Conformations of Isolated Fragments of Pancreatic Polypeptide†

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ABSTRACT: In spite of its short polypeptide chain, the pancreatic polypeptide molecule consists of a polyproline II type helix and α-helix. To understand the stability and formation of the α-helical region, we prepared some peptide fragments including the helical segment of chicken pancreatic polypeptide and studied their conformations by circular dichroism (CD). PP_{7-36} (a peptide fragment corresponding to residues 7–36 of chicken pancreatic polypeptide) showed a CD spectrum characteristic of the helix at pH 4.6 and at peptide concentrations as low as 1 μM. PP_{11-36} was able to form a helical conformation only at high peptide concentrations and not at concentrations lower than 10 μM. However, acetyl PP_{11-36} (in which the α-amino group is acetylated so that no positive charge exists at the N terminus) was able to form the helical conformation at pH 4.6 and at the peptide concentrations where PP_{11-36} could not. Succinyl PP_{12-36} (in which the α-amino group is succinylated to introduce a negative charge) was also able to form the helical conformation. The CD spectra of PP_{12-36} and PP_{13-36} were not characteristic of the helical conformation at all the pH values and peptide concentrations studied. Acetyl PP_{13-36}, which has no charge at the N terminus, did not form the helix. On the other hand, succinyl PP_{13-36}, which has a negative charge at the N-terminal end, did form the helix at pH 4.6. These findings indicate that the presence of the negative charge of carboxylate at the N-terminal region of a peptide fragment is important for helix formation. When the carboxyl-terminal amide group of succinyl PP_{13-36} was converted to a carboxylate group, the resulting peptide was no longer able to form the helix, indicating that the presence of a negative charge at the C terminus of the peptide is unfavorable for helix formation. These observations suggest that the interactions of a negative charge at the N-terminal region and a positive charge at the C-terminal region with the helix dipole are important for stabilizing the helix.

Pancreatic polypeptide (PP)[1] is a single-chain peptide consisting of 36 amino acid residues. Since it was discovered in the chicken pancreas (Kimmel & Pollock, 1968; Kimmel et al., 1975), homologous polypeptides have been found in a variety of vertebrates (Chance et al., 1979). In spite of the fact that it is a short polypeptide, the PP molecule assumes a globular conformation and consists of a polyproline II type helix (from the N-terminal residue to residue 8) and an α-helix (from residue 14 to residue 32) (Blundell et al., 1981; Glover et al., 1983). These are linked by a reverse turn (from residue 9 to residue 13) and are closely packed through hydrophobic interactions (see Figure 1).

The PP molecules exist in a monomer–dimer equilibrium, which depends on the protein concentration and pH (Noelken et al., 1980; Chang et al., 1980). Previously, Kanazawa and Hamaguchi (1986) studied the stability of chicken PP against heat and guanidine hydrochloride. They found that although the stability of the PP molecule differs depending on the PP concentration, the PP molecule assumes a compact globular conformation even in the monomer state. The PP molecule is thus considered to be the smallest one that can form a regular tertiary structure and can be used as a good model for studying the mechanism of folding and stabilization of bigger and more intricate protein molecules.

In this study we attempted to clarify the helix stability of the PP molecule in terms of its constituent amino acid residues. For this purpose, we prepared various peptide fragments including the helical region of the PP molecule and studied their conformations using circular dichroism. We found that the presence of a negative charge in the N-terminal region and the presence of a positive charge in the C-terminal region of the helix are important for the stabilization of the α-helix.

Materials and Methods

Preparation of Chicken Pancreatic Polypeptide and Its Fragments. Chicken PP was isolated and purified according to the methods of Kimmel et al. (1975) and Wood et al. (1977). The purity of the PP was checked by HPLC and amino acid analysis.

