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MICROSOMES AS SITES OF α-AMYLASE SYNTHESIS IN THE RAT-PAROTID GLAND

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SUMMARY

The biosynthesis of a specific protein, α-amylase, was studied in the rat parotid gland. The cell proteins were labeled *in vivo* by injecting [^{14}C]amino acids. Subsequently the specific radioactivity, as well as the total counts incorporated into amylase and into the total proteins of the various cell fractions, were recorded for different time intervals.

The following results were obtained.

1. The specific radioactivity at short time intervals (5–15 min after the injection), was highest in the amylase of the microsomal fraction, and lowest in the zymogen fraction. A rather high ^{14}C-concentration was also found in the amylase of the supernatant fraction.

2. A twenty-fold increase in the specific radioactivity of amylase in the zymogen granules was recorded after 120 min, while in the other cell components only a moderate rise occurred. In the microsomes there was even a drop in ^{14}C-content.

3. The distribution of the total counts incorporated into amylase 15 min after the injection was lowest in the zymogen granules. However, after 120 min the ^{14}C accumulated to a rather high extent in this fraction, while its concentration in the microsomal fraction decreased.

It is concluded that amylase is synthesized in the microsomes, rapidly released to the soluble portion of the cell, and subsequently transferred to the zymogen granules, where it is concentrated and stored.

INTRODUCTION

The digestive glands are particularly suited for the study of protein synthesis. They have a high protein output[1], their secretory activity is cyclic and can be triggered by various means[1–4], and the proteins produced are enzymes; these circumstances facilitate quantitative studies of well-characterized proteins.

The work on the synthesis of secretory enzymes was hitherto limited mainly to the pancreas. However, for the study of synthesis of a specific enzyme, the parotid gland seems to have several advantages over the pancreas. It produces large amounts of one typical secretory enzyme, α-amylase[1,5]. This enzyme is very stable, is easily and accurately assayed, and can be purified in small amounts to a very high degree with good recoveries[6].

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The synthesis of amylase was studied *in vivo* in the pancreas[3,7] and in the submaxillary gland[8]. Studies *in vitro* were also reported in the pancreas with slices[9–12], or with isolated cell fractions[13,14]. In all these studies, amylase synthesis was measured by increase in enzyme activity without concomitant net protein synthesis. Incorporation of radioactive amino acids into amylase of pancreatic slices was also reported[15,16]. However, the interpretation of all these studies remains controversial, and no conclusive evidence about the intracellular site of amylase synthesis has been presented.

This communication describes studies on the incorporation of labeled amino acids into the total proteins and into purified amylase of the rat-parotid gland and the determination of the most active period of protein synthesis in this gland. Evidence is provided that the microsomes represent the intracellular locus of amylase formation, and that this enzyme is subsequently transferred and stored in zymogen granules inside the cell. A preliminary account of this work has been published[17].

EXPERIMENTAL

Preparation of animals

Female rats weighing about 170 g were used for this study. In order to bring the exocrine cell population of the gland to a synchronized functional stage, the rats were starved for 48 h (see refs. 5, 18). For studying the cycle of synthesis and secretion, the latter process was stimulated by feeding for 30 min, followed by one intraperitoneal injection of 6 mg of pilocarpino hydrochloride in 1 ml of isotonic saline. The animals were sacrificed at various times after the pilocarpine injection. In experiments designed to determine the period in the cycle in which protein synthesis proceeded at the highest rate, animals after starvation, or at specific time intervals after pilocarpine injection, received intraperitoneally 3 μC of algal protein hydrolysate (uniformally labeled with ^{14}C) in 1 ml of isotonic saline. The animals were sacrificed 20 min after the ^{14}C injection. In these experiments 2 rats were used for each time interval.

For following the distribution of enzyme activity and protein-synthesizing capacity in various cell fractions, the starved rats received a meal and a pilocarpine injection, and 16–20 h later they were given an intraperitoneal injection of the radioactive algal protein hydrolysate in 1 ml of isotonic saline. The amount of the μC injected varied in different experiments and is indicated in the tables and figures. At specified intervals after the ^{14}C injection the rats were killed. Four rats were used in each time interval when total protein synthesis was studied. The number of animals was increased to 20 or 22 when the synthesis of α-amylase was investigated. Feeding and injections were scheduled to allow exact timing for the labeling with each group *in vivo*.

