RIBONUCLEIC ACID AND THE FORMATION OF AMYLASE IN CELL FREE PREPARATIONS FROM PIGEON PANCREAS

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SUMMARY

1. The transfer of radioactivity from [^{14}C]precursor protein into amylase enzyme protein in a pancreas extract depends upon the presence of adenosinetriphosphate, arginine and threonine; it is abolished if ribonuclease is added.

2. The transformation of the precursor protein into amylase has been separated into two processes. In the first, a fraction of the RNA of the pancreas is changed into a specific RNA. This process requires ATP, arginine and threonine and is not inhibited by chloramphenicol. The second process, which is inhibited by chloramphenicol, requires only the specific RNA and a pancreas extract and leads to the formation of amylase.

3. The RNA which has this specific action is formed only from the RNA of the larger cytoplasmic granules (mitochondria etc.), the soluble and the microsomal RNA being inactive in this reaction.

INTRODUCTION

Discussion of the interconnection between RNA metabolism and the formation of secretory enzymes[1-3] has led to no conclusions, mainly because the formation of some proteins and the turnover of the total RNA* have been compared[3].

The first definite connection between RNA and protein synthesis was only recently established when the soluble RNA was recognized as being the primary acceptor of activated amino acid residues[4,5]. It is rather difficult to visualize the formation of the fine structure of a protein by supposing that all the information is already provided when the s-RNA–amino acid complexes are assembled in the correct order.

In our previous work we observed that amylase is formed in cell free preparations of pancreas and that then no incorporation of amino acids occurs. Such preparations lose their ability to form amylase after treatment with ribonuclease. According to our hypothesis, amylase is formed in these cell free systems from a protein precursor and, if this is true, it is to be expected that the process is essentially a change in the secondary or tertiary structure of the protein. The inactivating effect of ribonuclease is by itself not sufficient proof of the participitation of RNA. In the present experi-

Abbreviations used: ATP, adenosinetriphosphate; RNA, ribonucleic acid; s-RNA, soluble RNA; g-RNA, RNA of the heavier cytoplasmic granules.

ments we have attempted to show in a more direct way that RNA plays a specific part in the transformation of precursor into amylase.

EXPERIMENTAL

Slices, mitochondrial fraction of pancreas, and acetone-dried powder from pigeon pancreas were prepared according to methods described earlier[6,7]. Enzyme activity was assayed by the method of SMITH AND ROE, slightly modified. Amylase was isolated from experimental mixtures by the method of STRAUB[8]. The radioactivity of the isolated and dialysed amylase was measured with a thin window counter, using very thin layers ($\ll$0.1 mg/cm^2). Protein content of these samples was determined according to LOWRY *et al.*[9]. RNA was measured optically by the absorption of the solution at 260 mμ. The method of GIERER AND SCHRAMM[10] was used for the isolation of RNA, the only modification being that deproteinization was carried out in a glass homogenizer by homogenizing the tissue with 50 % phenol for 10 min at 0°. pH 5 enzyme was prepared from the 105.000 $\times$ *g* supernatant of a sucrose homogenate of pigeon liver, according to the method of HOAGLAND *et al.*[11].

RESULTS

Effect of ribonuclease on amylase formation and transfer of label from precursor protein to amylase

Previous experiments have shown that the amylase content of a mitochondrial fraction, or of a freshly prepared extract from acetone dried pancreas, is increased upon incubation with ATP, arginine and threonine[7]. As there is no incorporation of free amino acids during this process, amylase may be formed from a protein precursor. Indeed, if the mitochondrial fraction is obtained from pancreas slices previously incubated with radioactive glycine, then upon incubation of the mitochondrial fraction with ATP, arginine and threonine, the radioactive label of the amylase protein is substantially increased[12].

We find that this transfer of label from precursor protein to amylase is inhibited by ribonuclease treatment.