PP_{7-36}, PP_{10-36}, PP_{13-36}, and PP_{1-36}–COOH were synthesized with a Model 430A peptide synthesizer (Applied Biosystems Japan Inc.). The first (*tert*-butyloxycarbonyl)tyrosine was anchored to benzhydrylamine or chloromethylstyrene divinylbenzene (1%) resin (100–200 mesh). Acidolysis of the protective groups of side chains and cleavage of peptides from the resin were performed in one step using anhydrous hydrogen fluoride (10 mL/g of resin) at 0 °C for 1 h in the presence of 10% anisole. Crude peptides were washed with *n*-hexane, extracted with 1% acetic acid, and lyophilized. Purification and a purity check of each peptide were carried out by re-

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[1] Abbreviations: CD, circular dichroism; $[\theta]_{222}$, mean residue ellipticity at 222 nm; HPLC, high-performance liquid chromatography; PP, chicken pancreatic polypeptide; PP_{X-Y}, fragment from *X* to *Y* in the sequence of the intact PP molecule; PP_{X-Y}–COOH, peptide PP_{X-Y} of which the C-terminal residue is the acid form; Ac-PP_{X-Y}, peptide PP_{X-Y} of which the α-amino group is acetylated; Suc-PP_{X-Y}, peptide PP_{X-Y} of which the α-amino group is succinylated.

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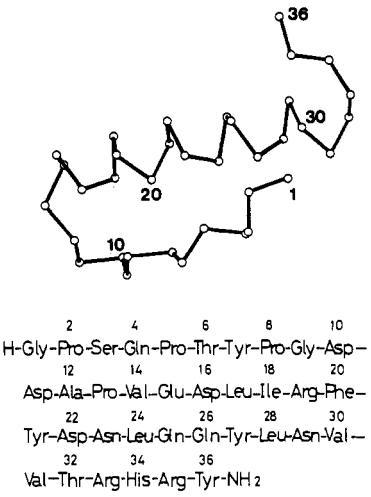


FIGURE 1: Primary structure and crystallographic structure represented by the α-carbon backbone of chicken pancreatic polypeptide (Glover et al., 1983).

verse-phase HPLC (see below). The amino acid composition of each peptide was determined and was confirmed to be within 2% of that expected. The whole amino acid sequence of each peptide was also confirmed by a gas-phase sequencer, model 470A (Applied Biosystems Inc.).

PP_{11-36} and PP_{12-36} were prepared by removing one and two amino acid residues, respectively, from the N terminus of PP_{10-36} using Edman degradation (Edman & Beggs, 1963). Because PP has no lysine residues, no modification by phenyl isothiocyanate occurs at sites other than the N-terminal α-amino group. Each peptide was purified by reverse-phase HPLC and then identified by amino acid analysis.

Acetylation of the α-amino groups of PP_{11-36} and PP_{13-36} was performed as follows: Twenty milligrams of the peptide was dissolved in 7 mL of 0.4 M sodium bicarbonate buffer at pH 8.5 containing 4 M guanidine hydrochloride and then reacted with a 3.5-fold molar excess of acetic anhydride at 0 °C with stirring. A total of 200 μL of acetic anhydride solution in dioxane [1% (v/v)] was added in 10 portions. The final dioxane concentration was 3%.

Succinylation of the N-terminal α-amino groups of PP_{12-36} and PP_{13-36} was performed by reacting 10 mg of the peptide with a 16-fold molar excess of succinic anhydride in 10 mL of 0.4 M sodium bicarbonate buffer at pH 8.5 containing 4 M guanidine hydrochloride at 15 °C with stirring. A total of 440 μL of succinic anhydride solution (1.2% (w/v)] in dioxane was added in 20 portions. The final dioxane concentration was 4%.

The extent of the modification reaction was followed by reverse-phase HPLC. A new peak that appeared on modification was isolated and identified by both amino acid analysis and electrophoresis in 15% polyacrylamide gel containing 8 M urea at pH 4.5 or 9.5 (Davis, 1964; Reisfeld et al., 1962). N-Terminal analysis by Edman degradation was also performed to confirm that no phenylthiohydantoin amino acid was present.

