Conservation of a Helix-Stabilizing Dipole Moment in the PP-fold Family of Regulatory Peptides†

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ABSTRACT: Investigation of the charge distribution for all known members of the PP-fold family of peptides reveals a common pattern characterized by a cluster of negative charges in the β-turn region and a cluster of positive charges in the receptor-binding region of the peptide. Detailed analysis of the electrostatic properties of five representative members of the PP-fold family of peptides (human neuropeptide Y, human peptide YY, human pancreatic polypeptide, avian PP, and lamprey peptide methionine tyrosine) shows that this characteristic charge clustering gives rise to a common dipole moment of 325–450 D directed from the β-turn region toward the receptor-binding region. This overall dipole moment is antiparallel to the dipole moment of the α-helix caused by alignment of the peptide dipoles parallel to the helix. Calculations of the stabilization energy for this antiparallel dipole moment arrangement were performed in two ways: (1) by the use of a Poisson–Boltzmann approach which allows for an estimate of the screening effect, and (2) by the use of a uniform dielectric model (Coulomb's law). It is found that the α-helix is stabilized by approximately 5–10 kcal/mol due to electrostatic forces alone when the screening effect is considered. This energy is of the same order of magnitude as the enthalpy change for the unfolding of avian PP (~30 kcal/mol), strongly indicating that the charge–dipole interactions are of significant importance for the stability of the three-dimentional structure of the PP-fold peptides.

Neuropeptide Y (NPY),[1] a neurotransmitter in the central and pehripheral nervous system, peptide YY (PYY), a hormone from the lower intestine, and pancreatic polypeptide (PP), a pancreatic hormone, are all members of the so-called PP-fold family of regulatory peptides. They contain 36 amino acids and differ from most other small- and medium-size peptides in holding a distinct tertiary structure, the PP-fold, which is stable, even in dilute aqueous solution, as characterized by circular dichroism (Clover et al., 1984; Krstenansky & Buck, 1987) and NMR (Li et al., 1992). One member of the family, avian PP, has been characterized in great detail by X-ray crystallography (Clover et al., 1984; Blundell et al., 1981; Clover et al., 1983). This polypeptide has a compact globular structure with a hydrophobic core comprising two antiparallel helices, an N-terminal polyproline-like helix (residues 2–8) and an amphipathic α-helix (residues 14–32), connected by a β-turn (Figure 1). The C-terminal residues (35, 36) are relatively mobile, even in the crystal (Clover et al., 1983). All members of the PP-fold family of peptides are C-terminally amidated, which has been shown to be of crucial importance for the biological effect of the peptides (Wahlestedt et al., 1986; Chance et al., 1981).

Extensive studies done by various groups support the assumption that the crystal structure of avian PP can be used as a model for the solution structure of avian PP itself, as well as for other PP-fold members (Clover et al., 1984; Krstenansky & Buck, 1987; Li et al., 1992; Wood et al., 1977; Schwartz et al., 1989; Fuhlendorff et al., 1990). It has been shown that the C-terminal hexapeptide and the N-terminal residue are directly involved in receptor binding (Li et al., 1992; Schwartz et al., 1989; Forest et al., 1990; Abens et al., 1989). The rest of the structure, the PP-fold as such, plays a significant role mainly in stabilizing the spatial relationship between the N- and C-terminal residues of the peptide (Li et al., 1992; Fuhlendorff et al., 1990; McLean et al., 1990). The structure of human NPY has previously been investigated by conformational search (Allen et al., 1987) and molecular dynamics (MacKerell, 1988). In these studies the overall tertiary structure is maintained, in agreement with the experimental results (Clover et al., 1984; Krstenansky & Buck, 1987; Li et al., 1992).

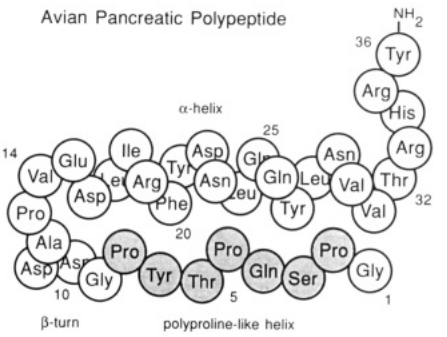

FIGURE 1: Schematic drawing of avian pancreatic polypeptide (avian PP) revealing the amino acid sequence of the peptide. The α-helix and the polyproline-like helix are emphasized.

The primary structure of more than 25 different PP-fold peptides from various species is known today. The overall sequence identity among all members of the PP-fold family varies between 25 and 30%, whereas the sequence identity between the three members within a given species varies

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[1] Abbreviations: PP, pancreatic polypeptide; NPY, neuropeptide tyrosine; PYY, peptide tyrosine tyrosine; PMY, peptide methionine tyrosine; P–B, Poisson–Boltzmann; RMS, root mean square.