Pigeon pancreas slices were incubated aerobically in Krebs bicarbonate saline solution at 37° with [1-^{14}C]glycine (specific activity 7.5 μC/μM) using 1.7·10^6 counts/min/g slice wet weight, in presence of 0.2 % glucose and 0.2 % caseine hydrolysate. After 10 min the slices were rinsed with cold saline and rapidly dried with cold acetone. The dry powder was extracted with 4 volumes of distilled water and the extract was centrifuged at 0° for 10 min at 20,000 $\times$ *g*. To 1 ml of the supernatant were added 50 μmoles ATP, 5 μmoles L-arginine, 10 μmoles of DL-threonine and Krebs saline solution in a final volume of 2 ml. Some of the salts were dissolved in glycerol to obtain a glycerol concentration of 40 % in the experimental mixture. The mixture was divided into two 1 ml portions, one of them receiving in addition 10 μg of crystalline ribonuclease and both were incubated at 37° for 1 h. Samples were withdrawn every 15 min for the determination of amylase enzyme content and aliquots were used for the micro-isolation of amylase[8] at zero time and after 60 min incubation. The radioactivity of the purified samples was determined and the total label in amylase was calculated from the specific radioactivity and amylase enzyme activity

of the experimental mixtures. Fig. 1 gives the result of an experiment which shows that the addition of ribonuclease inhibits both the increase in amylase enzyme activity and the transfer of radioactivity.

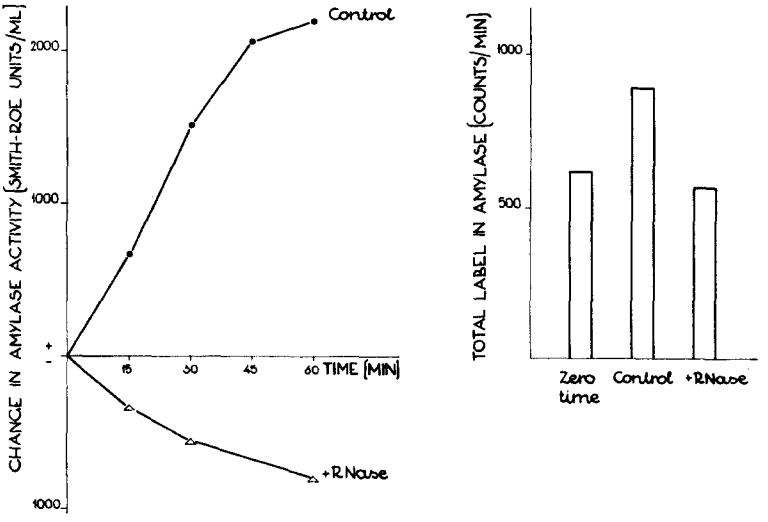


Fig. 1. Effect of ribonuclease on amylase formation and amylase labelling in a soluble system from pigeon pancreas pre-incubated with [1-^{14}C]glycine. Initial amylase activity: 9000 units/ml. Left: change in amylase content. Right: total label in amylase.

The role of "specific" RNA in the transformation of precursor protein into amylase

The fact that ribonuclease inhibits amylase formation in the soluble system[6] suggests that RNA plays some part in this process. We found the "mitochondrial fraction" to be the most adequate system for the study of this problem. It has the advantage that it is able to form relatively more amylase than the soluble system can. Like the "soluble system", the suspension of the heavier cytoplasmic granules does not incorporate free amino acids into the amylase protein, yet it is able to transfer the radioactivity from the labelled precursor into amylase, if ATP, arginine and threonine are present[12].