Preparation of homogenate

The rats were killed under ether anesthesia by cutting the heart. After rapid bleeding the two parotid glands were removed, collected in ice-cold 0.25 M sucrose, and dissected free from extraneous fat, fasciae and small lymph nodes. The glands were weighed (usually about 150 mg tissue for a 150-g rat) and divided into amounts of 300 mg of tissue, which were cut to small pieces into a pyrex test tube (13.0 × 150 mm). 0.25 M sucrose was added in a proportion of 1 ml/100 mg of tissue (0.5 ml/100 mg of

tissue when studying amylase synthesis). Homogenization was completed in 1 min employing a losely fitting teflon pestle (12.6 mm) to ensure a high yield of zymogen granules[5]. The homogenate was filtered through a fine plastic screen and the remaining masses of tissue were recollected, an equal volume of sucrose was added and homogenization was continued for another 1 min. This was again filtered through the plastic screen and added to the rest of the homogenate, while the remaining masses of connective tissues and whole cells were discarded.

Cell fractionation

The homogenate was fractionated according to the procedure of SCHRAMM AND DANON[5], but using a refrigerated International centrifuge Model PR-2 for the isolation of the nuclear and zymogen fractions, a Serval model SS-1 centrifuge operated at 4° and 14000 × *g* for mitochondria, and a 60-min run in the Model L Spinco ultracentrifuge for sedimenting microsomes. The first fraction (nuclei + zymogen granules) was finally suspended in a volume of 0.25 M sucrose equivalent to 0.2 of the volume of the original homogenate. The other fractions were each suspended in sucrose solution equivalent to 0.1 of the original volume.

α-Amylase isolation and purification

The procedure of LOYTER AND SCHRAMM[6] was used with slight modifications. Homogenization and cell fractionation were done in 0.25 M sucrose. Each fraction was diluted with H_2O to a concentration of 2 mg protein/ml, and then subjected to the first two steps of purification: ethanol precipitation and specific adsorption on glycogen. The washed glycogen–amylase precipitate was suspended in 0.02 M Na,K phosphate buffer (pH 6.9) containing 0.0067 M NaCl. The suspension was incubated at 30° for 2 h, and dialyzed for 24 h against H_2O. In this way about 85 % of the glycogen was digested and removed, with no appreciable loss in enzyme activity. Amylase from the various cell fractions was thus purified to almost the same degree (Table I), reaching a specific activity identical with that of the crystalline enzyme[6]. The amylase content of the microsomes was very low initially (see Table II), and the purification, although quite efficient, was not as effective as with the other cell fractions.

TABLE I

PURIFICATION OF AMYLASE FROM VARIOUS CELL FRACTIONS OF THE RAT-PAROTID GLAND

Expt. A was performed with 2.33 g of tissue (243 mg of protein) from 20 rats, while Expt. B was done with 3.09 g of glands (270 mg of protein) from 22 animals.

Fraction	*Amylase (units/mg protein)*				*Recovery** (%)	
	Before purification		*After purification*			
	A	*B*	*A*	*B*	*A*	*B*
Homogenate	328	300	3380	3280	89	93
Nuclei + zymogen granules	400	386	4000	4130	87	85
Zymogen granules	700	662	4380	3850	76	85
Mitochondria	160	206	4000	4400	89	95
Microsomes	27**	38**	1640	2840	66	89
Final supernatant	310	280	4170	3400	94	86

* The total amount of enzyme in each fraction before purification = 100.
** Values obtained after treatment with Triton X-100.

Counting of total protein and of α-amylase

The proteins of the various cell fractions were prepared, plated, counted and weighed according to SIEKEVITZ[19]. Purified amylase, which still contained about 50 % glycogen by weight, was plated and counted directly. In this case the specific radioactivity was calculated on the basis of direct protein determination on an aliquot of the same preparation. When the washing procedure described by SIEKEVITZ[19] was applied to this pure amylase, all the remaining glycogen was removed, but the specific radioactivity was the same as that obtained by the direct method.

Assay of α-amylase activity

Amylase activity was measured by reductometry with dinitrosalicylate, as described by BERNFELD[20]. A unit of amylase was defined as the amount which catalyses the formation of 1 mg equivalent of maltose hydrate in 3 min at 30°. Cell fractions, except microsomes, exhibited full amylase activity after ten-fold dilution in 0.02 M phosphate buffer (pH 6.9). In the case of the microsomes, treatment with Triton X-100 (0.1 %) caused a two fold rise in enzyme activity; but as the total activity of this fraction was always very low, measurements were done without triton, unless otherwise specified.