Suc-PP_{13-36}–COOH was prepared by releasing the amide group of Suc-PP_{13-36} with carboxypeptidase Y. Twenty milligrams of Suc-PP_{13-36} was digested with 2 mg of carboxypeptidase Y in 20 mL of 50 mM sodium 2-(*N*-morpholino)ethanesulfonate buffer at pH 6.0 for 18 min at 37 °C. Suc-PP_{13-35}–COOH was prepared by carboxypeptidase A digestion of Suc-PP_{13-36}–COOH. Six milligrams of Suc-PP_{13-36}–COOH in 12 mL of sodium phosphate buffer at pH 7.5 was digested with 0.3 mg of carboxypeptidase A for 3 h at 37 °C. Reactions with these carboxypeptidases were stopped by lowering the pH to 2 with 1 N HCl. The reaction products were purified easily by reverse-phase HPLC. Each purified peptide fragment was identified by amino acid analysis, and C-terminal amino acid analysis was performed by hydrazinolysis (Schroeder, 1972).

PP_{1-35}–COOH was prepared by carboxypeptidase A digestion of PP_{1-36}–COOH, PP_{1-34}–COOH by carboxypeptidase B digestion of PP_{1-35}–COOH, PP_{1-33}–COOH by carboxypeptidase A digestion of PP_{1-34}–COOH, and PP_{1-32}–COOH by carboxypeptidase B digestion of PP_{1-33}–COOH.

Reagents. Acetic anhydride and dioxane were purchased from Wako Pure Chemicals, and guanidine hydrochloride (specially purified grade) and succinic anhydride were from Nacalai Tesque Inc. All other reagents were of the highest grade commercially available. Carboxypeptidases A and B were obtained from Worthington Biochemicals Co., and carboxypeptidase Y was from Oriental Yeast Co.

HPLC Separation. Peptides synthesized by the solid-phase method were purified by a Gilson HPLC system on a preparative reverse-phase HPLC column (YMC D-ODS-5, 20 × 250 mm) at a flow rate of 7 mL/min. Chemically or enzymatically modified peptides were separated with an analytical reverse-phase column (YMC R-ODS-5, 4.6 × 250 mm) at a flow rate of 1 mL/min. Reverse-phase chromatography was performed with a linear concentration gradient of acetonitrile (0.05% trifluoroacetic acid in H_2O–0.05% trifluoroacetic acid in acetonitrile).

CD Measurement. CD measurements were carried out with a Jasco spectropolarimeter, Model J-500A, equipped with a data processor (DP-501). The results are expressed as mean residue ellipticity, $[\theta]$. The details of the CD measurements have been described previously (Goto & Hamaguchi, 1986). The temperature was controlled by using a thermostatically controlled cell holder. Unless otherwise specified, CD measurements were always carried out at 4 °C.

Peptide Concentration. The concentration of each stock peptide solution had been determined by amino acid analysis beforehand, and then sample solutions were prepared by dilution.

Buffer. The buffers used here were sodium formate buffers between pH 3 and 4, acetate buffers between pH 4 and 5.5, 2-(*N*-morpholino)ethanesulfonate buffers between pH 5.5 and 7, *N*-[tris(hydroxymethyl)methyl]-2-aminomethanesulfonate buffers between pH 7 and 8.5, and 2-(cyclohexylamino)-ethanesulfonate buffers between pH 8.5 and 10. The concentration of each buffer component was 5 mM, and all buffer solutions contained 20 mM NaCl. pH was measured with a Radiometer pHM26c meter at 4 °C.

Amino Acid Analysis. The amino acid compositions of the peptides were determined with an Irica amino acid analyzer, Model A-5500 (Kyoto, Japan). The samples were hydrolyzed in evacuated, sealed tubes with 6 N HCl containing 0.2% phenol at 110 °C for 24 h.

RESULTS

Intact PP. Figure 2 shows the CD spectra of the intact PP at 4, 45, and 80 °C at pH 4.6. The CD spectrum at 4 °C had two negative maxima at 208 and 222 nm, which is characteristic of the α-helix. The CD spectrum changed greatly on heating to 80 °C.

Figure 3 shows the pH dependence of the ellipticity at 222 nm ($[\theta]_{222}$) for the intact PP at 4 °C. A sharp transition was observed between pH 4.5 and 3.5.

