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THE BIOSYNTHESIS OF UBIQUITIN BY PARATHYROID GLAND

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SUMMARY

Data are presented on the isolation, biosynthesis, and identification of a small peptide (H) from parathyroid gland. Under our experimental conditions this peptide (H) represents, in addition to secretory protein-I and proparathyroid hormone, the other major protein which is rapidly synthesized during short term incubations of tissue slices. N-terminal sequence analysis was performed on samples of peptide H and the resulting data used to conduct a search of the sequence data bank. The search established the identity of peptide H as ubiquitin. These findings establish parathyroid gland as another system which rapidly produces ubiquitin in vitro, in addition to the systems employing hypothalamus and pituitary where ubiquitin biosynthesis was initially observed by Seidah et al and Scherrer et al.

INTRODUCTION

During the past several years our laboratory has been investigating the biosynthesis, processing and secretion of parathyroid hormone (for reviews see 1,2). Throughout the course of some of these studies, which initially focused on the hormone and prohormone, we have observed the presence of an unidentified peptide in extracts of tissue homogenates. Our attention was first drawn to this peptide because of its rapid labeling with radioactive amino acids, included to monitor the synthesis of prohormone. This report describes the purification of this peptide and presents sequence data which identifies it as ubiquitin.

METHODS

Fresh bovine parathyroid glands were trimmed, sliced and incubated at 37°C in a Krebs-Ringer buffer for 1 hour as described previously (3). The slices were then transferred to fresh medium containing one or more ^{3}H- or ^{14}C-labeled amino acids (New England Nuclear) and incubated for various time periods. Batches of incubated slices were homogenized in 10 volumes of 8 M

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urea, 0.2 M HCl, 0.1 M cysteine in a Polytron homogenizer. The filtered homogenate was centrifuged and processed by established methods (4) to yield a crude hormone preparation (trichloroacetic acid powder, TCA). TCA powders were next chromatographed on 2.5 x 45 cm G-100 Sephadex columns to crudely separate the radioactively labeled proteins into 3 components (void volume region, hormone-prohormone region and small peptide region). The small peptide region which contained the unidentified peptide H was pooled, lyophilized, and rechromatographed on a carboxymethyl-cellulose column (0.5 x 12 cm) using a linear salt gradient (5). Some fractions were first gel filtered through a Sephadex G-75 column prior to the cellulose column step. The peptide H material eluting from the cellulose column was monitored by aliquoting fractions for radioactivity measurement.

Molecular weight estimates were performed on a calibrated G-75 Sephadex column in guanidine-HCl using standards ranging from 25,000 to 6,000 daltons, as reported earlier (6).

Sequencing was performed on a Beckman model 890C instrument using either a DMAA (dimethylallylamine) program or an updated dilute Quadrol program (7). Identification of PTH-amino acids was accomplished using a Waters high pressure liquid chromatograph (8) and by amino acid analysis following back hydrolysis at 150°C for 4 hours (9). Amino acid compositions were performed on samples hydrolyzed in 5.7 N HCl (containing phenol) in evacuated sealed tubes at 110° for 20 hours using a Beckman 121 MB analyzer.

RESULTS

Figure 1 shows a gel filtration pattern of a TCA powder. The tissue in this example was incubated 30' with ^{3}H-leucine. Typically, we observe significant incorporation into large molecular weight proteins, (V_o fraction) the hormone-prohormone region, (fractions 52-65) and a lesser amount into a late eluting fraction (72-80) which we have labeled peptide H. The peak of radioactivity in this fraction is not coincident with the absorbance but rather occurs on the decending side of the absorbance peak. This elution position suggests a small molecular size for peptide H.