Analytical methods

Protein was determined by the method of LOWRY *et al.*[21], calibrated with crystalline serum albumin. Glycogen was estimated with the phenol–sulfuric acid reagent of DUBOIS *et al.*[22].

Materials

^{14}C-labeled algal protein hydrolysate was purchased from the Radiochemical Centre, Amersham (Great Britain). The nonionic detergent Triton X-100 was provided by Dr. M. SCHRAMM.

RESULTS

Cycle of secretion and synthesis in the rat-parotid gland

The secretory activity of the digestive glands is known to be cyclic. DALY AND MIRSKY[3], working with the pancreas, stated that one can initiate and control the synthesis of proteins in such a cycle because any excretion of enzyme during the secretory phase leads to an accelerated rate of protein synthesis, such as to compensate for the lost protein. SCHUCHER AND HOKIN[11], working with pancreatic slices, found, however, that the rate of enzyme synthesis was independent of the secretory activity. Therefore, they concluded that "the cycle of secretion and resynthesis may merely reflect a constant rate of enzyme synthesis with superimposed variations in the secretory rate".

In an attempt to determine the period of most active protein synthesis in the rat-parotid gland, the relations between secretion and synthesis were studied during a complete secretory cycle. The secretion was stimulated by both food and pilocarpine, a procedure which gave a reproducible 50–60 % drop in enzyme activity (Fig. 1). By using each of these triggers alone it was found, in accordance with the observations of DALY AND MIRSKY[3], that while pilocarpine produced a reproducible but small drop (20–30 %) in enzyme activity, feeding caused variable changes in activity. Also, with

the adopted procedure for induction of secretion, the course of restoration of enzyme activity was always reproducible.

A complete secretory cycle in the rat-parotid gland is shown in Fig. 1. There was a high level of enzyme activity at starvation. After stimulation of secretion, the activity dropped during a period of 4–5 h to less than half the initial value; it remained at this low level for another 5–6 h, and then rose slowly back to the initial value. We never observed a rise above the initial level of activity found in rats starved for 48 h. Such a rise has been reported when short fasting periods of less than 12 h were used[3,23], or when control animals were not starved[24].

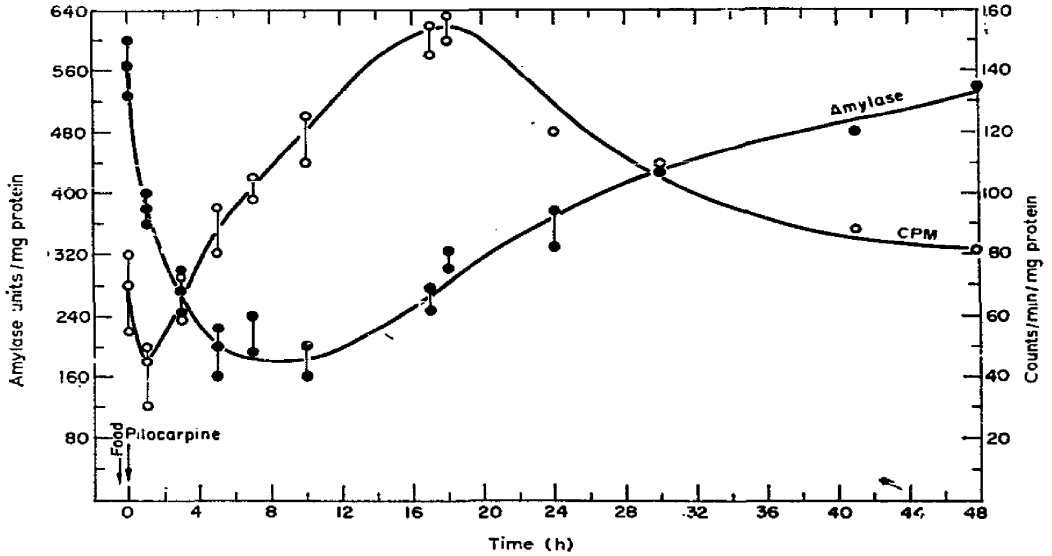

Fig. 1. The cycle of secretion of amylase and synthesis of proteins in a whole homogenate of the rat-parotid gland. Each circle represents one experiment with a pool of 2 rats.

Fig. 1 also shows changes in the rate of total protein synthesis during the secretory cycle, as determined by the rate of ^{14}C incorporation into proteins of parotid tissue within a defined time interval (20 min). There was a certain steady rate of synthesis at starvation, which dropped at the onset of secretion to a minimal value. The rate of synthesis stayed below the steady state level during the period of active secretion, then rose to a maximum at about 16–20 h after the onset of secretion, and finally dropped slowly back to the initial starvation level.