PP_{7-36}. The CD spectrum of PP_{7-36} was characteristic of the α-helical conformation at pH 4.6, but not at pH 2.8 or

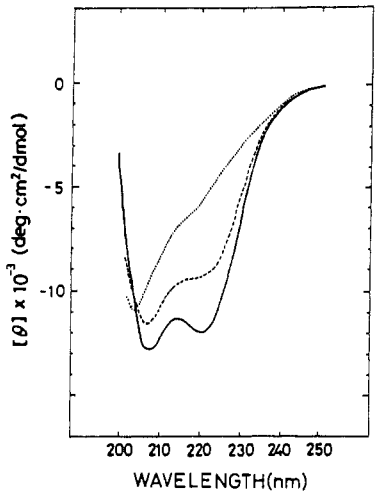


FIGURE 2: CD spectra of chicken PP at pH 4.6 and 4 (—), 45, (---), and 80 °C (···). The concentration of PP was 2 μM.

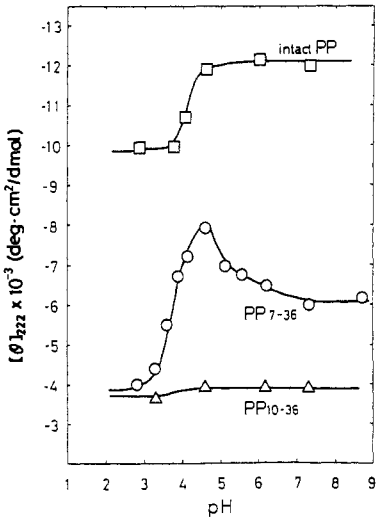


FIGURE 3: pH dependence of the ellipticity at 222 nm ($[\theta]_{222}$) for intact PP (□), PP_{7-36} (○), and PP_{10-36} (△) at 4 °C. The concentration of intact PP was 2 μM, and the concentration of each peptide was 1 μM.

7.4. The pH dependence of $[\theta]_{222}$ for PP_{7-36} (Figure 3) indicates that the stability of the α-helical conformation of PP_{7-36} is maximal at pH 4.5 and that ionizable groups with a pK_a of about 4 (probably the carboxyl group) and about 6 (probably His) are involved in the stabilization of the peptide helix.

Figure 4 shows the dependence of the ellipticity at 222 nm on the concentration of PP_{7-36} at pH 2.8, 4.6, and 7.4. At pH 4.6, the value of $[\theta]_{222}$ was not dependent on the peptide concentration below 5 μM. We failed to determine the apparent molecular weight of PP_{7-36} by gel filtration on Sephadex G-50 superfine under the same conditions, because the peptide adsorbed strongly to the gel. Assuming that the contribution of the polyproline helix segment to the ellipticity at 222 nm is almost the same as that of the random conformation, the α-helical content of PP_{7-36} under this condition is calculated to be about 30% compared with that of the intact PP. At pH 7.4 and 2.8, no appreciable dependence on the peptide concentration of the ellipticity at 222 nm was observed in the concentration range studied.

The helix stability of PP_{7-36} was decreased when the concentration of NaCl was increased from 0.005 to 0.1 M.

PP_{10-36} and PP_{11-36}. The CD spectra of PP_{10-36} were measured at three peptide concentrations at pH 4.6. PP_{10-36} did not form the α-helix below 5 μM. PP_{7-36} with three residues longer than PP_{10-36} formed the helix under the same conditions. At peptide concentrations above 12 μM, the CD spectrum became similar to the spectrum characteristic of the α-helix.

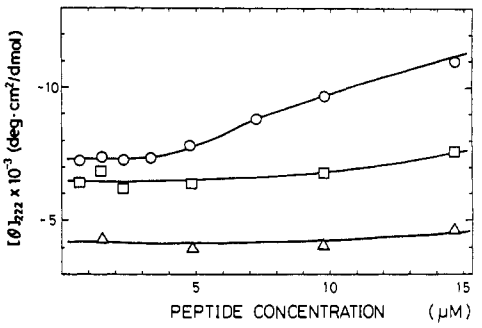


FIGURE 4: Dependence of the ellipticity at 222 nm ($[\theta]_{222}$) on the concentration of PP_{7-36} at pH 2.8 (△), 4.6 (○), and 7.4 (□) at 4 °C.