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between 45 and 70% (Schwartz et al., 1989; Krstenansky et al., 1989). The identical residues are mainly the core residues, which are believed to be important for the intramolecular, hydrophobic stabilization of the PP-fold, and the C-terminal hexapeptide, commonly accepted to be directly involved in receptor binding. The charged residues in the peptides are not significantly conserved; however, a closer analysis of the actual charge distribution reveals a common pattern for the whole family. This has motivated us to investigate in more detail the electrostatic characteristics of the family of peptides, including the influence of the electrostatics on molecular stability and biological activity. We have chosen to perform electrostatic energy calculations on five representative members of the family: avian PP, for which the crystal structure is known; human NPY; human PP; human PYY; and a peptide from the sea lamprey, peptide methionine tyrosine (PMY),[2] an extant representative of the earliest group of jawless vertebrates (Conlon et al., 1991).

EXPERIMENTAL PROCEDURES

Molecular Modeling. Coordinates for avian pancreatic polypeptide (avian PP) at 1.4-Å resolution were taken from the Brookhaven Protein Data Bank and used as the starting structure for molecular modeling of human NPY, human PYY, human PP, and lamprey PMY using the Discover and InsightII programs (Biosym Technologies Inc., San Diego). The valence force field was used during the Discover calculations (Hagler, 1985) excluding cross-term energies and Morse potentials. The nonbonded and valence force constants are described elsewhere (Dauper-Osguthorpe et al., 1988; Hagler & Lifson, 1974; Hagler et al., 1979). The steepest descent minimization algorithm and the conjugated gradient algorithm were applied, alternating with 1000 steps of each minimizer and always ending with conjugate gradient minimization until a maximum derivative less than 0.05 kcal/(mol·Å) was achieved. The cutoff for generating the neighbor list used in calculating nonbonded interactions was set to 12 Å (Hagler et al., 1979).

The following procedure for obtaining and relaxing the structure of human NPY is representative for all four PP-fold peptides: Initially, residues in avian PP were sequentially mutated to those in human NPY while the positions of the backbone atoms were maintained. The side chains of each new residue were positioned to preserve the side chain torsion angles for the original residue. When proline was exchanged for another residue, the side chain torsion angles were not preserved; in this case a minimum-energy conformation of the side chain was incorporated in the structure. To prepare the structure for relaxation, both polar and nonpolar hydrogen atoms were added, and the structure was soaked in a water box with the dimensions $40 \times 35 \times 35$ Å^3. Periodic boundary conditions were applied. This structure was considered the starting structure of human NPY.

In order to prevent large distortions due to bad initial contacts between atoms, the protein–water structure was gradually annealed (Dauper-Osguthorpe et al., 1988). First, the water molecules were energy minimized with respect to the protein structure while the coordinates of the protein structure were fixed. Then, in order to adjust the positions of the hydrogens added to the protein structure, all heavy atoms in the protein were gently constrained to their original positions, allowing only the protein hydrogens and the water molecules to move. The side chains were then energy minimized while the backbone atoms were constrained to relieve unfavorable interactions between mutated residues with bad initial orientations. Finally, the energy was minimized with respect to all Cartesian coordinates of the system. The avian PP crystal structure was soaked and relaxed analogously. The structural changes caused by the relaxation are summarized in Table I. In the relaxation process we chose not to apply conformational search or dynamics in order to overcome rotational barriers, since previous studies of PP-fold peptides have shown no significant changes for the overall structure (Allen et al., 1987; MacKerell, 1988). Furthermore, a small change in the conformation of a side chain has only minimal consequences for the electrostatic calculations. The resulting structure was used as input for the electrostatic potential map calculations using the DelPhi program (Biosym Technologies Inc., San Diego) as well as for dipole moment calculations and total electrostatic energy calculations using both DelPhi and Discover (see below).

Electrostatic Calculations. Electrostatic potential maps for the PP-fold peptides were calculated using DelPhi. DelPhi uses a finite-difference algorithm to solve the linearized Poisson–Boltzmann equation, which enables one to include the screening effect of a solvent including electrolytes (Klapper et al., 1986). A dielectric constant of 2 was used for the protein interior, which was defined by a Connolly surface using a probe of 1.8 Å. The solvent was represented by a continuum with a dielectric constant of 80 and an electrolyte concentration of 0.145 mol/L including ions with an average radius of 2.0 Å, which corresponds to physiological conditions with a pH of 7.4. A high resolution of the potential maps used for calculating the total electrostatic energy was obtained by using a grid containing $125 \times 103 \times 91$ grid points. For the potential maps displayed in Figure 3, a maximum of 65 grid points was applied. Boundary conditions were accounted for by using a Coulombic approach. The reported results are based on a "full-charge" model where the nitrogen of each lysine and the N-terminal amino group are assigned a charge of +1 and the guanidinium nitrogens are assigned charges of +0.5 each, while each carboxylate oxygen is assigned a charge of –0.5. All histidines are assumed to be neutral. The same calculations were performed using the default charge set from the Discover residue library (Dauper-Osguthorpe et al., 1988). The overall electrostatic potential maps obtained by using the two different charge sets show no significant differences. Only the former maps will be discussed here.

