SYNTHESIS OF PROTEIN IN THE PANCREAS

III. Uptake of Glycine-N^{15} by the Trypsinogen and Chymotrypsinogen of Mouse Pancreas

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In this paper further observations are presented concerning the role of the protein component of ribonucleoprotein in protein synthesis. Evidence that ribonucleoprotein takes part in the general process of protein synthesis in the cell was given in a recent paper (1).

Concerning the RNA component of the nucleoprotein, the most direct indication that it takes part in protein synthesis was the demonstration that uptake of labelled amino acid by liver ribonucleoprotein is impaired when this material is incubated with ribonuclease (1). This observation has been confirmed by Zamecnik and Keller (2). A protein component of the nucleoprotein complex that is closely associated with RNA can be isolated when the RNA of the complex prepared from mouse pancreas is decomposed by ribonuclease. The ribonucleoprotein complex is prepared by sedimentation in the ultracentrifuge. After treatment of this material with ribonuclease 80 per cent of the protein still is sedimentable while 20 per cent remains in the supernatant, and this fraction is also found to be non-dialyzable. The non-sedimentable fraction shows an incorporation of labelled amino acid two or three times as high as that of the total protein in the ribonucleoprotein complex. Thus that part of the protein which can be associated most definitely with ribonucleic acid is most active with respect to amino acid uptake.

A study of the time course of incorporation of glycine-N^{15} by various protein fractions of mouse pancreas provides evidence that the non-sedimentable protein component derived from the ribonucleoprotein complex serves as precursor material for synthesis of some other proteins of the cell. The fractions studied were the mixed proteins of the whole tissue, the total protein of the ribonucleoprotein complex (frequently referred to by other investigators as the microsome fraction), the non-sedimentable protein derived from the ribonucleoprotein complex by the action of ribonuclease, and the supernatant protein remaining in the supernatant after sedimentation of the ribonucleoprotein complex. When curves for the N^{15} uptake of the nucleoprotein complex and supernatant proteins were compared, it was found that the initial rapid rate of uptake and subsequent early decline of the former combined with the extremely high

maximum achieved by its non-sedimentable component made it possible to suppose that the protein of the non-sedimentable component serves as precursor material in the synthesis of proteins in the supernatant fraction.

Since some of the digestive enzymes synthesized by the pancreas are present in the supernatant fraction, it is of interest to inquire whether the non-sedimentable component can function as a precursor in their synthesis. We have, therefore, recently studied the incorporation of glycine-N^{15} into a purified preparation of trypsinogen and chymotrypsinogen of the mouse pancreas.

Attempts to purify mouse pancreas chymotrypsinogen by a chromatographic procedure described for beef chymotrypsinogen (3) were not successful. Mouse trypsinogen and chymotrypsinogen could, however, be purified by zone electrophoresis on starch. The proteolytic activity was concentrated in one peak, as the enzymes could not be separated from each other by this procedure. The specific activity of the purified enzyme was approximately twice that of a comparable mixture of crystalline beef trypsin and chymotrypsin. The specific activity of the enzyme preparation was the same whether electrophoresis was carried out at pH 3 or 5 and was unchanged when a preparation already purified by electrophoresis was subjected to electrophoresis at a different pH.

When uptakes of glycine into the purified enzyme preparations were measured they were found to be much higher than the isotope incorporations into any other fraction of mouse pancreas considered so far. The magnitude of the differences can be made clear by an examination of the data in Table I. Table I summarizes the N^{15} concentrations in the total protein and in the different protein fractions of the pancreas under a variety of experimental conditions. In all experiments the animals were fasted before administering the isotopic glycine, and in some of the experiments the animals were fed shortly before injecting the labelled amino acid. It is evident that the duration of the fast has an influence on the subsequent incorporation of glycine-N^{15}, that feeding increases the uptake, and that maximal incorporations occur 90 minutes after administering the isotopic amino acid. It is also evident that the N^{15} concentration of the purified proteases is far greater (3 to 6 times) than that of the total protein of the nucleoprotein complex. The incorporation into the non-sedimentable fraction of the complex has not been measured under precisely the same conditions, but from the relationship between incorporation into this fraction and into the total protein of the complex in other experiments it is clear that N^{15} uptake by the non-sedimentable fraction would fall far below that of the purified proteases. As uptake of labelled glycine by the purified enzymes was at all times much higher than uptake by the proteins of the ribonucleoprotein complex the results do not support the hypothesis that these proteins serve as precursor material for the digestive enzymes synthesized by mouse pancreas. If there is some component of the ribonucleoprotein which is a precursor of these enzymes, it must be present in very small quantities at any given time.

