Conformation and Association of Pancreatic Polypeptide from Three Species†

Milton E. Noelken, Paul J. Chang,‡ and Joe R. Kimmel*

ABSTRACT: The 36-residue peptide hormone pancreatic polypeptide (PP) from avian, bovine, and canine sources has been studied by molecular sieve chromatography and shown to undergo an increase in Stokes' radius on going from pH 4 to pH 8. Circular dichroism measurements and determination of frictional ratios show that no major conformation changes occur with these pH changes, thereby indicating the Stokes' radius increases are due to association. This conclusion is supported by molecular weight determinations. Evidence is presented to support a pH-dependent monomer–dimer equilibrium for avian PP. Sequence-based predictions of secondary structure when applied to the three peptides identify an uninterrupted region of α helix in bovine PP and canine PP beginning at residue 13 and extending for ~16 residues. A helical segment of similar location and length is also identified in avian PP, but this helix is predicted to be disrupted approximately in the center. The helical content determined by circular dichroism measurements is in good agreement with the sequence-based predictions of conformation. In the case of all three hormones, the predicted α helix exhibits a hydrophobic face. It is suggested that this face is involved in dimer formation. The determination of Stokes' radii of molecules with molecular weights of ~4200 has extended the usefulness of molecular sieve chromatography.

Pancreatic polypeptide (PP)[1] is a single-chain peptide of 36 amino acid residues. Since its initial isolation from chicken pancreas (Kimmel & Pollock, 1968; Kimmel et al., 1975), homologous polypeptides from various mammalian species, including humans, have been purified and sequenced (Lin & Chance, 1972; Chance et al., 1979).

Evidence has accumulated which suggests that PP represents a new pancreatic hormone (Kimmel et al., 1975). Workers have found that blood levels of the peptide increase after feeding in chickens (Kimmel & Pollock, 1975) and humans (Floyd et al., 1977), and secretion of the peptide appears to be mediated by the parasympathetic nervous system (Hedo et al., 1978; Schwartz et al., 1978). Although the hormonal function of PP is unknown, administration of avian PP in microgram amounts by injection results in hepatic glycogenolysis, increased plasma triglyceride levels, and decreased plasma glycerol and amino acid levels (Kimmel et al., 1978). Recently, workers have observed the ability of the peptide to return to normal the hyperglycemia, hyperinsulinemia, and weight gain of New Zealand obese mice (Gates & Lazarus, 1977).

It is believed that peptide hormone action is mediated by specific membrane receptors, and studies of glucagon (Sasaki et al., 1975) and insulin (Pullen et al., 1976) have suggested that hydrophobic residues involved in the self-association of these hormones are also involved in receptor binding. Preliminary X-ray studies have indicated that the avian PP molecule has significant α-helical structure and is arranged as a compact dimer in the crystal (Wood et al., 1977). Gel chromatography data derived from the hormone purification indicate a pH-dependent change in avian PP's elution volume which suggests that the peptide may also polymerize in solution (Kimmel et al., 1975). It is therefore possible that an understanding of the nature of PP's self-association will be useful in relating its structure to its function.

In this paper we used small-zone molecular sieve chromatography and analytical ultracentrifugation to study the self-association of PP. In addition, conformation studies were made by means of circular dichroism measurements and sequence-based conformation predictions.

Materials and Methods

Materials. Avian pancreatic polypeptide was prepared by the method of Kimmel et al. (1975). The method of R. Chance (personal communication) was used to isolate canine PP, and bovine PP was a gift from Dr. Chance, Eli Lilly Co. Cytochrome *c* (bovine heart, Type V) and myoglobin (horse heart, Type III) were obtained from Sigma Chemical Co. Bovine pancreatic trypsin inhibitor (Kunitz) and soybean trypsin inhibitor (Bowman-Birk) were purchased from Worthington Biochemical Corp. All other chemicals were of reagent grade or the equivalent.

Small-Zone Analytical Molecular Sieve Chromatography. A 0.9 × 150 cm jacketed chromatographic column was packed with buffer-swollen and degassed Bio-Gel P-30 polyacrylamide. Columns were operated at a flow rate of 4 mL/h and at 25 °C. Each column was equilibrated for 48 h with the appropriate buffer prior to use.

Samples were applied to the column in 0.5-mL aliquots at a concentration of 1 mg/mL. Fractions (2 mL) were continuously collected, and effluent volumes were calculated by subtracting the weight of the empty fraction tubes from the weight of the tubes containing the collected fractions and dividing the result by the specific gravity of the buffer used. In this way, volume measurements could be made to within ±0.05 mL. Reproducibility of elution volumes, determined from peak position measurements of calibrating proteins, was ±0.18 mL. The concentration of eluted protein was monitored spectrophotometrically at 276 nm with a Zeiss single-beam spectrophotometer, using quartz cuvettes with 1.0-cm light paths.