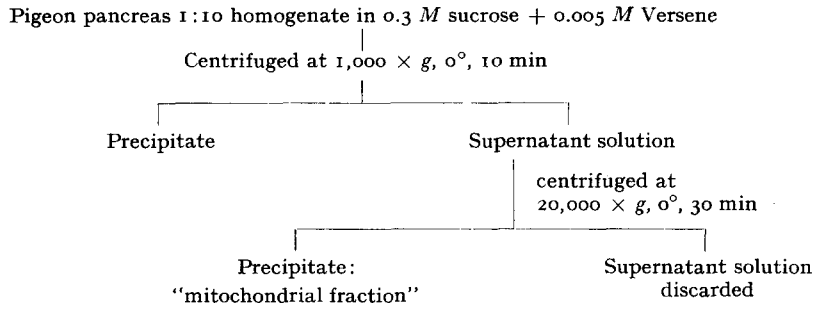
We assumed, as a working hypothesis, that the transformation of the precursor into amylase is preceded by some kind of alteration of the RNA, and that ATP, arginine and threonine are required to bring about this transformation. Using the "mitochondrial fraction", we were indeed able to separate the process into these two steps.

In these experiments we have used an experimental procedure which is summarized and described in detail in Diagram I. The procedure consists of two steps. First, the "mitochondrial fraction" is incubated with ATP, arginine and threonine. In presence of these ingredients, amylase would be formed in this system. However, in the present instance the incubation is carried out in presence of chloramphenicol, which inhibits the formation of amylase. After this incubation period, the RNA of the mixture is isolated and in Step 2. this RNA preparation is added to a freshly prepared "soluble system".

DIAGRAM I

Step 1. Formation of specific g-RNA in the "mitochondrial system"

a. Preparation of "mitochondrial system":



b. Incubation: "Mitochondrial fraction" obtained in the previous operation from 1 g pancreas suspended in Versene free 0.3 *M* sucrose, yielding about 1 ml of suspension. An equal volume of correspondingly concentrated Krebs' bicarbonate saline solution containing 50 μmoles ATP, 5 μmoles L-arginine and 10 μmoles DL-threonine added, + 0.1 mg/ml chloramphenicol. The mixture is incubated for 20 min at 37°.

c. Isolation of specific g-RNA. RNA was isolated from the reaction mixture of the previous operation by the phenol method of GIERER AND SCHRAMM[10]. After removing the phenol with ether and the ether with a stream of air, the RNA was precipitated with 60% ethanol after addition of 0.1 volume of 20% K-acetate. The precipitated RNA was dissolved in the minimum amount of water.

Step 2. Test for the effect of RNA on the formation of amylase.

a. Preparation of the "soluble system". Pigeon pancreas is rapidly homogenized twice successsively with an excess of cold acetone and rapidly dried. The powder is extracted with 4 volumes of dist. water for 10 min, the mixture is centrifuged at 0° for 10 min at 20,000 × *g*. The precipitate is discarded, the supernatant solution is the "soluble system". It is always freshly prepared immediately before use.

b. Incubation. The RNA isolated in Step 1.c., is mixed with 0.5 ml of the soluble system and supplemented with Krebs' bicarbonate saline solution and glycerol, in order to give the correct isotonic salt concentration and a glycerol concentration of 40%. Incubation is carried out for 45 min at 37° and 100 μl samples are withdrawn every 15 min for the determination of amylase enzyme content. These samples are immediately diluted 1:200 with ice-cold distilled water.

Such a "soluble system" alone gives no increase in amylase enzyme content upon incubation at 37° (Fig. 2, curve 4), but its amylase enzyme content increases if it is incubated in the presence of ATP, arginine and threonine (Fig. 2, curve 1). Fig. 2, curve 3, represents the result of an experiment in which the soluble system was incubated with the RNA isolated from the incubation mixture of a "mitochondrial fraction", as described in Diagram I, Step 1. It shows that this RNA preparation is able to replace the ATP, arginine and threonine. If this RNA preparation alone is added to the soluble system it leads to the formation of amylase. Fig. 2 curve 2 shows the result of a control experiment, in which the soluble system was supplemented with an RNA preparation, which went through the procedure described in Diagram I, except that the incubation mixture in Step 1 was not incubated at all, but the RNA was isolated immediately after mixing all the ingredients. It is evident that the RNA originally present in the "mitochondrial fraction" is inactive and that it acquires its specificity only upon incubation with ATP, arginine and threonine.