The possibility that a protein component of the ribonucleoprotein complex is involved in the production of digestive enzymes should not, however, be dismissed. The fact remains that uptake of labelled amino acid into the proteins of ribonucleoprotein is increased when synthesis of enzymes is stimulated by secretion (1). A final point concerning the relationship between the nucleoprotein and the digestive enzymes should be mentioned. When the ribonucleoprotein complex is prepared from mouse pancreas, 13 per cent of the protease and

TABLE I

Glycine-N^{15} Incorporation into Pancreas Proteins

Time	Duration of fast	Homogenate		Trypsinogen and chymotrypsinogen		Ribonucleoprotein complex		Non-sedimentable component of nucleoprotein complex	
		Not fed	Fed	Not fed	Fed	Not fed	Fed	Not fed	Fed
	hrs.								
0.5	20	0.315				0.550			
	48		0.550						1.473
	96	0.288	0.429	1.787	2.584	0.579	0.778		
1.0	20	0.834			4.822	1.320			
	48		1.088						1.913
	96	0.460	0.597	3.628		0.781	1.138		
1.5	20	1.119				1.596		3.426	
	48		1.568						3.050
	96	0.703	1.063		7.823	1.114	1.229		
3.0	20	1.086				1.017		2.240	
	48								
	96	1.129	1.343		6.035	0.889	1.166		

N^{15} concentration in protein expressed relative to that of administered glycine taken as 100. (N^{15} concentration in glycine administered is 33.3 atom per cent excess.)

Mice were fasted for the indicated intervals prior to glycine administration. Glycine was injected intraperitoneally at zero time, each animal receiving 1 mg. glycine per gm. of the body weight as measured before fasting. In feeding experiments animals were fed 30 minutes before injection of glycine.

4 per cent of the amylase of the tissue remain associated with it and cannot be removed by washing with sucrose or by treatment with ribonuclease. For an understanding of the role of ribonucleoprotein in protein synthesis it might be significant to investigate the incorporation of labelled amino acid into a fraction of digestive enzymes so closely associated with ribonucleoprotein.

Purification Technique

For studies of the uptake of glycine-N^{15} into trypsinogen and chymotrypsinogen the cytoplasmic fraction of mouse pancreas, which had been saved from preparations

of citric acid nuclei, was used. Each sample was obtained from the tissues of about 50 mice which had been injected with N^{15}-glycine.

All operations were carried out in a cold room at 2°C. The suspension of cytoplasm was dialyzed against 0.05 per cent citric acid and then lyophilized. The lyophilized material was stored in the deep freeze for a few months before preparation of the enzyme. The powder was extracted in the blendor with about 10 parts of 0.25 N H_2SO_4 for a few minutes, allowed to stand ½ hour with occasional stirring, and then centrifuged. The extract was dialyzed against acetate buffer (pH 5, μ = 0.2) and then concentrated by precipitation with $(NH_4)_2SO_4$ to 0.4 saturation. To insure completeness of precipitation, the suspension was allowed to stand for a few hours before centrifuging. The precipitate was redissolved in about 2 ml. of water and dialyzed against the solution to be used for electrophoresis in order to remove ammonium sulfate. The extract was then purified by zone electrophoresis on starch as described by Kunkel and Slater (4). The starch was first washed several times with warm buffer over a period of 24 hours in order to remove nitrogen-containing substances which might contaminate the product. Thymol was added as a preservative. The pancreas extract was applied to a starch block 70 cm. long, 4 cm. wide, and 1 cm. deep, and 600 volts were applied across the electrodes.

Electrophoresis was usually carried out at pH 5 in acetate buffer (μ = 0.2) for 3 days. The proteases moved much more quickly at pH 3 in a solution of acetic acid 0.1 N and NaCl 0.2 N, adequate separation being achieved in 18 hours, but variable losses of enzyme activity occurred under these conditions. Later it was found that by reducing the acetic acid concentration to 0.02 N no appreciable losses resulted, and electrophoretic separation could be achieved in 18 hours.

After electrophoresis the starch block was cut into 2 cm. segments and each was extracted with 4 ml. of water. The protein was located by means of its absorption at 280 mμ, and enzyme activities were measured by the casein digestion method of Kunitz (5) after activation of the solutions with a trace of trypsin. The fractions containing the enzyme were precipitated with TCA (final concentration 5 per cent), washed with TCA, alcohol and ether, and dried. N^{15} concentrations were measured in the mass spectrometer after the usual conversion of organic nitrogen to gaseous N_2 by Kjeldahl digestion and treatment with hypobromite.

SUMMARY

The uptake of glycine-N^{15} into the trypsinogen and chymotrypsinogen of mouse pancreas is much higher than that into any ribonucleoprotein component of the pancreas that has so far been investigated.

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