Partition coefficients K_{av} were calculated from the measured elution volume V_e according to the equation (Ackers, 1970)

† From the Department of Biochemistry, University of Kansas Medical Center, Kansas City, Kansas 66103. *Received September 13, 1979.* This work was supported by National Institutes of Health Grant AM 09072 and by a grant from Eli Lilly Co.

‡ Taken in part from the undergraduate honors thesis of P.J.C., Department of Biology, Harvard College, Cambridge, MA.

[1] Abbreviations used: PP, pancreatic polypeptide; CD, circular dichroism.

0006-2960/80/0419-1838$01.00/0

$$K_{av} = \frac{V_e - V_0}{V_t - V_0} \quad (1)$$

where V_0, the void volume, was determined as the elution volume of bovine serum albumin, which is totally excluded from the P-30 gel matrix, and V_t, the total bed volume, was calculated by water calibration of the column prior to packing. The column was calibrated with proteins with known Stokes' radii.

To demonstrate the reversibility of the self-association of the peptide hormone and to minimize the loss of the very limited amounts of purified hormone available, it was necessary to reconcentrate the diluted hormone fractions after each column run. Fractions from chromatography experiments that contained peptide hormone were pooled together and concentrated by flash evaporation, followed by 48-h exhaustive dialysis of the concentration against the appropriate buffer.

Analytical Ultracentrifugation. A Beckman Model E analytical ultracentrifuge equipped with interference and Schlieren optics was used for sedimentation equilibrium and diffusion experiments.

Molecular weight determinations were made by the meniscus depletion sedimentation equilibrium method of Yphantis (1964) using an AN-D rotor with a 12-mm double-sector cell equipped with sapphire windows. Interference patterns were recorded on Kodak II-G spectrographic plates and fringe displacements were measured on a Nikon microcomparator. By use of 0.5-cm columns, initial protein concentrations of about 0.15 mg/mL, and 42 040 rpm running velocity, equilibrium was reached in about 5 days as verified by essentially identical fringe patterns observed at 5, 6, and 7 days of centrifugation. Only fringe displacements greater than 100 μm were used in the calculation of molecular weight. Blank corrections for cell distortion were made by exhaustively rinsing the cell with buffer after the sample had been run and measuring the fringe pattern obtained with buffer in both compartments with the rotor speed and temperature the same as those of the sample run. Partial specific volumes were calculated from the amino acid compositions (Kimmel et al., 1975; Chance et al., 1979) by the method of McMeekin et al. (1949). The values found were 0.719, 0.720, and 0.726 mL/g for the avian, bovine, and canine hormones, respectively.

Diffusion coefficients were measured in the analytical ultracentrifuge according to the height–area method described by Chervenka (1969). Runs were carried out at 16 200 rpm in a 12-mm double-sector cell equipped with an aluminum-filled Epon capillary-type synthetic boundary centerpiece. The Schlieren optical system was used, and patterns were recorded on Kodak metallographic plates. Areas under peaks were calculated by use of Simpson's approximate integration method involving discrete intervals. Values of D were obtained from plots of $(A_s/Y)^2$ vs. time in seconds where A_s is the area under the peak in cm^2 and Y is the peak height in cm. The slope of the plot has the value $4\pi D$. Diffusion coefficients were corrected to water at 20 °C by use of standard equations (Chervenka, 1969). Values for the viscosity of water and the 0.1 ionic strength Tris-HCl buffer were found in the literature (Weast, 1974–1975; Brewer et al., 1974). The viscosity of the 0.1 ionic strength sodium acetate buffer, pH 4.0, was measured at 24.7 ± 0.02 °C by using Cannon-Fenske viscometers, with flow times for water of ~100 s; the value found was 1.118 ± 0.001 cP.

Circular Dichroism. Circular dichroism measurements were made at room temperature in a Jasco J-20 spectropolarimeter using 1-cm path length cells in the 250–300-nm region and 1- and 0.5-mm cells at lower wavelengths. Polypeptide concentrations used were routinely about 0.2 mg/mL and were determined by amino acid analysis. The data are expressed as the mean residue ellipticity $[\Theta]_\lambda$ in deg cm^2 dmol^{-1}, as calculated from the expression $[\Theta]_\lambda = \Theta_\lambda M_0/10lc$, in which Θ_λ is the observed ellipticity at wavelength λ, M_0 is the mean residue weight of the polypeptide, l is the path length in centimeters, and c is the protein concentration in grams per milliliter. Values of M_0 were obtained from the amino acid compositions and are 117.7, 117.4, and 116.6 for the avian, bovine and canine peptides, respectively.