In the above experiment, we did not use all of the RNA of the pancreas, but only the fraction which is included in the heavier granules. This RNA of the heavier granules (g-RNA) can be transformed into a specific g-RNA, which takes part in the formation of amylase.

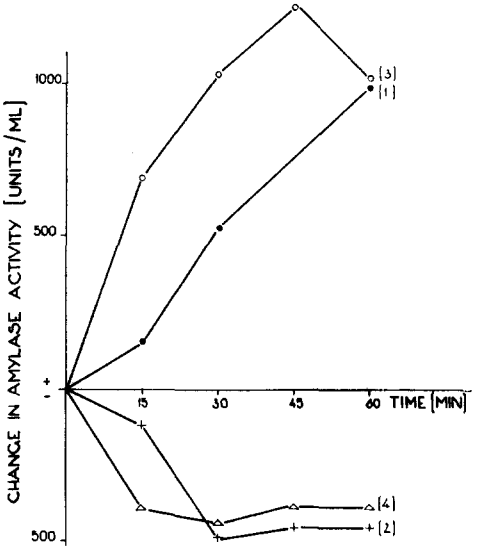


Fig. 2. Amylase formation in "soluble system", effect of specific g-RNA on amylase formation. The experimental technique is described in detail in Diagram I. 1: ATP, arginine and threonine added, 2: g-RNA, from non incubated sample added (about 5 mg), 3: "specific" g-RNA added (about 5 mg), 4: no addition. Initial amylase activity: 4000 units/ml.

It appears that this "specific g-RNA" is accumulated only in the presence of chloramphenicol. If the experiment is carried out as in Diagram I, but in Step i the incubation is carried out without chloramphenicol, the RNA isolated from such a mixture gives no effect in the soluble system. Similar negative results were obtained in experiments in which, in Step 1, the ATP, arginine or threonine were left out: the g-RNA isolated from such mixtures was always inactive in Step 2.

During the usual isolation of RNA by the phenol method, some of the ATP is precipitated along with the RNA. The question arises whether this ATP is perhaps needed for Step 2. In some experiments we have therefore washed the precipitated RNA with a slightly acid (pH 3) calcium chloride solution. The RNA is not dissolved in this solution, but all traces of ATP are removed by it. (This was controlled by precipitating and washing the RNA after addition of [^{32}P]ATP.) We were able to show that the carefully washed RNA, free of ATP, was active in Step 2.

Addition of ATP, arginine and threonine, together with specific RNA, in Step 2 did not substantially increase the amylase formation. The active RNA preparations have been shown to contain, after hydrolysis, only traces of these amino acids. Such traces do not give any amylase formation. It can therefore be concluded that in Step 2 it is the specific RNA which effects the transformation of precursor into amylase and that ATP, free arginine and threonine play no further part in Step 2, provided that enough specific RNA is added.

The next problem investigated was the nature of Step 1, namely, the reaction

in which g-RNA is changed into the specific g-RNA. As this reaction requires the presence of ATP and two amino acids, the possibility exists that an amino acid activation process is somehow involved. To examine this possibility we have devised another experimental procedure, summarized in Diagram II. In this procedure Step 1 is much simpler. Instead of using the "mitochondrial fraction" of the pigeon pancreas, we use only the RNA of the pancreas and do the incubation with ATP, arginine and

DIAGRAM II

Step 1. Formation of specific RNA from pancreas RNA by the action of liver pH 5 enzyme system.

a. Preparation of RNA. RNA was isolated from fresh pigeon pancreas by the phenol method, precipitated after addition of 0.1 volume of 20% K-acetate by alcohol (60%) and the precipitate was dissolved in water. 1 ml of this solution contains the RNA of 1 g pancreas.

b. Incubation: To 1 ml RNA solution, obtained in the previous operation, 0.5 ml of a pH 5 enzyme solution was added, containing 5–10 mg protein dissolved in medium A of KELLER AND ZAMECNIK[13], plus 50 μmoles of ATP, 10 μmoles of DL-threonine, 5 μmoles of L-arginine and 0.1 mg/ml of chloramphenicol, final volume: 2 ml. The mixture is incubated for 20 min at 37°. The pH 5 enzyme preparation was obtained from pigeon liver, using the method of HOAGLAND[4].

c. Isolation of specific RNA. After incubation, the RNA of the experimental mixture was isolated again by the phenol method and, after precipitation with alcohol, it was dissolved in the minimum amount of water. This specific RNA was used in Step 2.