In contrast with the considerable changes in the rate of protein synthesis during the secretory cycle, no significant alteration in the total protein content of the homogenate could be observed. Small changes in the amount of total protein, as compared to quite pronounced changes in enzyme activities, were reported in the pancreas[3,23,24].

From the curves in Fig. 1, it can be concluded that the relation, if any, between enzyme secretion and protein synthesis in the rat-parotid gland stimulated by food and pilocarpine is not a direct one. There was neither a constant rate of synthesis during the secretory cycle, nor an acceleration of synthesis at the onset of secretion, but rather an inhibition. As already mentioned, the most active period of protein synthesis was between 16 and 20 h after the onset of secretion. FARBER AND SI-

DRANSKY[23] similarly observed an increased rate of incorporation of radioactive amino acids into proteins of rat pancreas in the period of enzyme restoration. All of the experiments dealing with ^{14}C-labeling of proteins in general, and α-amylase in particular, were therefore performed with rats whose parotid glands were at this stage of the secretory cycle.

Distribution of amylase activity in cellular components at various stages of the secretory cycle

Table II shows the distribution of amylase activity and protein in cell fractions of a homogenate prepared from glands excised before, and at various intervals after feeding and pilocarpine injection. Similar results were obtained after fasting periods of 12 and 48 h. The same distribution was also reported after a 24-h fast[5]. SIEKEVITZ AND PALADE[18] proposed that a long fasting period might change the distribution of enzyme activity, and might especially lower the microsomal activity. The results presented here indicate that, in the rat-parotid at least, prolonged starvation does not change the pattern of enzyme distribution.

The first two fractions, representing mainly large and medium sized zymogen granules[5,25], contained about 50 % of the enzyme activity, while most of the remainder was in the soluble fraction. After the onset of secretion, amylase activity dropped in the first fraction and rose in the mitochondria and, to a smaller extent, in the final supernatant. This decline and rise in activity was most pronounced in the period of active protein synthesis. No significant rise could be observed in the activity of the microsomes at any time during the complete secretory cycle. The activity measured at ½, 1, 2, 4, 5 and 7 h after pilocarpine injection was the same as that recorded in Table II.

TABLE II

DISTRIBUTION OF AMYLASE AND PROTEIN IN CELL FRACTIONS AT DIFFERENT TIME INTERVALS AFTER PILOCARPINE INJECTION

Fraction	*Amylase Relative distribution**			*Protein Relative distribution**		
	*0 h***	*3 h*	*18 h*	*0 h***	*3 h*	*18 h*
Nuclei + zymogen granules	38	31	24	26	20	16
Zymogen granules	13	14	15	9	8	8.5
Mitochondria	2	4	10	7	10	11.5
Microsomes	0.4	0.6	0.6	9	9	10
Final supernatant	36	38	40	39	42	45
Recovery (%)	89	88	90	90	89	91

* Homogenate = 100.
** After a 48-h fast.

The changes in the distribution of protein in the various cell fractions (Table II) reflect the same picture as those for amylase activity.

The results of these studies present no conclusive evidence regarding the site of protein and amylase synthesis in the rat-parotid gland. The only fraction which showed an increase in amylase activity and protein content were the mitochondria. However, this was also the most heterogeneous fraction and contained small zymogen granules as well as large microsomes.

Further experiments designed to determine the intracellular loci for protein synthesis in general, and α-amylase formation in particular, were therefore carried out

by tagging the cell proteins *in vivo* with isotopic amino acids, and following with time the labeling of proteins of the various cellular components.

Incorporation of radioactive algal protein hydrolysate into proteins of cell fractions

Fig. 2 shows the isotopic content of the cellular proteins at different times after the injection of [^{14}C] amino acids. Initially the highest specific activity was found in the microsomes, which, however, leveled off after about 45 min. The curve of the mitochondria rose linearly to a relatively high peak at 45 min, and then declined. The zymogen granules, on the other hand, exhibited a pronounced lag during the first 15 min. The specific activity then rose very rapidly to equal that of the mitochondria at 45 min. This pronounced rise continued until the end of the experiment. The lowest radioactivities were in the nuclear and supernatant fractions.