Prediction of Conformation. Sequenced-based predictive methods were used to assign secondary structure to PP molecules. The method of Chou and Fasman (Chou & Fasman, 1974; Chou et al., 1975; Fasman et al., 1976) and that of Maxfield & Scheraga (1976) have empirical foundation and were developed by correlation of regions in proteins of known three-dimensional structure with the amino acid sequence of those regions. The method of Lim (1974) involves a priori theory which takes into account general principles of protein structure such as compactness of form, the dimensions and geometry of side chains, and the presence of a tightly packed hydrophobic core and that of a hydrophilic external layer. In Lim's procedure, amino acid residues are first classified according to size and hydrophobicity or hydrophilicity: H_L = large hydrophobic, H_s = small hydrophobic, G_L = large hydrophilic, and G_s = small hydrophilic. Segments with α-helical structure are characterized by H_L residues in a 1,5 relationship in the sequence. These helical segments can be extended by linkage of 1,5 hydrophobic pairs, 1,4 terminal H_L pairs, or terminal H_L,G_L pairs 1,2, 1,4, or 1,5. Rules defining the requirements for helicity in these segments are given by Lim and include such considerations as the relative inability of some amino acid side chains (e.g., Tyr) to participate in tight packing in a hydrophobic core.

In general, each of these methods involves inspection of the amino acid sequence for regions of high probability of being either α helical or folded into β structure; the Chou–Fasman method allows prediction of β bends as well. In spite of this similarity, they differ enough from each other in details that it is useful to employ the three of them.

Results

Calculation of Stokes' Radii. (1) Diffusion. Due to the relative paucity of literature describing proteins with known Stokes' radii within the desired range, R_S values for the two calibration proteins soybean trypsin inhibitor (Bowman–Birk dimer) and bovine pancreatic trypsin inhibitor (Kunitz) were calculated from analytical ultracentrifuge diffusion experiments performed in 0.1 ionic strength sodium acetate buffer, pH 4.0. In addition, diffusion data were used to derive R_S values for avian PP, pH 8.0 (Tris-HCl, 0.1 ionic strength), and canine PP, pH 4.0 (sodium acetate buffer, 0.1 ionic strength).

Stokes' radii were calculated from the observed diffusion coefficient $D_{20,w}$ by the relation

$$R_S = \frac{(1 \times 10^{15})kT}{6\pi\eta_{20,w}D_{20,w}} \quad (2)$$

where k is the Boltzman constant, expressed in joules per kelvin, $\eta_{20,w}$ is the viscosity of water at 20 °C, and R_S is expressed in angstroms.

The observed diffusion coefficients and the calculated R_S values for avian PP, pH 8.0, and canine PP, pH 4.0, as well as for the trypsin inhibitors used for the column calibration, are presented in Table I.

Table I: Stokes' Radii Calculated from Diffusion Data[a]

protein	$D_{20,w} \times 10^6 \pm$ SD ($cm^2\ s^{-1}$)	$R_S \pm$ SD (A)
avian PP, pH 8.0	1.26 ± 0.02	17.1 ± 0.2
canine PP, pH 4.0 (acetate)	1.62 ± 0.03	13.4 ± 0.3
soybean trypsin inhibitor (Bowman-Birk dimer), pH 8.0	1.24 ± 0.03	17.5 ± 0.3
bovine pancreatic trypsin inhibitor, pH 8.0	1.46 ± 0.02	14.9 ± 0.2

[a] R_S values were calculated from diffusion coefficients by using eq 2.

Table II: Stokes' Radii Calculated from Gel Chromatography Data[a]

hormone	pH	$K_{av} \pm$ SD	$R_S \pm$ SD (A)
avian PP	4.0 (formate)	0.398 ± 0.008	14.1 ± 0.3
	4.0 (acetate)	0.408 ± 0.004	14.0 ± 0.2
	8.0 (Tris)	0.271 ± 0.003	16.0 ± 0.2
canine PP	4.0 (formate)	0.480 ± 0.003	13.0 ± 0.2
	4.0 (acetate)	0.442 ± 0.006	13.5 ± 0.3
	8.0 (Tris)	0.360 ± 0.004	14.7 ± 0.3
bovine PP	4.0 (formate)	0.474 ± 0.003	13.1 ± 0.2
	4.0 (acetate)	0.428 ± 0.004	13.7 ± 0.2
	8.0 (Tris)	0.313 ± 0.004	15.4 ± 0.3

[a] K_{av} values were calculated from elution volume data by using eq 1, while R_S values were determined by interpolation of the gel calibration curve, by using eq 3.

(2) *Molecular Sieve Chromatography.* Preliminary gel chromatography data derived from purification studies of avian PP (Kimmel et al., 1975) indicated that the elution volume at pH 4.0 was significantly greater than that observed for the peptide at pH 8.0. In this present study, the change in pH-dependent elution volume was represented in terms of change in Stokes' radius by use of analytical gel chromatography of proteins of known R_S values. When the logarithm of R_S values for these proteins was plotted against K_{av} values, a linear relationship was observed which by least-squares regression followed the equation

$$\log R_S = -AK_{av} + B \qquad (3)$$

where the values A and B were found to be 0.438 and 1.323, respectively.