Step 2. Test for the effect of RNA on the formation of amylase. This is performed in exactly the same way as in Step 2 of Diagram I.

TABLE I

AMYLASE FORMATION IN "SOLUBLE SYSTEM" FROM PIGEON PANCREAS AFTER ADDITION OF "SPECIFIC" RNA OBTAINED THROUGH THE ACTION OF LIVER pH 5 ENZYME

The experimental technique is summarised in Diagram II. Step 1: the formation of the "specific" RNA. Step 2: amylase formation in the "soluble system". Results are expressed as the change in amylase enzyme content in Step 2, after the addition of the RNA formed in Step 1. In all the experiments recorded here, the zero time amylase content was between 4,500 and 5,800 units/ml.

Expt. No.	*Factor omitted (—) or added (+) Step 1*	*Added in Step 2*	*Change in amylase units/ml*
1 a	(Complete system)		+ 1000
b	— RNA		+ 150
c	— threonine, — arginine		— 50
d	— pH 5 enzyme		+ 150
e	— ATP		— 500
2 a	(Complete system)		+ 1080
b	— threonine, — arginine + serine + lysine + tryptophan + leucine + isoleucine + proline*		— 380
3 a	(Complete system)		+ 1150
b	— threonine		+ 500
c	— arginine		+ 400
d	— threonine — arginine + 16 other amino acids*		— 250
4 a	(Complete system)	No addition	+ 1800
b	(Complete system)	0.1 mg/ml chloramphenicol	— 200

* The concentration of amino acids other than arginine and threonine was in all cases 1 m*M*.

threonine in the presence of pH 5 enzyme from pigeon liver. After incubation, the RNA of the reaction mixture is isolated again and this RNA is tested in Step 2, its ability to form amylase in a "soluble system" being investigated.

The results of such experiments are summarized in Table I. It is seen that amylase is formed in the "soluble system" upon addition of the specific RNA which was obtained according to Step 1 of Diagram II. In other words, the specific g-RNA formed in pancreas "mitochondrial fraction" can be replaced by a specific RNA, obtained from pancreas RNA through the action of the pH 5 enzyme complex,— provided that ATP, arginine and threonine are present and that chloramphenicol is added. Arginine and threonine are necessary constituents in Step 1 and cannot be replaced by other amino acids (Table I).

Other experiments recorded in Table I show that the pancreas-RNA and ATP are absolutely necessary ingredients in Step 1. It should be remembered that the pH 5 enzyme from liver contains most of the s-RNA and this is naturally isolated from the incubation mixture of Step 1. But the negative result obtained in Expt. 1 of Table I* shows that this liver s-RNA does not acquire specificity as the pancreas RNA.

The last experiment in Table I gives the expected result: chloramphenicol, which does not inhibit Step 1, *i.e.* the formation of the specific RNA, inhibits the second step, *i.e.* the formation of amylase.