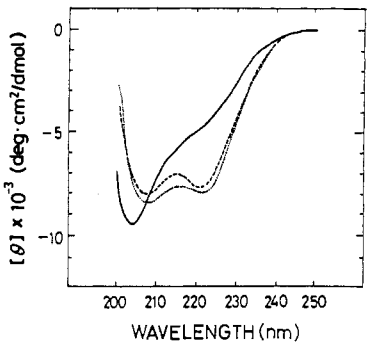
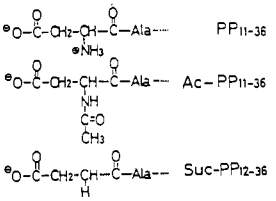


FIGURE 5: CD spectra of PP_{11-36} (—), Ac-PP_{11-36} (---), and Suc-



PP_{12-36} (···) at pH 4.6 and 4 °C. The concentrations of PP_{11-36}, Ac-PP_{11-36}, and Suc-PP_{12-36} were 2.7, 1.2, and 1.2 μM, respectively.

The change with the peptide concentration in the CD spectrum of PP_{11-36} was very similar to that for PP_{10-36}; below 5 μM PP_{11-36} did not form the helix (see Figure 5), but when the peptide concentration was increased, a CD spectrum characteristic of the helix was obtained. These findings indicate that PP_{10-36} and PP_{11-36} can form the helix only when the peptide molecules associate intermolecularly.

PP_{12-36} and PP_{13-36}. We measured the CD spectra of PP_{12-36} and PP_{13-36} under the conditions in which PP_{11-36} was able to form the helix. PP_{12-36} and PP_{13-36} did not form the helix even at much higher concentrations than those used for PP_{10-36} and PP_{11-36} (not shown).

PP_{11-36}, Ac-PP_{11-36}, and Suc-PP_{12-36}. To clarify the effect of the N-terminal negative charge on helix stability, we measured the CD spectra of Ac-PP_{11-36} and Suc-PP_{12-36} at pH 4.6. The N-terminal residue of PP_{11-36} is Asp, which has one negative β-COO^- and one positive α-NH_3^+ at this pH value. Thus, the net charge of the N-terminal residue of the peptide is zero. Ac-PP_{11-36} and Suc-PP_{12-36} have one negative charge each at the N terminus. These three peptides are isosteric, and the length between the carboxylate and Ala 12 is the same in all cases with the same number of single C–C bonds (see legend to Figure 5). Figure 5 shows the CD spectra of these three peptides. As can be seen, Ac-PP_{11-36} and Suc-PP_{12-36} were able to form the helical conformation at the concentration

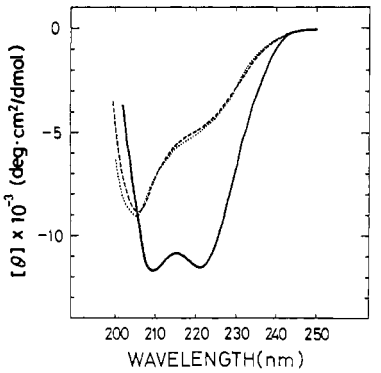


FIGURE 6: CD spectra of PP_{13-36} (···), Ac-PP_{13-36} (---), and Suc-PP_{13-36} (—) at pH 4.6 and 4 °C. The concentrations of PP_{13-36}, Ac-PP_{13-36}, and Suc-PP_{13-36} were 16.9, 3.0, and 1.2 μM, respectively.

where PP_{11-36} could not. This finding strongly suggests that the presence of a net negative charge at the N terminus of the helix is essential for helix formation in the PP fragments and that the presence of a positive charge in addition to a negative charge destabilizes the helix.

