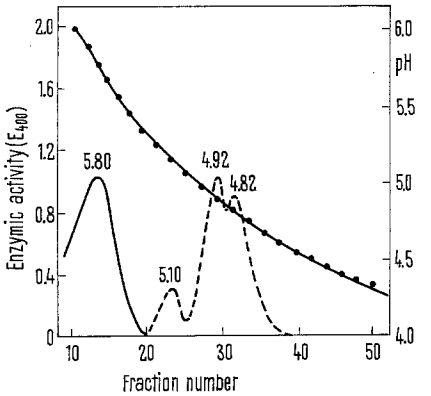

Fig. 1. Isoelectric focusing of native (---) and neuraminidase treated (—) acid phosphatase I. The diagram is a composition of 2 separate experiments carried out under the same conditions. The electrolysis (600 V/2 mA/48 h) was carried out according to SVENSSON[6] in a 26-ml apparatus[3] filled with 1% ampholine solution (LKB, Stockholm) in 0–50% sucrose gradient. ●—●, pH gradient. Protein concentration paralleled with enzymic activity.

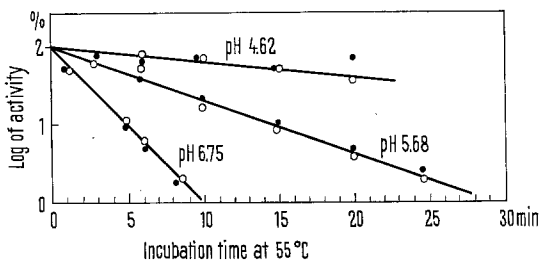

Fig. 2. Thermal denaturation of native (○—○) and modified (●—●) enzyme at various pH. The reaction mixture of 100 µl of buffer solution and 1 µl of enzyme solution (1 mg/ml) was heated at 55 °C for indicated periods of time, then cooled to 0 °C and activity determined with *p*-NPP as a substrate in standard conditions. For pH 4.62 and 5.68 citrate and for pH 6.75 *Tris*-maleic acid-NaOH buffer solutions were used.

Values of K_m, V_{max} and $\Delta(\Delta F°)$ of native phosphatase I (E) and treated with neuraminidase (En)

Substrate	K_m	V_{max}	$-\Delta(\Delta F°)$
pA	E 1.85×10^{-3}	9.5	
	E_n 1.05×10^{-3}	4.25	364
d-pGp	E 3.0×10^{-3}	10.5	
	E_n 2.35×10^{-3}	5.0	34
p-NPP	E 7.25×10^{-4}	4.0	
	E_n 7.05×10^{-4}	3.5	28

All the assays were carried out in 0.1 *M* citrate buffer of pH 5.5, with incubation at 37 °C for 10 min. The values of K_m are expressed in moles of substrate per liter and V_{max} in µg/ml of Pi or *p*-nitrophenol. Values of $\Delta(\Delta F°)$ are in cal/mole.

enzyme. This suggestion is supported by the changes in the decrease of free energy calculated for different substrates[8]:

$$\Delta F° = -RT \ln \frac{1}{K_m}$$

hence

$$\Delta(\Delta F°) = \Delta F°_{En} - \Delta F°_{E}$$

where E-native enzyme, and En-enzyme treated with neuraminidase. The Table shows that values of $\Delta(\Delta F°)$ decrease in proportion to increasing acidity of the substrate molecule, i.e. from pA, through d-pGp to *p*-NPP[9,10].

The phosphatase digested with neuraminidase was more strongly inhibited by L(+)-tartrate as competitive inhibitor[11], and the calculated value of K_i for p-NPP as substrate is 3.6×10^{-4}, compared with 6.2×10^{-4} for native enzyme. This is probably due to presence of neuraminic acid residues on the surface of the protein molecule presenting an obstacle to penetration of strongly polar molecules of the inhibitor into the active center of the enzyme.

It should be emphasized that, according to Moss[12] and SARASWATHI and BACHHAWAT[13], neuraminic acid in alkaline phosphatases from animal and human tissues plays a similar role in heterogeneity and activity as in prostatic acid phosphatase.

Résumé. On a comparé certaines propriétés physicochimiques et enzymatiques de la phosphomonoestérase acide native de la prostate de l'homme à celle traitée par la neuraminidase. L'enzyme dépourvue d'acide neuraminique atteint son optimum à un pH élevé, elle présente une plus grande affinité avec certains substrats et est inhibé d'une manière plus intense par le L(+)-tartrate que la phosphomonoestérase native.