R_S values for avian PP at pH 8.0 and 4.0 were calculated by interpolating from the above calibration curve. For comparison, R_S values for the homologous mammalian hormones, bovine PP and canine PP, were also calculated at pH 8.0 and 4.0. At pH 4.0, a significant concentration of undissociated acetic acid (~0.7 M) is present in the acetate–acetic acid buffer. Since this relatively apolar solvent could conceivably interfere with any peptide hydrophobic interactions, the hormones were also run at pH 4.0 by using a sodium formate–acid buffer, 0.1 ionic strength, which is more polar than the acetate buffer at this pH.

Table II summarizes the results obtained from the gel chromatography experiments. As can be seen from a comparison of Tables I and II, the R_S values of the peptides derived from gel chromatography and diffusion experiments are in excellent agreement with each other.

Molecular Weight Determination. Molecular weights for the pancreatic peptides were calculated from sedimentation equilibrium data by using the equation

$$M_r = \frac{2RT}{(1 - \bar{v}\rho)\omega^2} \frac{(\mathrm{d} \ln c)}{\mathrm{d}(r^2)} \qquad (4)$$

where R is the gas constant, expressed in erg/(K mol), T is the temperature, $\bar{v}$ is the partial specific volume of the peptide, ρ is the density of the solvent, and ω^2 is the angular velocity of the rotor.

Table III: Molecular Weight Determination by Sedimentation Equilibrium

hormone	M_r (obsd)	M_r of dimer (theor)[a]
avian PP, pH 8.0	8372	8476
canine PP, pH 8.0	7725	8452
bovine PP, pH 8.0	7136	8392

[a] Theoretical molecular weights for hormone dimers were determined from primary sequence data (Kimmel et al., 1975; Lin & Chance, 1972).

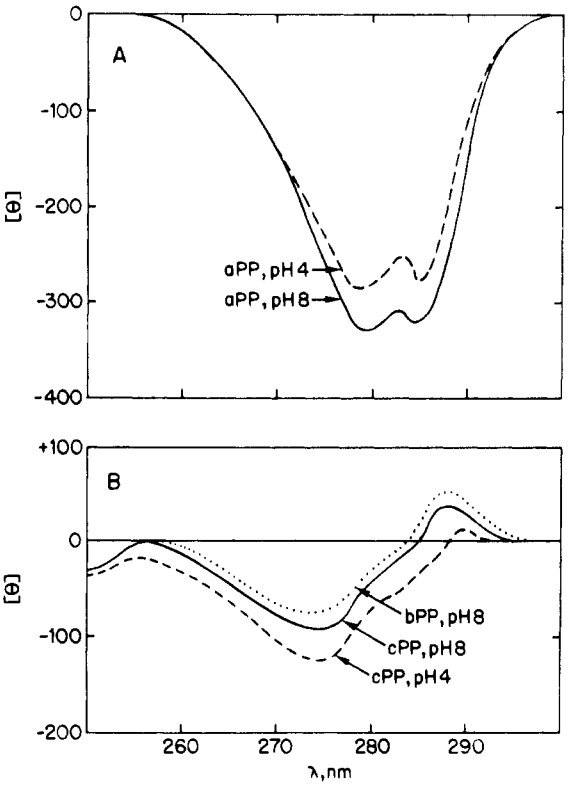

FIGURE 1: Near-ultraviolet circular dichroism spectra of pancreatic polypeptides measured at room temperature. Panel A: avian pancreatic polypeptide (aPP) in 0.1 ionic strength sodium formate buffer, pH 4, and in 0.1 ionic strength Tris-HCl buffer, pH 8. Panel B: bovine pancreatic polypeptide (bPP) and canine pancreatic polypeptide (cPP). Buffers are those described for panel A.

Although the sedimentation equilibrium experiments were successful at pH 8.0, data from the hormones at pH 4.0 were unsatisfactory due to the fact that meniscus depletion was never attained, even after 7 days, possibly due to the lower molecular weight of the peptides at this pH. The molecular weight results are listed in Table III.

Reversibility of Association. To demonstrate the reversibility of avian PP's observed pH-dependent change in R_S, we determined the partition coefficient of a sample of avian PP by chromatography at pH 8.0. Fractions containing the hormone were then pooled, concentrated, and dialyzed exhaustively against pH 4.0 (formate) buffer. The K_{av} value for this concentrated sample was determined. After reconcentrating the sample and bringing it back to pH 8.0, we repeated the above cycle several times. The constancy of the results shown in Table IV clearly demonstrates the reversibility of the pH-dependent change in the Stokes' radius of avian PP.