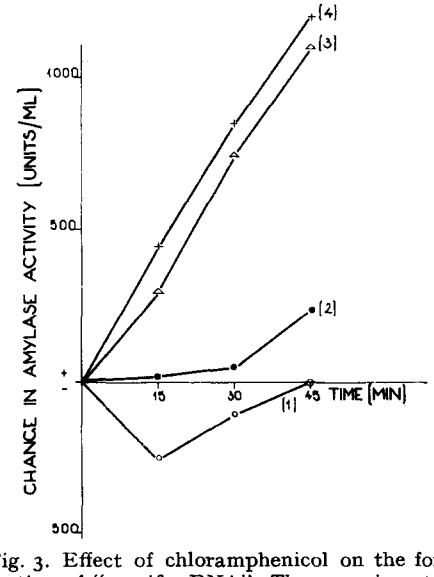


Fig. 3. Effect of chloramphenicol on the formation of "specific RNA". The experimental points indicate amylase formation in the soluble system after addition of different RNA preparations, which were obtained according to the experimental technique described in Diagram II, but using different concentrations of chloramphenicol in Step 1. Curve 1: effect of RNA obtained in the absence of chloramphenicol, Curves 2–4: effect of RNA obtained in the presence of 1 μg/ml, 10 μg/ml and 100 μg/ml of chloramphenicol respectively. The amount of RNA added was in all cases between 5–6 mg. Initial amylase activity: 5800 units/ml.

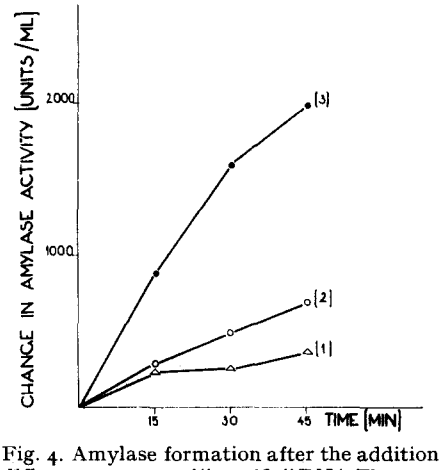


Fig. 4. Amylase formation after the addition of different amounts of "specific" RNA. The experimental technique is described in Diagram II. Additions to the soluble system from the same "specific" RNA preparation: 1: 0.85 mg, 2: 2.85 mg, 3: 8.5 mg "specific" RNA. Initial amylase activity: 6,500 units/ml.

* It will be shown later (Fig. 5) that even the pancreas s-RNA fraction is not transformed into a specific RNA; this fraction is inactive in the amylase formation.

Fig. 3 shows an experiment, also performed according to Diagram II, in which the chloramphenicol concentration of the incubation mixture in Step 1 was varied. It is very interesting that chloramphenicol proves to be an essential component of the system in which the specific RNA is accumulated (Step 1), regardless of whether this is a mitochondrial fraction from pancreas, or a pH 5 enzyme preparation.

Fig. 4 gives the results of an experiment which was also performed according to the plan of Diagram II. In this instance, in Step 2, the amount of specific-RNA added to the "soluble system" was varied. It follows that the amount of RNA is still rate limiting. From these experiments it does not follow that the absolute amount of specific-RNA is identical with the amount of RNA added. We have yet no idea how much of the RNA is transformed into a specific RNA and, because our knowledge of the fractionation of RNA is rather rudimentary, we have not attempted to investigate this problem.

There is, however, an indication that not all of the pancreas RNA may be transformed into specific RNA. In the first type of experiments—those done according to Diagram I—we obtained positive results with an RNA which was formed within the "mitochondrial fraction" and this fraction contains less than half of the cytoplasmic RNA of the pancreas. It contains only traces of the microsomal and s-RNA. We have investigated the question whether the microsomal and s-RNA can also be transformed into specific RNA or not. To test this problem, we made the following experiment:

A sucrose homogenate of pigeon pancreas was prepared and, after discarding the sediment obtained after 10 min centrifugation at 1,000 × *g*, the homogenate was centrifuged for 30 min at 0° at 20,000 × *g*. The sediment is the "mitochondrial