PP_{13-36}, Ac-PP_{13-36}, and Suc-PP_{13-36}. The effect of the N-terminal charge on the helix formation was further studied by using PP_{13-36}, Ac-PP_{13-36}, and Suc-PP_{13-36}. The net charges on the N-terminal residues of PP_{13-36}, Ac-PP_{13-36}, and Suc-PP_{13-36} at pH 4.6 are +1, 0, and −1, respectively (see legend to Figure 6). Figure 6 shows the CD spectra of these three peptides. PP_{13-36}, which has one positive charge at the N terminus, did not form a helix even at a very high concentration of 16.9 μM. Ac-PP_{13-36}, which has no charge at the N terminus, did not form a helix either. On the other hand, Suc-PP_{13-36}, which has one negative charge at the N terminus, did form the α-helix even at a low concentration of 1.2 μM. Thus, the importance of the presence of a negative charge at the N terminus for helix formation is strongly suggested.

PP Fragments with Modified C-Terminal Residues. Figure 7 shows the CD spectra of Suc-PP_{13-36}, Suc-PP_{13-36}–COOH, and Suc-PP_{13-35}–COOH at pH 4.6. Suc-PP_{13-36}–COOH is a peptide in which the C-terminal amide group of Suc-PP_{13-36} was converted to carboxylate, and Suc-PP_{13-35}–COOH is a peptide in which the C-terminal residue, Tyr 36, was removed from Suc-PP_{13-36}–COOH. Whereas Suc-PP_{13-36} was able to form a stable α-helix, Suc-PP_{13-36}–COOH and Suc-PP_{13-35}–COOH could not. This clearly shows the importance of the amide group of the C-terminal residue for helix formation.

PP with Modified C-Terminal Residues. As described above, conversion of the C-terminal amide group of Suc-PP_{13-36} to carboxylate destabilizes the helical conformation. To determine whether the same effect is observed for the intact PP, we compared the CD spectrum of the intact PP with that of PP_{1-36}–COOH at pH 9.0. At this pH, while all the peptide fragments studied above did not form the α-helix, the intact PP did so (Figure 3). No appreciable change was observed for the CD spectrum when the C-terminal amide group of the intact PP was converted to carboxylate at pH 9.0. On removal of the C-terminal residues one by one to PP_{1-33}–COOH, the depth of the double minima at 208 and 222 nm decreased gradually, but the CD spectra remained characteristic of the α-helix. PP_{1-32}–COOH, which has no positive charge in the C-terminal region, did not form the α-helix.

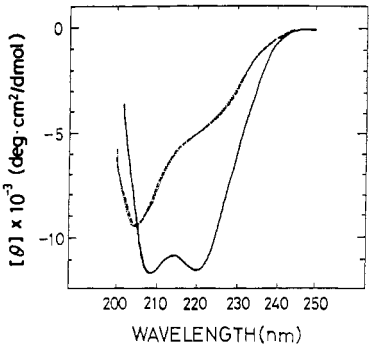


FIGURE 7: CD spectra of Suc-PP_{13-36} (—), Suc-PP_{13-36}–COOH (---), and Suc-PP_{13-35}–COOH (···) at pH 4.6 and 4 °C. The concentration of each peptide was 1.2 μM.

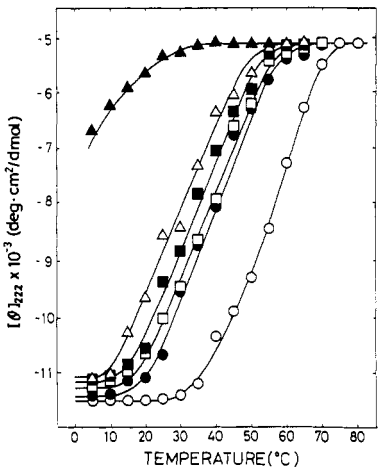


FIGURE 8: Thermal unfolding curves of intact PP (○), PP_{1-36}–COOH (●), PP_{1-35}–COOH (□), PP_{1-34}–COOH (■), PP_{1-33}–COOH (△), and PP_{1-32}–COOH (▲) at pH 9.0 as measured by CD at 222 nm. The concentration of each peptide was 2 μM.