Figure 2 illustrates the ion exchange chromatography of the peptide H sample obtained by the gel filtration step. Essentially all of the radioactivity eluted from the cellulose column at a conductivity of 2.5 m Mho. Separation of some nonradioactive protein from the peptide H fraction was achieved. Occasionally, it was advantageous to first remove larger quantities of this nonlabeled component by a second gel filtration through G-75 prior to the ion exchange column step. The peptide H fractions obtained from the ion exchange column were homogeneous as evaluated by polyacrylamide gel

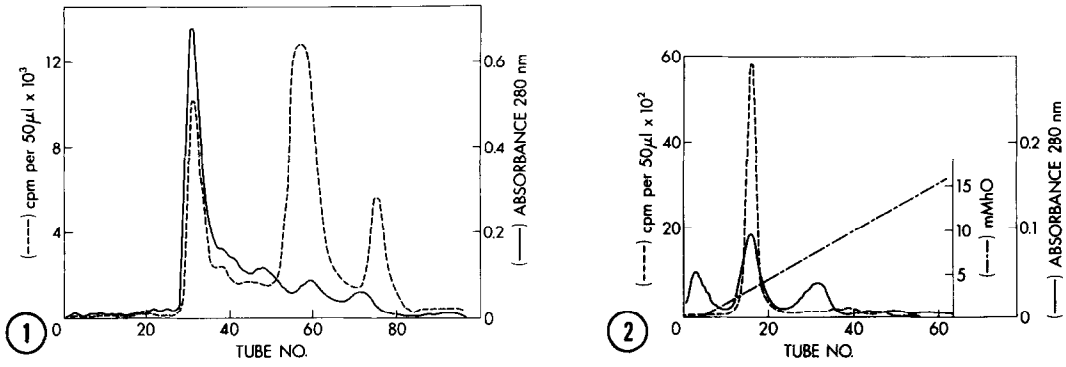

Figure 1. G-100 Sephadex elution profile of a T.C.A. powder. Sample was prepared from 10 g of tissue slices which were incubated with ^{3}H-leucine for 30 min. The column was eluted with 0.1 M ammonium acetate pH 4.8. 2.0 ml fractions were collected and aliquots of 50 µl were removed for counting. The peak of radioactivity at tubes 72 to 80 contains the peptide H material.

Figure 2. Carboxymethyl-cellulose chromatography of the peptide H fraction obtained from the gel filtration step. The column was eluted with a linear gradient formed from 0.01 M ammonium acetate pH 5.0 (120 ml) and 0.33 M ammonium acetate pH 7 (120 ml). 2.0 ml fractions were collected and 50 µl aliquots removed for counting. Conductivity was measured on every fifth fraction. Fractions 13 to 18 were pooled as peptide H.

electrophoresis and amino acid analysis through the peak. Molecular size estimates of the purified peptide H resulted in values between 5,000 and 6,000 daltons.

There are no unusual features found in the amino acid composition of peptide H. The molecule lacks cysteine and tryptophan. Upon comparison of this composition with that of parathyroid hormone (or proparathyroid hormone) there are no obvious similarities. However, it was interesting to find that the amino acid compositions of the hormone and peptide H are similar if like amino acids are grouped and then expressed as percentages for each grouping (10).

In examining the time course of incorporation of amino acid into peptide H we have observed that its biosynthetic rate parallels that of proparathyroid hormone. One such experiment is shown in Figure 3. Thus under these conditions parathyroid tissue synthesizes primarily 3 proteins, 2 of which were identified earlier as proparathyroid hormone (which begins to be

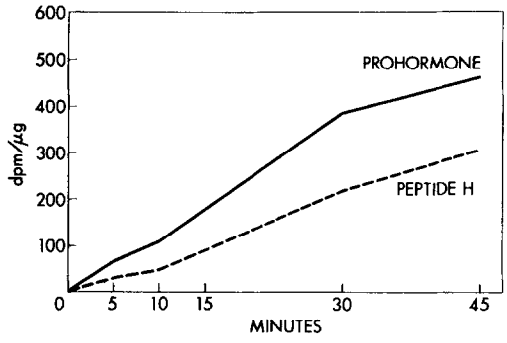

Figure 3. Biosynthetic time course of peptide H and proparathyroid hormone. 5 g portions of tissue slices were incubated with ^{3}H-leucine for the indicated times. Then prohormone and peptide H were isolated for each time point by adding 10 g of non-radioactive slices to each flask (as carrier) and processing the resulting T.C.A. powders as described.

converted to hormone after about 20 min) (5,11,12), and the 70,000 dalton secretory protein-I (13,14), and the third being peptide H. The synthetic rate of secretory protein is not shown in this data but it too displays a rapid rate of synthesis.

