AMINO ACID COMPOSITION AND N-TERMINAL AMINO ACID SEQUENCE OF PORCINE SECRETIN[1)]

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The preparation of porcine secretin with a biological activity of $2x10^4$ clinical units (= $4x10^5$ Hammarsten cat units) per mg. air dry weight was recently reported (Jorpes and Mutt 1961). Preliminary amino acid analysis of the small amount of polypeptide material available at that time seemed to indicate that secretin was a polypeptide with the composition alanine (1), arginine (3), aspartic acid (1), glutamic acid (2), glycine (2), leucine (5), serine (2), threonine (1) and valine (1).

This type of secretin material has now been obtained a number of times by the same technique except that the counter-current distribution has been carried through 200 transfers in an all-glass automatic distribution apparatus (H.O. Post, Inc., Middle Village 79, N.Y., U.S.A.).

300 mgs. of the material purified by chromatography on carboxymethyl cellulose was loaded into the first three tubes of the apparatus and the distribution carried out during 70 hours in an atmosphere of argon at $19^{\circ}C \pm 1^{\circ}C$. After the distribution was completed aliquot samples were removed from the tubes and assayed for peptide material with the ninhydrin reaction after hydrolysis according to von Hoffsten (1956) and for secretin activity according to Mutt and Söderberg (1959). In all experiments the bulk of peptide material was found in the first fifteen tubes of the

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series and secretin activity in tubes 60 through 100. The contents of tubes 60 through 100 were combined and the phases made to coalesce by the addition of 180 ml. of ethanol. The solution was kept at -15°C overnight and then warmed to +4°C. The phosphates that had precipitated were filtered off and discarded. The clear filtrate was dissolved in 15 volumes of water at room temperature and the pH of the solution brought to 3±0.1 with hydrochloric acid. Then 2.5 grams of alginic acid (dry weight) prewashed with ethanol, 0.2 M hydrochloric acid and water were suspended in the solution and the mixture stirred for one hour at room temperature. The alginic acid, bearing the adsorbed secretin, was collected on a Büchner funnel (5.5 cm. in diameter) and washed repeatedly on the filter paper with ice-cold 0.001 M HCl. The secretin was eluted from the alginic acid with portions of 0.2 M HCl, 50 ml. in all. The chloride in the eluate was exchanged for acetate on a column of DEAE-Sephadex (A 25, Coarse, obtained from Pharmacia, Uppsala, Sweden). The effluent from the Sephadex column was lyophilized. The weight of the lyophilized product was, in three experiments, 24, 21 and 30 mgs., respectively. The secretin activity was in each instance practically the same, 2×10^4 clinical units per mg.

Amino acid analyses of the material according to Spackmann et al. (1958) after hydrolyzing for 22 hours, 110°C in an atmosphere of argon confirmed the absence of sulfur-containing amino acids, tyrosine, isoleucine, lysine, tryptophan and proline. However, phenylalanine and histidine were found in amounts sufficient to account for one mole residue of each per mole of secretin. Quantitative results of the amino acid analyses are shown in the table. From these the minimum molecular weight was calculated to be 3200 - 3500. The absence of tryptophan was also confirmed by a modification of the Voisenet-Rhode p-dimethylaminobenzaldehyde reaction (Spies et al. 1950). The absence of tyrosine was confirmed by the 1-nitroso-2-naphthol method of Gerngross et al. (1933) as modified by Ceriotti et al. (1957). Further evidence that both tyrosine and tryptophan are absent was the lack of a relative maximum in the light absorbancy curve around 2800 Å. Quantitative sulfur analysis, performed by Dr. W. Kirsten, Microchemical Laboratory, Uppsala University, Sweden, gave the figure 0.04%, which is compatible with the amino acid analyses, indicating complete absence of sulfur-containing amino acids.

TABLE

Amino acid analysis of porcine secretin (400,000 HCU/mg		
Amino acid residue	Amount in micromoles per mg	Probable number of residues per 3200-3500 grams
Ala	0.272	1
Arg	0.988	4
Asp	0.489	2
Glu	0.683	3
Gly	0.486	2
His	0.202	1
Ileu	0.031	0
Leu	1.400	6
Lys	0.045	0
Phe	0.262	1
Pro	0.007	0
Ser	0.844	4
Thr	0.444	2
Val	0.246	1
NH_3	1.063	(5)
	Total number:	27

N-terminal amino acid sequence. Using the phenylisothiocyanate method of Edman (Eriksson et al. 1960) the reaction mixture after coupling was extracted 5 times with benzene, the residue freeze-dried, phenylthiohydantoins formed at 40°C, for 2 hours in glacial acetic acid saturated with HCl gas. Those PTH-amino acids that are soluble in ethyl acetate were extracted and chromatographed in solvents I, II and III (Sjöquist 1960). Part of the extracted aqueous phase was chromatographed in solvent IV, the rest of this phase was reprocessed with phenylisothiocyanate to couple the amino acid residue next in sequence. The identity of the PTH-derivatives was established by chromatographing known PTH-amino acids parallel to the sample and by determination of the light absorbancy spectrum in the ultraviolet after eluting the chromatogram spots with 95% ethanol. The results of two different experiments on separate preparations indicated that the first three residues from the N-terminal end are his.ser.asp(NH_2) or his.ser.asp. This sequence resembles the his.ser.glu. sequence at the N-terminal end of glucagon (Bromer

et al. 1957). Qualitative and quantitative confirmation of the N-terminal histidyl residue was obtained by dinitrophenylation according to Sanger's method (Fraenkel-Conrat et al. 1954). DNP-histidine was identified in the paper chromatography system of Mellon et al. (1953).

Trypsin digestion of the secretin followed by descending chromatogra on Whatman 3 HR paper in the butanol, acetic acid, pyridine, water system of Waley and Watson (1954) led to the separation of four components with R_f values of 0.2, 0.4, 0.5 and 0.8, respectively, under the conditions of the experiments.

Amino acid analysis of the "tryptic peptides" was performed preliminarily according to the method of Redfield (1953) and showed that three components contained arginine, whereas the fourth, with R_f 0.8 did not. This latter component, on the assumption that trypsin had split only arginyl peptide bonds, should represent the C-terminal fragment of secretin. The amino acids in this fragment are leucine, valine, glycine and glutamic acid. The fragment with R_f 0.2 contains arginine, serine, alanine and aspartic acid. Histidine could be demonstrated by Pauly's reaction (Smith 1960) in both the components with R_f 0.4 and 0.5, which probably indicates unsatisfactory separation of these peptides. Alanine was found only in the component with R_f 0.2 and valine in that with R_f 0.8

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