Circular Dichroism. The near-UV CD results are shown in Figure 1. The spectra of the bovine and canine hormones are quite similar, which is a reflection of their almost identical

Table IV: Reversibility of Change in K_{av} and R_S[a]

pH	8.0	→ 4.0	→ 8.0	→ 4.0	→ 8.0
K_{av}	0.270	0.399	0.272	0.397	0.269
R_S (Å)	16.0	14.1	16.0	14.1	16.0

[a] A particular sample of hormone was studied alternately at pH 8.0 and 4.0 as described in the text. R_S values were calculated by using eq 3.

amino acid sequences and thus comparable conformations. On the other hand, the pattern of the avian hormone is strikingly different from those of the mammalian species. This is in large part due to differences in location of the tyrosine residues within the amino acid sequence and related differences in the electronic environment of the tyrosyl residues due to differences in the nature of the neighboring residues and in local tertiary structure (Schellman, 1968; Strickland, 1974). The mammalian hormones have tyrosyl residues at positions 7, 20, and 27 and a COOH-terminal tyrosinamide. In contrast, the avian hormone has phenylalanine at position 20 which is followed by a tyrosine at position 21, a position occupied by alanine in bovine and canine PP. The aromatic side chains of the phenylalanine–tyrosine pair have electronic transition dipoles which could interact strongly due to their proximity and have profound effects on the CD spectrum (Strickland, 1974).

The far-UV CD spectra of the avian, bovine and canine hormones are presented in Figure 2. As in the case of the near-UV CD spectra, the bovine and canine hormones have far-UV spectra similar in magnitude and shape, but they clearly differ in shape from that of the avian hormone. The latter has a minimum at about 208 nm which is of greater magnitude than that at 222 nm, whereas the reverse is true for the other hormones. These differences may be due in large part to contribution of aromatic residues to the CD spectrum in this wavelength region (Sears & Beychok, 1973). In addition, secondary structure contributions are likely in view of the pronounced sequence differences between the avian and mammalian hormones in certain regions of the molecule. The double minimum at 208 and 222 nm is indicative of the presence of α helix. From the value of $[\Theta]_{222}$, estimates for α helix of ~40% for avian PP, and 55–60% for bovine PP and canine PP, can be made, based on the value of $[\Theta]_{222}$ of $-30\,000$ deg cm^2 dmol^{-1} found by Chen et al. (1974) for α helices in five proteins of known three-dimensional structure. The spectrum of the avian hormone could be fitted quite well in the wavelength region 206–240 nm by the method of Chen et al. (1974) with values of ~40% α helix, ~15% β structure, and 45% "random" structure at pH 8 and with similar values at pH 4. The value of the α-helix content is probably more accurate than those of β and random structures since the CD curve is more sensitive to the presence of α helix because of its larger CD signal.

To determine if the pH-induced association of avian PP was accompanied by a conformation change, we measured the CD spectra of two solutions, one at pH 4 and the other at pH 8. These were derived from the same stock solution of avian PP. The resulting spectra, shown in Figure 2A, are very similar to each other and indicate that no significant conformation change occurs upon aggregation of avian PP. The same conclusion appears to hold for canine PP as well (Figure 2B), although the spectra at pH 4 and 8 were obtained on solutions prepared independently.

Conformation Predictions. The results of sequence-based predictions of secondary structure are summarized in Table V for both avian PP and bovine PP. All three methods identify an α-helical segment in avian PP beginning at residue 13 and

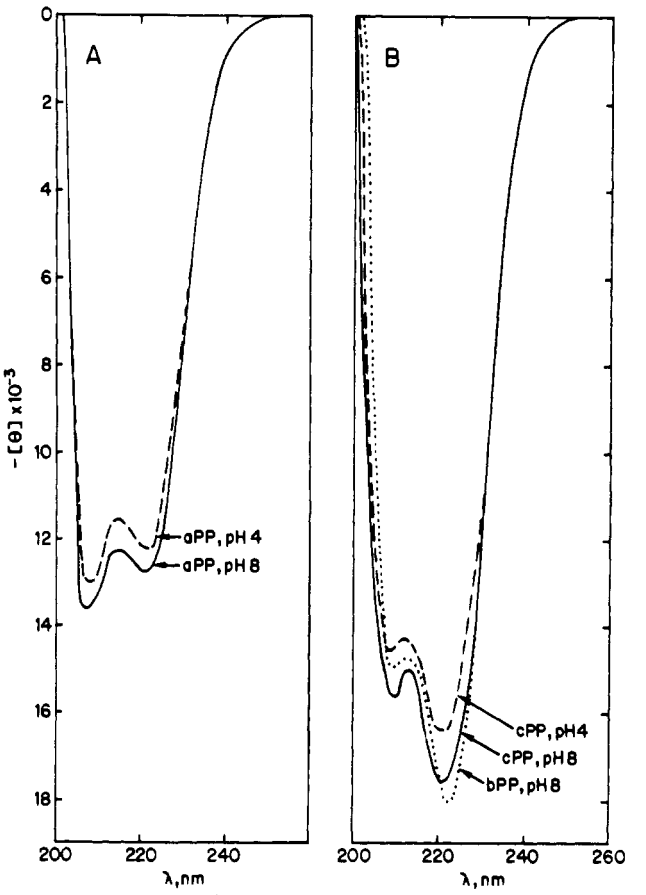

FIGURE 2: Far-ultraviolet circular dichroism spectra of pancreatic polypeptides measured at room temperature. Panel A: avian polypeptide (aPP) in 0.1 ionic strength sodium formate buffer, pH 4, and in 0.1 ionic strength Tris-HCl buffer, pH 8. Panel B: bovine pancreatic polypeptide (bPP) and canine pancreatic polypeptide (cPP). Buffers are those described for panel A.

