyme synthesis in a solubilised system

Previous communications from this laboratory have shown that a net synthesis of amylase occurs in a homogenate of pigeon pancreas if this is complemented with an appropriate amino acid mixture and a surprisingly high concentration of adenosinetriphosphate (ATP)[1]. The amylase synthetising activity is concentrated in the granular fraction which sediments at 0° C from a 0.3 *M* sucrose solution between 1000–20,000 *g* in 30 minutes[2].

We have observed that a solubilised preparation with amylase synthetising activity can be obtained from pigeon or pig pancreas. The fresh organ is homogenised in cold acetone (10 vol.), the treatment with acetone is repeated and the residue dried *in vacuo* in the cold. The water extract (16 volumes) of this powder is brought to pH 5 with the addition of acetate buffer. The precipitate, centrifuged in the cold, is suspended with water in 1/5 of the original volume, and taken up in the buffered incubation medium as indicated in Table I. When supplemented with the amino acid mixture and ATP in the same proportion as previously described, and incubated at 37° C, the amylase activity increases up to 30–60 minutes and then declines owing to the proteolytic activity of the preparation.

TABLE I

AMYLASE SYNTHESIS IN SOLUBILISED PREPARATION

The complete mixture is prepared in the following way: 0.4 ml of the suspension of the acetate precipitate, as described in the text is mixed with 0.8 ml of the following solution: 0.02 *M* ATP, 0.02 *M* ascorbic acid, 0.6% casein hydrolysate in KREBS' bicarbonate-saline pH 7.2. The mixture is incubated at 37*g*. Amylase determined according to SMITH AND ROE and expressed in their unit. Amylase was determined at time zero of incubation and every 15 minutes thereafter. For the sake of simplicity only the results obtained after 30 minutes are shown in the Table. The last column gives the difference of column 3 and 2, *i.e.* the synthesis or destruction of amylase.

Experiment No.	*Amylase units/ml reaction mixture*		
	0′	*30′*	*Change in 30′*
1 (a) Complete system	1730	2570	+ 840
(b) as (a) without ATP	2100	1730	— 370
2 (a) Complete system	1280	1550	+ 270
(b) as (a) + 100 μg/ml *p*-fluorophenylalanine	1280	1080	— 200
(c) as (b) + 200 μg/ml phenylalanine	1030	1250	+ 220
3 (a) Complete system	7660	8900	+1240
(b) as (a) + 0.4 μg/ml ribonuclease	7810	7000	— 810

It is easy to show that the observed increase in amylase activity is due to the synthesis of new enzyme protein. No increase in amylase activity occurs if either the amino acid mixture or the ATP is omitted from the test. Moreover, the increase of amylase activity is inhibited by 100 μg of the amino acid analogue DL-*p*-fluorophenylalanine. This inhibition is reversed by increasing the concentration of DL-phenylalanine in the test system. The role of ribonucleic acid in protein synthesis is indicated by the fact that the addition of 0.4 μg ribonuclease at time zero completely inhibited the increase of amylase activity.

It is expected that such solubilised preparations will advance the solution of many problems invoved in the study of the mechanism of protein synthesis.

The detailed account of this work will be published in *Acta Physiologica Hungarica.*

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