More information on the incorporation of [^{14}C] amino acids into proteins of the rat-parotid gland is given in Table III, which records variations with time in the total labeling of proteins of the various cell fractions. At 5 min after injection of the algal protein hydrolysate, the microsomal fraction contained 36 % of the total counts, although this fraction comprises only 10 % of the total protein (Table II, last column). At longer time intervals the ^{14}C content of this fraction dropped rapidly. This decline reflects not only a decline in relative radioactivity, but also in the actual total counts, which dropped from 2400 at 45 min to 1990 at 120 min, although the total ^{14}C in the homogenate was still rising during the 45–120-min interval. The zymogen and final supernatant fractions, on the other hand, increased their share of the total incorporation with time.

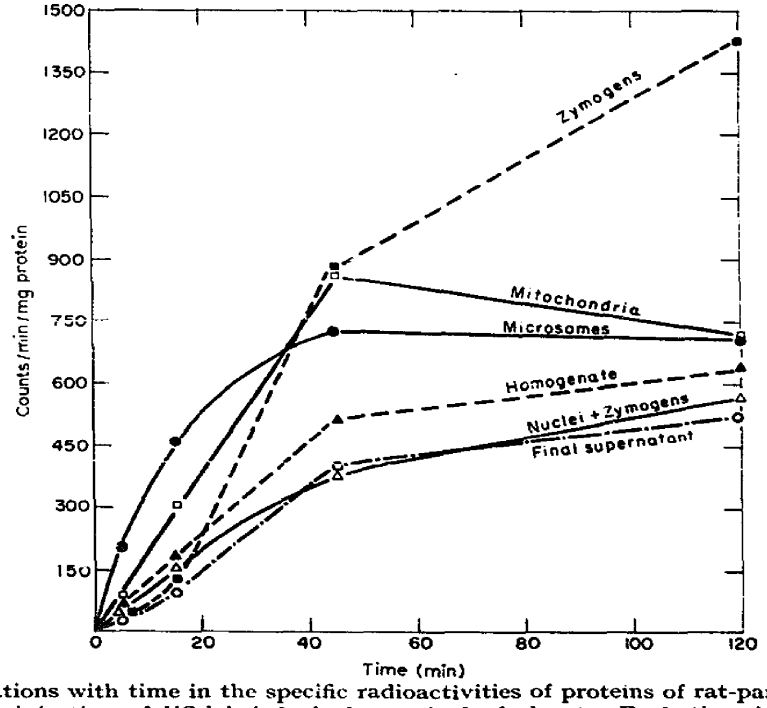

Fig. 2. Variations with time in the specific radioactivities of proteins of rat-parotid cell fractions after *in vivo* injection of ^{14}C-labeled algal protein hydrolysate. Each time interval represents a pooled homogenate from 4 rats, that received a total of 19 μC of labeled protein hydrolysate.

TABLE III

TOTAL LABELING OF PROTEINS FROM CELL FRACTIONS AT DIFFERENT TIME INTERVALS AFTER *in vivo* INJECTION OF ^{14}C-LABELED ALGAL PROTEIN HYDROLYSATE

The values were obtained by multiplying the specific activities in Fig. 2 by the weights of protein, determined on aliquots of the respective cell fractions. The results are expressed as per cent of counts in the initial homogenate. The latter were 1995, 6134, 15872 and 21148 at 5, 15, 45 and 120 min respectively.

Fraction	*Time after injection (min)*			
	5	*15*	*45*	*120*
Nuclei + zymogen granules	11	14	13	12
Zymogen granules	3	3.5	10.5	15.5
Mitochondria	18	19	17	15
Microsomes	36	28	15	9.5
Final supernatant	23	24	34.5	36
Recovery (%)	91	88.5	90	88

According to these results the microsomes differ from other cell components by their higher initial incorporative rates. Similar results were obtained by various workers with the pancreas[23,26,27]. The pronounced decline with time in the share of the total incorporation into microsomes, and the rise in the labeling of the zymogen and supernatant fractions is compatible with the concept of a transfer of newly synthesized labeled proteins from the microsomes, in a soluble form, to the zymogen granules.

Incorporation of labeled algal protein hydrolysate into amylase of cell fractions

To further clarify the situation encountered in the incorporation into total proteins described in the preceding section, and to evaluate the relative importance of α-amylase production within the general process of protein synthesis by the rat-parotid gland, the incorporation of isotopic amino acids into α-amylase was compared with that into total proteins in the same experiments.