extending to residue 19 or 20. The Lim method predicts a second α helix beginning at residue 24 and extending almost to the carboxyl terminus. The Chou–Fasman procedure recognizes secondary structure in this same region but predicts that α helix and β structure are almost equally probable. The Maxfield–Scheraga prediction does not recognize a second α-helical segment and instead predicts a more extended conformation in this portion of avian PP. It is noteworthy that all three methods predict α-helix disruption at residue 19 or 20. The predicted α-helical content is 50% by Lim's method, 20–44% according to Chou–Fasman predictions, and 19% by the Maxfield–Scheraga procedure.

In the case of bovine PP, an uninterrupted α helix of 14 or 15 residues is predicted by each method, and this helix is located in essentially the same part of the molecule as in avian PP (Table V). The helical content by all three methods is about 42%.

Chou–Fasman analysis predicts β bends at several locations in the NH_2-terminal portion of both avian and bovine PP. Some of these possibilities conflict with each other (Table V), and there is no clear reason to choose among them except to note that bends at identical locations are predicted for both peptides.

Conformation predictions applied to canine PP give results essentially identical with those for bovine PP.

A helical wheel model (Schiffer & Edmundson, 1967) for both avian and bovine PP encompassing the residues predicted to be in α-helical conformation is shown in Figure 3. In both cases, the helices exhibit a hydrophobic face, a property which

Table V: Conformation Predictions from Three Methods

Chicken Hormone

Residue					5					10					15					20					25					30					35		
Code	G	P	S	Q	P	T	Y	P	G	D	D	A	P	V	E	D	L	I	R	F	Y	D	N	L	Q	Q	Y	L	N	V	V	T	R	H	R	Y	- NH_2
C-F[b]		⌒	⌒		⌒	⌒				⌒	⌒		H	H	H	H	H	H	H	H	⌒	⌒		H	H	H	H	H	H	H	H						
																	B	B	B	B	B			B	B	B	B	B	B	B	B	B					
Lim													H	H	H	H	H	H	H					H	H	H	H	H	H	H	H	H	H	H			
M-S	E	E	E	E	E	E	E	E	E				H	H	H	H	H	H	H						E	E	E	E	E	E	E	E	E				

Bovine Hormone[a]

Residue					5					10					15					20					25					30					35		
Code	A	P	L	E	P	G[c]	Y	P	G	N	D	A	T	P	E	Q	M	A	Q	Y	A	A	G	L	R	R	Y	I	N	M	L	T	R	P	R	Y	- NH_2
C-F					⌒	⌒				⌒	⌒		H	H	H	H	H	H	H	H	H	H	H	H	H	H	H										
																											B	B	B	B	B	B					
Lim																H	H	H	H	H	H	H	H	H	H	H	H	H	H	H							
M-S	E	E	E	E	E	E	E	E	E					H	H	H	H	H	H	H	H	H	H	H	H	H	H	E	E	E	E	E	E	E			

[a] Chance et al. (1979). [b] C-F, Chou and Fasman; M-S, Maxfield and Scheraga. B represents residues in the β conformation; H, α helical; E, extended conformation; ⌒, β bends. [c] This residue is valine in the canine hormone (Chance et al., 1979).

may be of some importance in connection with the tendency of these molecules to dimerize. The hydrophobic face shown for avian PP represents the maximum number of residues in the α helix. All the predictive methods indicate a discontinuity or distortion at residues 20–23, and therefore the hydrophobic face of avian PP may differ from that shown.

Discussion

The results of this study show that avian, bovine, and canine PPs are compact, globular polypeptides at pH 4 and 8 and that each of them has a tendency to associate in a reversible manner at the higher pH. Circular dichroism results on avian and canine PP indicate that little, if any, change in secondary structure attends association.

Two approaches were used to estimate the secondary structure of these peptides, one based on CD and the other based on primary structure. In the case of avian PP, ~40% α helix was estimated from CD, which compares favorably with the value 50% from the Lim predictive method and a maximum of 44% by the Chou–Fasman method. In the case of the mammalian peptides, the CD results could not be analyzed by more rigorous techniques, and the α-helix content of 55–60% calculated from $[\Theta]_{222}$ is somewhat uncertain. However, the value is in reasonable agreement with the value 42% obtained from all three predictive methods used.