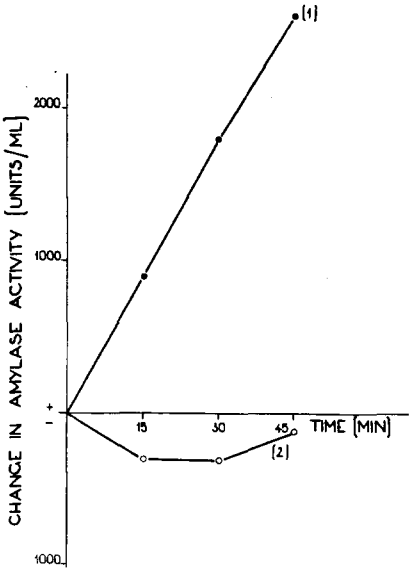


Fig. 5. Specific RNA formation from different RNA fractions of the pancreas. For a description of the experimental technique, see the text. The experimental points show the amylase content of the soluble system, after addition of pre-incubated RNA, 1: 4.6 mg RNA from the "mitochondrial fraction", 2: 6.2 mg from the microsome + s-RNA fraction. Initial amylase activity: 8,200 units/ml.

fraction" and the supernatant contains the microsomes and the soluble RNA. The supernatant was then acidified to pH 5.2 and the precipitate was collected, neutralized and dissolved. This contains most of the microsomes and the s-RNA, together with the acyl-activating enzymes.

The "mitochondrial fraction" was worked up according to Diagram I and the RNA isolated after Step 1 was tested in the soluble system. The effect of this g-RNA is shown in curve 1 of Fig. 5.

The pH 5 fraction, containing the microsomes the s-RNA and the amino acid-activating enzymes, was incubated with the usual concentration of ATP, arginine, threonine and chloramphenicol. In this instance neither RNA nor pH 5 enzyme was added. The latter was already present in sufficient quantity and the RNA in it was the RNA of the microsomes and the s-RNA. After 20 min of incubation the RNA of this mixture was isolated and tested in Step 2. As curve 2 of Fig. 5 shows, this RNA has no activity on amylase formation. The amount of RNA used in the two parallel experiments was of the same order of magnitude (see Fig. 5).

DISCUSSION

From our previous investigations[6,7,12] we concluded that the formation of amylase in the pancreas proceeds in several steps: in the intact cell a precursor protein is first formed *de novo* from amino acids and this in turn is transformed into the enzymically active amylase protein. The latter process may be regarded as the last phase of the formation of a specific protein. Whereas the formation of the amylase-precursor from amino acids occurs only in intact cells, the transformation of the precursor protein into amylase proceeds also in the isolated heavier cytoplasmic granules of a cell homogenate. The bulk of this fraction consists of mitochondria. When such isolated granules are incubated with ATP, arginine and threonine, amylase is formed from the precursor. In this system, however, no incorporation of arginine or threonine into the amylase molecule is observed. Their action is therefore indirect.

An extract of acetone dried pigeon pancreas behaves in a similar way. This "soluble system" is forming amylase in the presence of ATP, arginine and threonine, just as the "mitochondrial system". It must be emphasized that the two systems are in principle equivalent; in both of them, amylase is formed and it is formed only from the precursor protein, not from free amino acids. There are, however, differences from the technical point of view. The mitochondrial system is relatively simple: it contains only part of the proteins and RNA of the pancreas and it contains less amylase. The percentage increase in amylase content is therefore relatively higher. The soluble system, on the other hand, which contains nearly all of the soluble proteins, all of the RNA and of the amylase of pancreas, has the great advantage that it contains no permeability barriers. For this reason, in spite of the fact that the percentage increase of amylase in the soluble system is smaller than it is in the mitochondrial system, the soluble system can be used by itself for experiments in which the addition of ribonuclease, or the addition of polymeric RNA, are to be investigated. The soluble system has the disadvantage of containing already some ribonuclease, so that during incubation its RNA content is more rapidly depolymerised than is that of the mitochondrial system.

These technical considerations have led us to an experimental procedure in

which the first phase of the transformation of the precursor protein into amylase is performed in a mitochondrial system and the specific RNA produced in this phase is tested in a soluble system. The use of chloramphenicol, which inhibits the second phase, makes it possible to separate these two steps.