TABLE IV

SPECIFIC RADIOACTIVITY OF PURIFIED α-AMYLASE AND OF TOTAL PROTEINS ISOLATED FROM CELL FRACTIONS AT DIFFERENT TIMES AFTER *in vivo* INJECTION OF ^{14}C-LABELED ALGAL PROTEIN HYDROLYSATE

The tissue for the 15- and 120-min time intervals was actually derived from Expts. A and B, respectively, of Table I. Each group of rats received a total of 130 μC of labeled protein hydrolysate. The specific radioactivity of the total proteins was determined on aliquots of each cell fraction before the purification of amylase.

Fraction	*Amylase radioactivity (counts/min/mg)*		*Total protein radioactivity, (counts/min/mg)*	
	15 min	*120 min*	*15 min*	*120 min*
Whole homogenate	300	1255	148	483
Nuclei + zymogen granules	260	1450	130	457
Zymogen granules	100	2040	100	858
Mitochondria	805	1970	276	550
Microsomes	3750	2433	410	473
Final supernatant	500	1330	80	374

The results presented in Table IV demonstrate that in all cell fractions at both time intervals studied (except in zymogen granules at 15 min) the specific activity of amylase was by 2–9 times higher than that of the total proteins. It follows, therefore, that a very active synthesis of amylase occurred in this system. 15 min after administration of isotopic amino acids, amylase of the microsomes had a specific activity 4–5 times higher than that of the mitochondria, the next most active component. Amylase of the zymogen granules had the lowest specific activity. It should be emphasized that the specific radioactivity of the microsomes was most probably even higher than the recorded one, since the degree of purification of amylase achieved in this fraction at 15 min was only about half that achieved in any other cell constituent (see Table I). The specific activity of amylase of the soluble supernatant was already quite high at 15 min, while the total protein of this fraction had a very low ^{14}C concentration (see also Fig. 2). This high specific activity of soluble amylase cannot be attributed to contamination of the supernatant phase by broken components of other cell fractions as suggested by HOKIN[25], since the zymogen granules in the present study had a much lower specific activity, while the mitochondria and microsomes contained much less amylase than the soluble fraction (Table II).

A twenty-fold increase in the specific activity of amylase in the zymogen granules was recorded after 120 min, while in the other cell fractions only a moderate rise occurred. In the microsomes there was even a drop of more than 30% in activity, although this fraction still had the highest specific amylase radioactivity. In the total proteins, the zymogen granules had the highest ^{14}C concentration at 120 min (see also Fig. 2). This was probably due to the vary high amylase content of this fraction.

The pronounced differences in the specific radioactivity of amylase in the different cell fractions might be due in part to dilution of the newly synthesized labeled amylase with the pool of non-labeled enzyme present in each component. As is shown in Table II, this pool was quite large in the soluble supernatant and zymogen fractions, and almost non-existant in the microsomes. Hence, dilution could significantly lower

TABLE V

TOTAL ^{14}C OF PURIFIED α-AMYLASE ISOLATED FROM CELL FRACTIONS AT DIFFERENT TIMES AFTER INJECTION OF LABELED PROTEIN HYDROLYSATE

The figures were calculated by multiplying the specific radioactivity values in Table IV by amylase protein content determined on the basis of percent recovery of amylase during purification, as given in Table I.

Fraction	*Time after injection (min)*			
	15		*120*	
	CPM	%	*CPM*	%
Homogenate*	7034	100	32 765	100
Nuclei + zymogen granules	1137	16	5 963	18
Zymogen granules	479	7	7 300	22
Mitochondria	693	10	3 533	11
Microsomes	612	9	416	1
Final supernatant	4050	57	14 740	45
Recovery	6971	99	31 952	97

* The counts incorporated into total protein in the whole homogenate were 35964 and 130410 counts/min at 15 and 120 min respectively.

the specific activity of amylase of the first two fractions, while in the microsomes no serious dilution might take place. To clarify this point, the total isotope incorporated into amylase of each cell fraction was calculated and recorded in Table V. Since the recovery of purified amylase ranged between 66 to 95% (Table I), the calculated figures are quite representative. It is evident that even the total counts incorporated into the amylase of the zymogen fraction at 15 min were less than those incorporated into the microsomes. At shorter time intervals the difference might be even more pronounced as is suggested by Fig. 2 and Table III.