Due to the similarities found in the synthetic rates of peptide H and proparathyroid hormone and the compositional match between peptide H and the hormone, we considered the possibility that this small peptide may be structurally related to the prohormone hormone structure. On this basis we initiated some N-terminal sequence analyses. After obtaining positive identification of approximately 50 residues it was readily determined that peptide H is not homologous with parathyroid hormone. However, a search of the sequence data bank by Dr. L. Hunt at the National Biomedical Research Foundation did permit identification of peptide H as ubiquitin (15). Table 1 is a compilation of the sequence data obtained for peptide H and for comparison includes the first 50 residues of bovine ubiquitin. Although we have not yet completed the total sequence of peptide H these data clearly establish it to be ubiquitin.

TABLE 1

Partial Sequence of Peptide H and Ubiquitin

	1				5					10					15					20
Peptide H ...	M	Q	I	F	V	K	T	L	T	G	K	T	I	T	L	E	V	E	P	_
Ubiquitin‡ ..	M	Q	I	F	V	K	T	L	T	G	K	T	I	T	L	E	V	E	P	S
	21				25					30					35					40
Peptide H ...	D	T	I	E	N	V	K	A	K	I	Q	D	K	Q	G	I	P	P	D	Q
Ubiquitin ...	D	T	I	E	N	V	K	A	K	I	Q	D	K	E	G	I	P	P	D	Q
	41				45					50										
Peptide H ...	E	R	L	G	F	A	G	K	S	L	-	-	-	-	-	-	-	X		
																		74		
Ubiquitin ...	Q	R	L	I	F	A	G	K	Q	L	-	-	-	-	-	-	-	R		

The data shown for peptide H were compiled from 3 separate runs of automated sequencing. Position 20 has not been positively identified.

‡Sequence data for bovine thymus ubiquitin taken from Schlesinger et al (15).

DISCUSSION

Ubiquitin has been known for several years and is reported to be present in many tissues (16). It was first obtained from bovine thymus and the sequence was established (15). The physiological function of ubiquitin is unknown. It is reported to induce differentiation of T and B lymphocytes and to activate adenylate cyclase (16). Recently, the sequence of the first 37 residues of the nonhistone component of the nuclear protein, A24, was found to be identical to ubiquitin (17). This suggests some possible function for ubiquitin in chromatin structure. Just how the findings described here relate to a possible nuclear role for ubiquitin is not clear at present.

The data presented in this report establish that parathyroid tissue incubated in vitro rapidly biosynthesizes the small peptide ubiquitin. Moreover, the ubiquitin synthesized represents a significant portion of the rapidly produced protein of parathyroid tissue as judged by incorporation of radioactive amino acids. The other two major protein products, previously identified (11-14) in this tissue, are proparathyroid hormone, the immediate precursor to hormone, and secretory protein I. The observed similarity in

the rates of production of prohormone and ubiquintin suggests the possiblity of some as yet undefined relationship between the two.

The biosynthesis of ubiquitin has already been described in rat brain, hypothalamus and human pituitary tissue, both normal and tumor (18,19). Those findings were, much like our own, somewhat fortuitous, in that the system was originally established to study production of beta-lipotropin and ACTH. However, these workers also observed a significant amount of radioactive amino acid incorporated into a small peptide, which they subsequently identified as ubiquitin. It remains to be determined just why the parathyroid gland and brain tissue and perhaps other tissues as well, exhibit such a rapid synthesis of this peptide, but this suggests that the ubiquitin is quickly turning over perhaps as a result of its role in the cell.

The disparity between our determined molecular weight of peptide H (6000) and the actual 8,500 daltons determined from the sequence for the 74 residue ubiquitin is puzzling. The possibility that we have only a fragment of ubiquitin is not likely because our compositional data indicate 1 histidine and 1 tyrosine which is the case with bovine ubiquitin, tyrosine being at residue 59 and histidine at residue 68. We have not yet encountered a tyrosine or histidine in the first 50 residues. A similar molecular weight discrepancy was also noted by Scherrer et al (19) who reported their brain ubiquitin peptide migrated with beta-endorphin (about 4000 on Sephadex). It is not known why this molecule and others behave anomalously in these systems. Interestingly, this same property is characteristic of parathyroid hormone also (6).

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