The change in the CD spectrum on conversion of the C-terminal amide of the intact PP to carboxylate was not so distinct at 4 °C. However, the thermal unfolding curves of the intact PP and PP_{1-36}–COOH were different, and the midpoint temperature of the thermal unfolding curve decreased on conversion of the amide to carboxylate (Figure 8). The unfolding curve of PP_{1-32}–COOH was further shifted to a lower temperature region.

Discussion

Stabilization of the α-Helix by a Negative Charge at the N Terminus and a Positive Charge at the C Terminus. On the basis of the following observations, we may conclude that the presence of a negative charge at the N terminus of a peptide is essential for stabilization of the α-helix.

(1) PP_{7-36} shows a CD spectrum characteristic of the α-helix at pH 4.6 but not at pH 2.8 (Figure 3). This suggests that an ionizable group with a pK_a value of about 4 (probably a

carboxyl group) is involved in the formation of the α-helix.

(2) PP_{11-36} cannot form a helix at pH 4.6 and peptide concentrations below 5 μM, whereas Ac-PP_{11-36} and Suc-PP_{12-36} can form a helix under the same conditions (Figure 5). Ac-PP_{11-36} and Suc-PP_{12-36} are isosteric to PP_{11-36}. PP_{11-36} has both a positive NH_3^+ group and a negative COO^- group in the N-terminal residue, and these charges may cancel out to yield zero net charge. Ac-PP_{11-36} and Suc-PP_{12-36} have only one negative charge each at the N terminus.

(3) Suc-PP_{13-36} with a negative COO^- group in the N terminus can form a helix, whereas PP_{13-36} with a positive NH_3^+ group and Ac-PP_{13-36} with no charge in the N terminus cannot form the helix. Although Suc-PP_{13-36} is longer than PP_{13-36}, PP_{12-36}, which is almost the same length as Suc-PP_{13-36}, cannot form the helix either.

PP_{7-36} is able to form the helix. In this peptide, the carboxyl groups of Asp 10 and Asp 11 are located distant from the N-terminal NH_3^+ group, and thus the cancellation of these charges may not be sufficient. Therefore, the helix formation of PP_{7-36} may be due to the presence of the negative carboxylate charges of Asp 10 and Asp 11 rather than to its long peptide length.

The following observations strongly suggest that the presence of a positive charge in the C-terminal region is essential in the formation of an α-helix.

(1) The pH dependence of the value of $[\theta]_{222}$ for PP_{7-36} suggests that an ionizable group with a pK_a value of about 6 is involved in helix formation (Figure 3). As observed for PP_{7-36}, Ac-PP_{11-36}, Suc-PP_{12-36}, and Suc-PP_{13-36} can form the α-helix at pH 4.6 but not at pH 7.4 (data not shown). An ionizable group with a pK_a of about 6 may be assigned to His, and there is only one His residue (His 34) in the peptide.

(2) When the C-terminal amide group of Suc-PP_{13-36} is converted to carboxylate, the resulting Suc-PP_{13-36}–COOH no longer forms an α-helix (Figure 7). Thus, not only the presence of a positive charge, His 34, but also the absence of a negative charge in the C-terminal residues seem to be essential for α-helix formation of PP fragments.

There are two basic residues, Arg 33 and Arg 35, in addition to His 34, in the C-terminal region of Suc-PP_{13-36}. Since the pK_a value of Arg is greater than 12, the change with pH in the helix stability in the pH region above pH 4.5 is due only to His 34. It is not clear at present whether Arg 33 and Arg 35 are also involved in the helix stability.

Peptides studied here can be classified into three groups on the basis of the peptide concentration dependence of their helicity.

Group 1 comprises peptides that can form an α-helix at a peptide concentration of 1 μM. PP_{7-36}, Ac-PP_{11-36}, Suc-PP_{12-36}, and Suc-PP_{13-36} belong to this group. All these peptides have only negative charge(s) in the N-terminal region and positive charge(s) at the C-terminal region at pH 4.6.

Group 2 comprises peptides that can form an α-helix at peptide concentrations above 10 μM but not below 1 μM at pH 4.6. PP_{10-36} and PP_{11-36} belong to this group. At pH 4.6, PP_{10-36} and PP_{11-36} have one positive α-NH_3^+ in addition to a negative β-COO^- at the N-terminal residue. As in the case of peptides in group 1, positive charges are also present in the C-terminal region. As in the case of the peptides in group 1, a negative charge exists at the N terminus.