After the first 15 min, however, the zymogen granules accumulated counts very rapidly while the microsomes were losing counts. This may indicate that the microsomes did not accumulate the newly synthesized amylase, but released it immediately. This rapid release was also indicated by the unchanging low level of enzyme activity found in this fraction at different stages of the secretory cycle (Table II). The same low level was found at all the time intervals studied in this, and in the preceding experiment. Because of the rapid release of newly synthesized amylase from the microsomes, their total radioactivity at each time interval studied need not have reflected the total amount of enzyme synthesized by them, but merely the small amount which was not yet released.

The results summarized in Table V also demonstrate that amylase was not only actively synthesized in the rat-parotid gland, but also comprised a rather large fraction of the total newly synthesized proteins in this system, since between 19–25% of the counts incorporated into the proteins of the whole homogenate were accounted for by ^{14}C incorporated into amylase.

DISCUSSION

Changes in the intracellular distribution of digestive enzymes in the pancreas of various animals during a secretory cycle were studied by SIEKEVITZ AND PALADE[18,28] with trypsin-activatable proteases (*i.e.* trypsinogen, chymotrypsinogen and procarboxy-peptidase) and ribonuclease, and by LAIRD AND BARTON[7,29] with amylase. Both groups found a rather high enzyme content in the microsomes of starved animals, and observed that the concentration rose significantly after the induction of secretion by food or pilocarpine. On the basis of their findings, they suggested that new enzyme protein was synthesized in association with microsomes.

Our results show that very little amylolytic activity was present in the microsomes of fasted rats and that this level did not change markedly during a complete secretory cycle. Low amylase activity in the microsomal fraction was also reported by HOKIN[25] in pancreas of fasted dogs, and by DALY, ALLFREY AND MIRSKY[30] in mouse pancreas. The discrepancies in the amounts of enzyme reported in microsomes of digestive glands were found not to be due to differences in the nutritive state of the animals. Neither can they be attributed to differences in fractionation technique, nor to the use of different sucrose concentrations (0.25 or 0.88 M), since with 0.25 M, both high[29,31] and low[25,30] amylase contents of pancreatic microsomes were reported. The most attractive explanation of the observed discrepancies seems to lie in species or organ differences in the rate of intracellular release and transport of the digestive enzymes. If the enzymes are assumed to be synthesized in the microsomes, a slow rate of release would imply a high enzyme content in this fraction, and a rise in its concentration

during a period of active synthesis. With a rapid rate of release, on the other hand, a low enzyme content and no significant change during synthesis should be found in the microsomes. Also, if the rate of transport of the enzymes to the zymogen granules is slower than the rate of release from the microsomes, there might appear a transient high enzyme content in the soluble supernatant.

This may be the case in the rat-parotid gland. The results of labeling the proteins in general, and amylase in particular, in each cell fraction at different time intervals, are entirely compatible with the hypothesis that the microsomes are the site of synthesis of α-amylase and of the total proteins of this system. However, on the basis of the enzymic as well as ^{14}C-labeling results, it is most probable that the newly synthesized amylase does not accumulate in the microsomes, but is very rapidly released from this fraction to the soluble phase of the cell, and subsequently transferred to the zymogen granules, where it is concentrated and stored.

Similar studies were recently reported with chymotrypsinogen of guinea-pig pancreas[32] and ribonuclease of mouse pancreas[23]. In both cases only specific radioactivities of the isolated enzymes were reported, since no quantitative recovery of the enzymes was attempted. These results were therefore not corrected for any dilution by non-radioactive enzyme pools. Nevertheless both investigations implicate the microsomes as the sites of biosynthesis of the pancreatic proteins. These enzymes did, however, accumulate to a rather large extent in the microsomal fraction, and only a comparatively small portion was released and transferred to other cell fractions during the time intervals studied.