Avian PP has been studied by X-ray crystallography and found to occur as a compact dimer containing substantial amounts of α helix when crystallized at pH 7 (Wood et al., 1977). Our results indicate that avian PP, as well as bovine and canine PP, at pH 8, undergoes association and also exhibits significant α-helical content in solution.

The association of avian PP was studied in greater detail than that of bovine or canine PP due to its greater availability. Normally, in order to demonstrate that a self-association equilibrium scheme does exist for a particular system, one takes advantage of the concentration dependence of the equilibrium. However, this approach was not practical in this present study

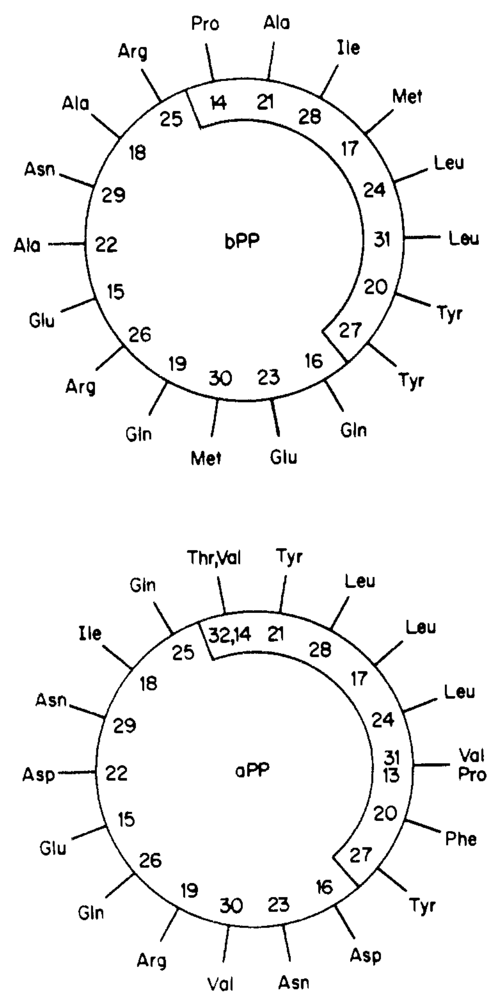

FIGURE 3: Schiffer–Edmundson helical wheels for avian (aPP) and bovine (bPP) pancreatic polypeptide. Numbers inside the circles represent the position of an amino acid residue in the sequence.

because the concentration range that could be achieved for the hormone would be too small: any change in K_{av} or R_S due to a shift in the equilibrium could not be detected with any confidence. Therefore, avian PP's pH-dependent change in elution volume was used to effect a greater perturbation of the association equilibrium. This pH-dependent change in elution volume was shown to be reversible and accompanied by a change in molecular weight, thus proving that hormone self-association occurred.

As can be seen from the results summarized in Table II, avian PP undergoes an increase in Stokes' radius of about 2 Å on going from pH 4.0 to pH 8.0, and it is clear from Table IV that this change in R_S is reversible. However, these observations alone are not sufficient to demonstrate self-association. Partial peptide unfolding or denaturation, for example, could also account for the observed change in R_S.

This alternative interpretation can be discounted if one compares the circular dichroism spectra of the peptide at the two pH values. As can be seen from Figures 1A and 2A, the CD spectra for avian PP are essentially identical for pH 4.0 and 8.0, except for slight differences which may be due to changes in tyrosine environment. The CD spectra indicate that any changes in the hormone's conformation are not sufficient to account for the change in R_S on going from pH 4.0 to pH 8.0.

Additional evidence for avian PP dimerization from pH 4.0 to pH 8.0 comes from the molecular weight determination derived from the sedimentation equilibrium studies. As Table III shows, the molecular weight for avian PP at pH 8.0 is within 2% of the value that would be expected for the dimer, and a dimerization scheme is the simplest and most probable model that accounts for the data.

No direct evidence is available to show that the hormone is predominantly monomer at pH 4.0, since technical difficulties prevented a molecular weight determination at that value. However, since avian PP seems to be essentially a dimer at pH 8.0 and the decrease in R_S in going from pH 8.0 to pH 4.0 is not due to conformation changes, this decrease must be due to dissociation of the peptide.

The above strongly suggests that avian PP undergoes a pH-dependent dimerization. It must be noted, however, that these studies do not give any hard quantitative information about the extent of hormone association at pH 8.0 or dissociation at pH 4.0. However, as can be seen from Table III, avian PP does seem to be more associated at pH 8.0 than the mammalian homologues since the observed molecular weight of avian PP is within 2% of that of the dimer, while the observed molecular weights of canine and bovine PP are only within 10 and 15% of what would be expected for their respective dimer. In addition, while R_S values for avian PP at pH 4.0 are essentially the same in the formate and acetate buffers, both mammalian peptides display an increase in R_S at pH 4.0 when acetate buffer is used (Table II). This may be due to the peptide's increased degree of association or, alternatively, to a more expanded structure in the acetate buffer. This latter possibility may be due to the fact that, at pH 4.0, the acetic acid of the acetate buffer is largely undissociated, and hydrophobic side chains might cluster less in this environment. In this context Cann (1971) observed an increase in volume of ribonuclease at pH 3 in acetic acid and proposed that this was the result of hydrogen bonding with carboxyl groups on the protein and hydrophobic interactions between the methyl group of acetic acid and the side chain of aspartic and/or glutamic acid.