Group 3 comprises peptides that cannot form an α-helix even at concentrations much higher than 10 μM. PP_{12-36}, PP_{13-36}, and Ac-PP_{13-36} belong to this group. PP_{12-36} and PP_{13-36} have one positive α-NH_3^+ at the N-terminal residue at pH 4.6. Ac-PP_{13-36} has no charge at the N terminus. As in the case of peptides in group 1, all these peptides have only positive charges at the C-terminal region.

These observations indicate that the difference in the ability of α-helix formation among these groups is due to the difference in the state of charge in both of the terminal regions. A negative charge at the N terminus and a positive charge at the C-terminal region may stabilize the monomeric α-helical conformation of the peptide, and this stability may be increased by intermolecular association.

As described above, PP_{7-36}, Ac-PP_{11-36}, Suc-PP_{12-36}, and Suc-Pp_{13-36} form stable helices at pH 4.6. The helices of these peptides are much more stable than predicted by the Zimm–Bragg equation (Zimm & Bragg, 1959). Baldwin and his co-workers reported that the C-peptide and S-peptide obtained from ribonuclease A form stable helices (Shoemaker et al., 1987). They interpreted the stabilities of the helices of these peptides in terms of the interactions of the helix dipole with charges in both terminal regions of the helix (Blagdon & Goodman, 1975; Wada, 1976; Hol, 1985). The stabilities of the helices of the peptides studied here are also well explained in a similar way. Nicholson et al. (1988) showed that the thermostability of T4 phage lysozyme was increased by replacement of Ser 38 and/or Asn 144, which are located at around the N-cap positions of helices (Richardson & Richardson, 1988), by Asp with a negative charge. Shoemaker et al. (1987) also pointed out the importance of an ion pair in the stabilization of the C-peptide helix. Recently, Goodman and Kim (1989) reported that a short peptide corresponding to the α-helical region of bovine pancreatic trypsin inhibitor shows partial folding and that the helix is stabilized by the interactions of the charged groups and the helix dipole and/or salt bridge formation. For the peptides studied here, it would be possible for an ion pair to be formed between Glu 15 (or Asp 16) and Arg 19 or between Arg 19 and Asp 22. However, we were unable to clarify the role of these ion pairs in helix stabilization.

The C-terminal residues of many peptide hormones are amidated. It is interesting to note that when the C-terminal amide group of Suc-PP_{13-36} is converted to carboxylate, the helix is no longer formed. When the C-terminal amide group of the intact PP was converted to carboxylate, its thermal stability was greatly decreased (Figure 8). Thus, amidation of the C-terminal residue of some peptide hormone molecules may contribute to the formation and stability of their α-helices, which in turn affect their activities. Very recently, Fairman et al. (1989) reported the importance of the C-terminal amide group in the stabilization of the α-helix of a short peptide.

When the stability and formation of an α-helix are related to the presence of charges at both termini of the helix, phosphorylation of a side chain, binding or access of an ion, or a change in the membrane potential of the cell membrane might trigger a helix–coil transition in the local region of the protein molecule. Such a local conformational change may lead to a drastic conformational change in the whole molecule, which may, in turn, modify the function of the protein. Recently, findings that modifications of protein function by phosphorylation are involved in cell transformation (Hunter & Cooper, 1985; Goustin et al., 1986) and in metabolic control with activation of a key enzyme (Hunter et al., 1984; Baraban et al., 1985; DeRiemer et al., 1985; Harano et al., 1985; Green & Lattimer, 1986; Kojima et al., 1986) have been reported. Sprang et al. (1988) reported that the activation of glycogen phosphorylase by phosphorylation of Ser 14 is attributable to formation of a helical structure around the phosphorylation site. Various models for opening and closing of ion channels

by a change in membrane potential have also been presented (Alberts et al., 1983). It is thus possible that a change in the ion potential alters the conformation and stability of the helices in protein molecules.

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