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Molecular Architecture of Peptide Hormones Optical Rotatory Dispersion of Cholecystokinin-Pancreozymin, Bradykinin and 6-Glycine Bradykinin

The significance of secondary and tertiary structure of proteins with highly specific biological activity is fully recognized: the clefts in the molecule of enzymes accommodate the substrates and oxygen fits perfectly into the cavities of myoglobin or hemoglobin. A well-defined conformation for smaller molecules is less obvious and the general impression prevailing about shorter peptide chains, at least in neutral aqueous solution, is that of randomness. Peptide hormones with cycles formed by disulfide bridges (oxytocin, vasopressin, insulin) must have

serious limitations in assuming different conformations, but no such limitations can a priori be postulated for single chain peptides such as angiotensin, bradykinin, caerulein, etc. It was somewhat surprising, therefore, to observe the optical rotatory dispersion (ORD) and circular dichroism (CD) spectra of the gastrointestinal hormone (porcine) secretin. The spectra showed[1] striking similarity with those of lysozyme, a protein with low but well-established[2] helix content. The ORD and CD spectra of secretin (Figures 1 and 2) were interpreted as indications for a part of the molecule being in a rigid form. A comparison of the spectra of the hormone itself with the spectra of a series of shorter peptides corresponding to partial sequences of secretin suggested that this rigidity in one part of the chain is a consequence of intramolecular cooperative interactions[3] between distant parts of the molecule. Addition of alcohol to the aqueous solution of the N-terminal half or the C-terminal half of secretin increased the values at the maxima but did not produce the characteristic spectra of the hormone itself. On the other hand, the conformation of the complete molecule was found sufficiently stable to allow heating of its aqueous solution. At a higher temperature the 'helicity' decreased but the spectra were restored on cooling (Figure 3).

While the spectra of the shorter fragments from secretin[1] do not show complete randomness, they either represent some preferred conformation for which no simple geometric description can be formulated, or they may correspond to several conformations simultaneously present in the population of individual molecules. Certain rigidity, however, could be present even in small peptides. The sequence of bradykinin[4] is suggestive in this respect because this nonapeptide contains 3 L-proline residues, 2 of them neighbors. Yet an examination of the ORD and CD spectra of bradykinin[4] and 6-glycine bradykinin[5] (Figures 4 and 5) failed to reveal characteristics that would point to helical or other well-defined conformations. This observation is in harmony with the diffusion studies of CRAIG[6] which also suggest considerable conformational freedom for bradykinin.

The conclusion that a peptide such as bradykinin consisting of 9 amino acids does not have a preferred conformation but that a well-defined geometry develops from a certain

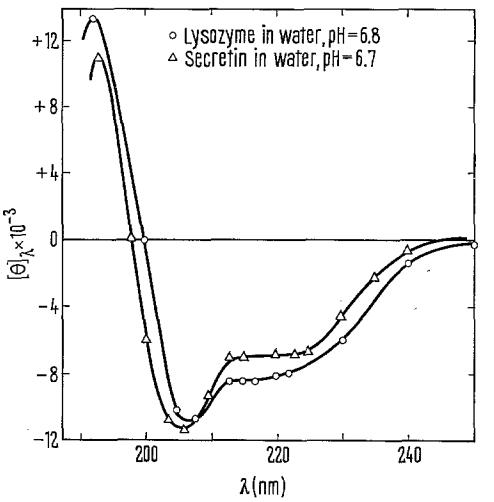

Fig. 2. Circular dichroism spectra of lysozyme and secretin.

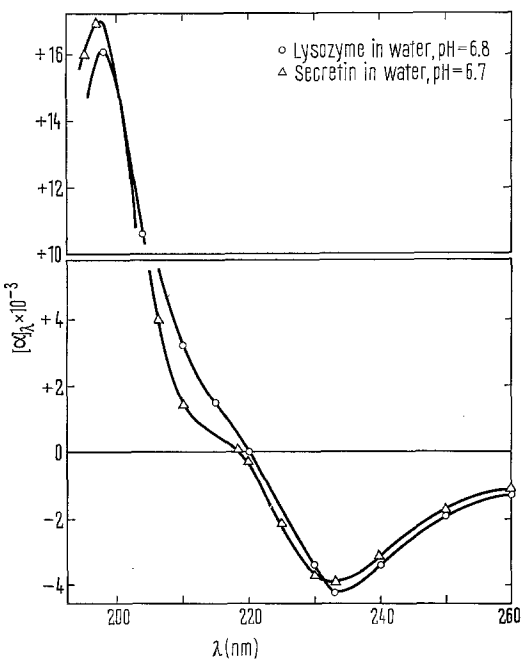

Fig. 1. Optical rotatory dispersion spectra of lysozyme and secretin.

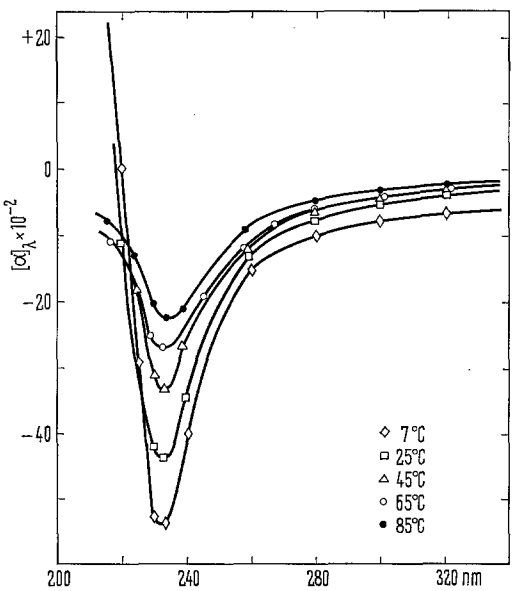

Fig. 3. Changes in the optical rotatory dispersion spectra of secretin on heating of its aqueous solutions (the changes are reversible).