While the main purpose of this study was to obtain information on the pancreatic polypeptides, the gel filtration results may be of general technical interest since we have greatly extended the range in which this technique can be used under nondenaturing conditions to include polypeptides with molecular weights as low as ~4200.

Acknowledgments

The authors are grateful to Joyce Konrardy and H. G. Pollock for their excellent technical assistance.

References

Ackers, G. K. (1970) *Adv. Protein Chem. 24*, 343.
Brewer, J. M., Pesce, A. J., & Ashworth, R. B. (1974) *Exp. Tech. Biochem. 1*, 355.
Cann, J. R. (1971) *Biochemistry 10*, 3707.
Chance, R. E., Moon, N. E., & Johnson, M. G. (1979) in *Methods of Hormone Radioimmunoassay* (Jaffe, B. M., & Behlman, H. R., Eds.) 2nd ed., p 657, Academic Press, New York.
Chen, Y.-H., Yang, J. T., & Chau, K. H. (1974) *Biochemistry 13*, 3350.
Chervenka, C. H. (1969) *A Manual of Methods for Analytical Ultracentrifuge*, Beckman, New York.
Chou, P. Y., & Fasman, G. D. (1974) *Biochemistry 13*, 211.
Chou, P. Y., Adler, A. J., & Fasman, G. D. (1975) *J. Mol. Biol. 96*, 29.
Fasman, G. D., Chou, P. Y., & Adler, A. J. (1976) *Biophys. J. 16*, 1201.
Floyd, J. C., Jr., Fajans, S. S., Pek, S., & Chance, R. E. (1977) *Recent Prog. Horm. Res. 33*, 519.
Gates, R. J., & Lazarus, N. R. (1977) *Horm. Res. 8*, 189.
Hedo, J. A., Villaneuva, M., & Marco, J. (1978) *J. Clin. Endocrinol. Metab. 47*, 366.
Kimmel, J. R., & Pollock, H. G. (1968) *Endocrinology 83*, 1323.
Kimmel, J. R., & Pollock, H. G. (1975) *Fed. Proc., Fed. Am. Soc. Exp. Biol. 34*, 454.
Kimmel, J. R., Hayden, L. J., & Pollock, H. G. (1975) *J. Biol. Chem. 250*, 9369.
Kimmel, J. R., Pollock, H. G., & Hayden, L. J. (1978) in *Gut Hormones* (Bloom, S. R., Ed.) p 234, Churchill-Livingstone, London.
Lim, V. I. (1974) *J. Mol. Biol. 88*, 857.
Lin, T. M., & Chance, R. E. (1972) *Gastroenterology 63*, 852.
Maxfield, F. R., & Scheraga, H. A. (1976) *Biochemistry 15*, 5138.
McMeekin, T. L., Groves, M. L., & Hipp, N. J. (1949) *J. Am. Chem. Soc. 71*, 3298.
Pullen, R. A., Lindsay, D. G., Wood, S. P., Tickle, I. J., & Blundell, T. L. (1976) *Nature (London) 259*, 369.
Sasaki, K., Dockerill, S., Adamiak, D. A., Tickle, I. J., & Blundell, T. L. (1975) *Nature (London) 257*, 751.
Schellman, J. A. (1968) *Acc. Chem. Res. 1*, 144.
Schiffer, M., & Edmundson, A. B. (1967) *Biophys. J. 7*, 121.
Schwartz, T. W., Holst, J. J., Fahrenkrug, J., Jensen, S. L., Nielsen, O. V., Rehfeld, J. F., Schaffalitzky de Muckadell, O. B., & Stadil, F. (1978) *J. Clin. Invest. 61*, 781.
Sears, D. W., & Beychok, S. (1973) in *Physical Principles and Techniques of Protein Chemistry* (Leach, S. J., Ed.) Part C, p 445, Academic Press, New York.
Strickland, E. H. (1974) *CRC Crit. Rev. Biochem. 2*, 113.
Weast, R. C., Ed. (1974–1975) *Handbook of Chemistry and Physics*, 55th ed., CRC Press, Cleveland, OH.
Wood, S. P., Pitts, J. E., Blundell, T. L., Tickle, I. J., & Jenkins, J. A. (1977) *Eur. J. Biochem. 78*, 119.
Yphantis, D. A. (1964) *Biochemistry 3*, 297.