We have already described circumstantial evidence suggesting that ribonucleic acid plays some part in the transformation of precursor protein into amylase. The present experiments provide the most direct evidence in favour of this assumption. In the system described above, the formation of amylase is dependent on the addition of a certain ribonucleic acid fraction and no formation of amylase occurs if other RNA fractions are added or if the RNA is omitted. It follows that the RNA involved in the process is a specific RNA. As Fig. 5 shows, if the cytoplasmic RNA of the pancreas is divided into two fractions, one of them containing the microsomal and soluble RNA, the other the RNA of the larger granules (g-RNA), it is only the latter fraction which leads to amylase formation. The complete inactivity of the microsomal and s-RNA in our system is particularly interesting in view of existing data, which show that these fractions play a very important part in the early phases of protein synthesis[4,5].

The specific g-RNA is not present in appreciable quantities in living pancreas tissue. But it is accumulated under specific conditions, if, in the presence of chloramphenicol, the heavier pancreas granules are incubated with arginine and threonine in the presence of ATP. The specific g-RNA is perhaps rapidly decomposed in the absence of chloramphenicol. Whether this is due to its being used up during its physiological function (formation of amylase), or by other (hydrolytic) action, or by both of these processes, cannot be yet decided.

According to the results described in this paper we may formulate the process of the transformation of precursor to amylase in the following way:

$$\text{g-RNA} \xrightarrow{\text{ATP, Thre, Arg}} \text{Specific g-RNA} \qquad (1)$$

$$\text{Precursor protein} \xrightarrow{\text{specific g-RNA}} \text{Amylase} \qquad (2)$$

The second reaction, but not the first, is inhibited by chloramphenicol. In view of the above considerations, the experimental data suggest the following picture. If we take a soluble system (or a mitochondrial preparation) and incubate it in the presence of ATP, arginine and threonine, then the two phases occur simultaneously. Specific g-RNA is constantly formed and used for the formation of amylase, because these systems contain the precursor protein, the g-RNA and the enzymes necessary for the formation of the specific g-RNA in the presence of ATP, arginine and threonine.

The nature of this specific g-RNA cannot yet be decided. Of great importance, however, is the fact that the formation of the specific RNA from g-RNA can be brought about, not only by the pancreas granules themselves, but also by the enzymes present in the pH 5 fraction from the liver, or by a purified fraction of amino acid activating enzymes*. The obvious implication of an RNA-amino acid complex has yet to be investigated.

When the precursor protein is transformed into the enzymically active amylase protein, no free amino acids are incorporated. Therefore the change effected by the

* Unpublished observation of Dr. I. MILE and the authors.

specific g-RNA occurs in the secondary or tertiary structure of the protein. It is generally supposed that the specific structure of a protein is transmitted by the RNA, which directs the amino acid sequence of a protein, *i.e.* by the RNA of the s-RNA and microsome-RNA fraction. Our results emphasize the multiplicity of the information, the information about the secondary or tertiary structure being transmitted again by another RNA.

It is hoped that the relatively simple change which leads from a precursor protein to an enzymically active protein is more useful for the study of the actual role that RNA may play as a template.

In all work on protein formation, the evaluation of the results becomes rather difficult if the possibility of a release must be considered. This is particularly true when, as in our investigation, the process studied occurs without the incorporation of free amino acids. PALADE AND SIEKEVITZ[14] have shown that amylase is released from incubated pancreas microsome granules and that this release is greatly increased by the addition of ATP. In our experience release of bound, preexisting amylase occurs in pancreas homogenates or in granular fractions of the pancreas. We have already summarized[7] our evidence, which suggested that such a release of bound enzyme and the formation of amylase from its precursor are two different processes which may be separated under definite experimental conditions. The results presented in this paper are taken as a decisive answer to this problem. In the experimental system devised by us, the increase in amylase enzyme activity is observed when neither ATP nor amino acid is present. In a positive and a negative experiment, the only difference deciding whether the amylase activity is increased or not, is the origin of the RNA added. We believe that in this way it is possible to distinguish between the ATP-induced release of amylase from microsomes[14] and the formation of amylase from a precursor protein observed in our experimental system.

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