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chain length on (secretin contains 27 amino acids, glucagon with fairly similar sequence and somewhat similar spectra[7] 29 amino acids), would be attractive but is not well founded. Examination of the ORD and CD spectra of the intestinal hormone cholecystokinin-pancreozymin (CCK-PZ)[8] (Figures 4 and 5), a single chain peptide of 33 amino acids, gives no evidence for structural rigidity which would express itself in spectra similar to those of helical molecules. While some trough-like depressions can be observed in the ORD spectra of bradykinin, 6-glycine bradykinin and CCK-PZ, this should not be interpreted as evidence for helicity. The rotatory bands characteristic of helical structures (trough at 233 nm, shoulder at 220 and peak at about 190 nm)[9] are absent in Figure 4, while a complete sequence of these characteristic features cannot be missed in the spectra of lysozyme and secretin (Figure 1). The CD spectra (Figure 5) lead to similar conclusions. (A trough at 222 nm is known[9] to be characteristic for helical structures, a positive band near 220 nm and a negative around 206 nm for randomly disordered polypeptides.) The spectra of bradykinin, 6-glycine bradykinin and CCK-PZ do not exclude some preferred conformation, but they show no evidence for rigidity similar to that found in helical molecules. Spectra of partial sequences from CCK-PZ were also studied and were found to lack features pointing to known secondary or tertiary structures.

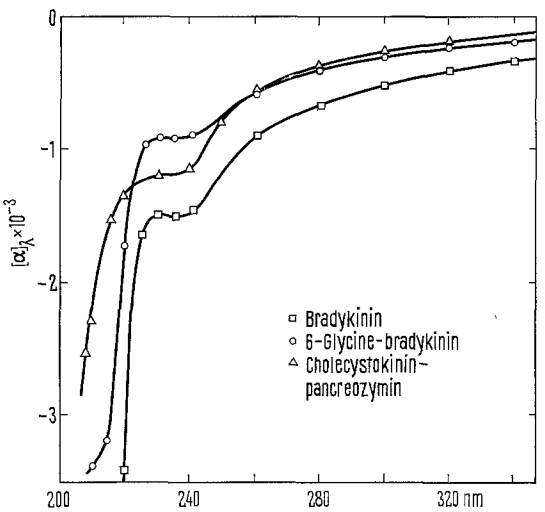

Fig. 4. Optical rotatory dispersion spectra of bradykinin, 6-glycine bradykinin and cholecystokinin-pancreozymin in water.

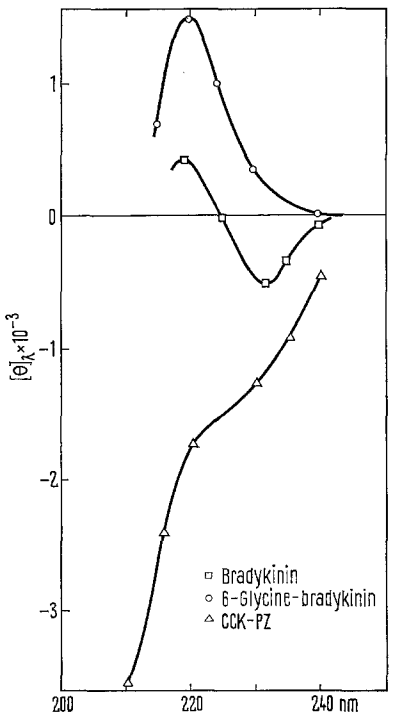

Fig. 5. Circular dichroism spectra of bradykinin, 6-glycine bradykinin and cholecystokinin-pancreozymin in water.

The contrast between the presence of well-defined conformation in secretin and the absence of rigid geometry in bradykinin or CCK-PZ prompts the speculation that the cooperative intramolecular interactions which determine the architecture of secretin might be replaced by similar but intermolecular interactions in CCK-PZ (perhaps also in bradykinin), that is by interactions between the hormone and the yet unknown receptor site. Some support for this speculation can be found in the remarkable fact that while with secretin practically the whole chain is needed for full biological activity[10], this is not true for CCK-PZ: already relatively short C-terminal sequences reveal the various hormonal activities of the CCK-PZ itself[11,12].

Zusammenfassung. Die optischen Rotationsspektren des intestinalen Hormons Cholecystokinin-Pancreozymin weisen, im Gegensatz zu denen des Secretins, auf das Fehlen bevorzugter Konformation und geben Einblick in den Zusammenhang zwischen molekularer Architektur und Hormonaktivität.

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