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REFERENCES

1 B. P. BABKIN, *Secretory Mechanisms of the Digestive Glands*, Paul. B. Hoeber Inc., New York, 1950, p. 838, 560.
2 R. HEIDENHAIN, *Arch. ges. Physiol. Pflüger's*, 10 (1875) 557.
3 M. M. DALY AND A. E. MIRSKY, *J. Gen. Physiol.*, 36 (1952) 243.
4 M. R. HOKIN AND L. E. HOKIN, *J. Biol. Chem.*, 203 (1953) 967.
5 M. SCHRAMM AND D. DANON, *Biochim. Biophys. Acta*, 50 (1961) 102.
6 A. LOYTER AND M. SCHRAMM, *Biochim. Biophys. Acta*, in the press.
7 A. K. LAIRD AND A. D. BARTON, *Biochim. Biophys. Acta*, 27 (1958) 12.
8 C. A. SCHNEYER AND L. H. SCHNEYER, *Am. J. Physiol.*, 198 (1960) 771.
9 L. E. HOKIN, *Biochem. J.*, 48 (1951) 320.
10 L. E. HOKIN, *Biochem. J.*, 50 (1951) 216.
11 R. SCHUCHER AND L. E. HOKIN, *J. Biol. Chem.*, 210 (1954) 551.
12 A. ULLMANN AND F. B. STRAUB, *Acta Physiol. Acad. Sci. Hung.*, 8 (1955) 279.
13 R. B. KHESIN, *Biokhimiya*, 18 (1953) 462.
14 A. ULLMANN AND F. B. STRAUB, *Acta Physiol. Acad. Sci. Hung.*, 11 (1957) 31.
15 M. T. SZABÓ AND T. GARZÓ, *Acta Physiol. Acad. Sci. Hung.*, 12 (1957) 303.
16 A. ULLMANN, T. GARZÓ AND F. B. STRAUB, *Acta Physiol. Acad. Sci. Hung.*, 13 (1958) 179.
17 Z. GROMET-ELHANAN AND T. WINNICK, *Bull. Research Council Israel*, 11A (1962) 69.
18 P. SIEKEVITZ AND G. E. PALADE, *J. Biophys. Biochem. Cytol.*, 4 (1958) 309.
19 P. SIEKEVITZ, *J. Biol. Chem.*, 195 (1952) 549.
20 P. BERNFELD, in S. P. COLOWICK AND N. O. KAPLAN, *Methods in Enzymology*, Vol. I, Academic Press Inc. New York, 1955, p. 149.
21 O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR AND R. J. RANDALL, *J. Biol. Chem.*, 193 (1951) 265.

[22] M. DUBOIS, K. A. GILLES, J. K. HAMILTON, P. A. REBERS AND F. SMITH, *Anal. Chem.*, 28 (1956) 350.
[23] A. J. MORRIS AND S. R. DICKMAN, *J. Biol. Chem.*, 235 (1960) 1404.
[24] E. FARBER AND H. SIDRANSKY, *J. Biol. Chem.*, 222 (1956) 237.
[25] L. E. HOKIN, *Biochim. Biophys. Acta*, 18 (1955) 379.
[26] P. SIEKEVITZ AND G. E. PALADE, *J. Biophys. Biochem. Cytol.*, 4 (1958) 557.
[27] V. G. ALLFREY, M. M. DALY AND A. E. MIRSKY, *J. Gen. Physiol.*, 37 (1953) 157.
[28] P. SIEKEVITZ AND G. E. PALADE, *J. Biophys. Biochem. Cytol.*, 4 (1958) 203.
[29] A. K. LAIRD AND A. D. BARTON, *Biochim. Biophys. Acta*, 25 (1957) 56.
[30] M. M. DALY, V. G. ALLFREY AND A. E. MIRSKY, *J. Gen. Physiol.*, 39 (1955) 207.
[31] J. L. VAN LANCKER AND R. L. HOLTZER, *J Biol. Chem.*, 234 (1959) 2359.
[32] P. SIEKEVITZ AND G. E. PALADE, *J. Biophys. Biochem. Cytol.*, 7 (1960) 619.

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STRUCTURE OF *HANSENULA CAPSULATA* NRRL Y-1842 PHOSPHOMANNAN

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SUMMARY

Di- and trisaccharide phosphomonoesters have been isolated after mild acid hydrolysis of the phosphomannan produced by *Hansenula capsulata* NRRL Y-1842. Periodate oxidation studies on both the oligosaccharide phosphates and the intact polymer have served to elucidate further the polyphosphodiester structure. The disaccharide ester has been characterized as 2-*O*-(6-*O*-phosphoryl-β-D-mannopyranosyl)-D-mannose; the trisaccharide ester appears to contain an additional mannosyl residue α-1,2-linked to the phosphorylated moiety of the disaccharide. The biosynthetic implications of these findings are discussed.

INTRODUCTION

Among the phosphorylated mannose polymers[1] produced by yeasts of *Hansenula* and related genera, those produced by *H. capsulata* strains are the most highly phosphorylated[2]. The latter polymers are further distinguished by their low or negative optical rotations, indicating the presence of mannosidic linkages of the β-configuration[2].

Previous work[3] on the phosphate linkages in phosphomannans has shown that these extracellular polymers are polyphosphodiesters containing the α-1,6'-phosphate ester bridge between mannose units as a characteristic structural feature. Also, mild

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