To obtain an estimate for the screening effect of the peptide system, an additional high-resolution calculation of the electrostatic potential map was performed in which no electrolytes were included and a dielectric constant of 2 was applied for both the peptide and the solvent. This calculation was based on the full-charge approximation; otherwise the same parameters for the protein as described above were used.

The dipole-moment calculations and the total electrostatic energy calculations were performed using Discover with a dielectric constant of 2 and no cutoff. The dipole-moment calculations were based on the default Discover charge set, while for the electrostatic-energy calculations a full-charge approximation was used. The default partial charges were used for all backbone atoms plus all side-chain atoms which were part of a neutral residue. For the charged residues the point charges of the side-chain atoms were set to 0, and a full-charge model like the one used by DelPhi was applied.

RESULTS AND DISCUSSION

An alignment of the primary structures for the five members of the PP-fold family is shown in Figure 2a. The charged

[2] PMY is named this way since it is still unknown to which of the three types of mammalian peptides it corresponds (Conlon et al., 1991).

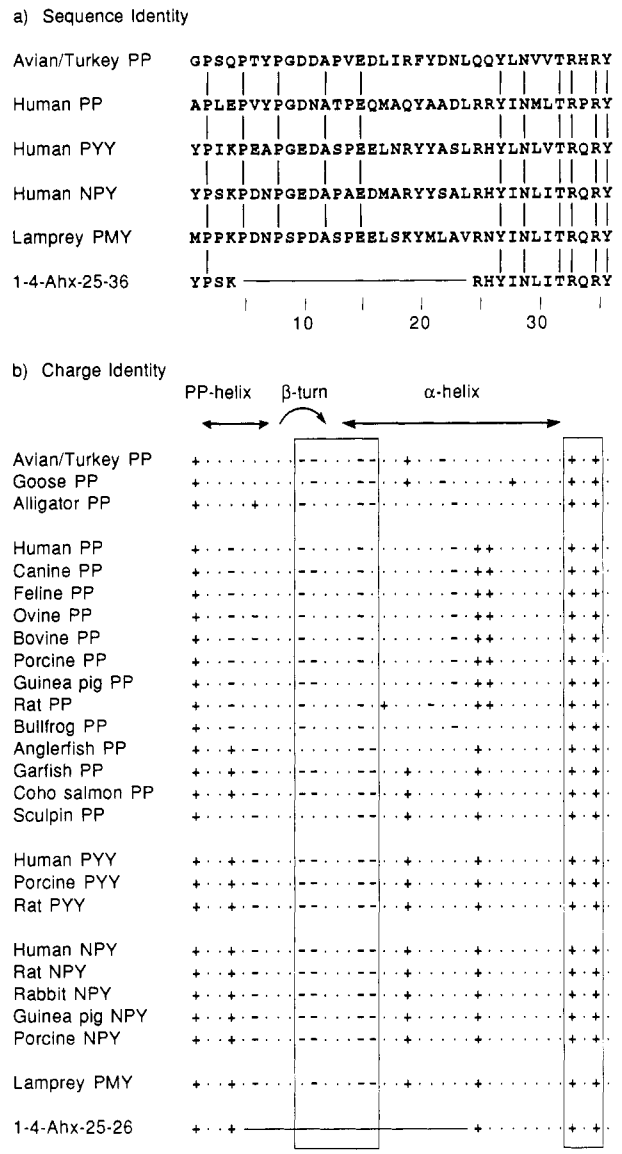

FIGURE 2: Sequence alignments for members of the PP-fold family. (a) Sequences in one-letter code for the six peptides included in this study. (b) Charge clustering (illustrated by boxes) for members of the PP-fold family. Asp and Glu are negative; Arg, Lys, and the N-terminal amino group are positive; and all other residues are considered neutral. All peptides are C-terminally amidated in their biologically active form. The sequences are taken from Schwartz et al. (1989) and Conlon et al. (1991).

residues do not appear to be conserved in comparison with the core residues. Only three out of ten residues are identical (Asp15, Arg33, and Arg35) among all five PP-fold peptides included in the present study. Of these, Arg33 and Arg35 are believed to be directly involved in receptor binding, at least in NPY (Beck-Sickinger et al., 1990a). However, a significant conservation of charges is found for all known members of the PP-fold family when only the charge status of a residue in a given position is considered (Figure 2b). A cluster of negatively charged residues is present in the sequence corresponding to the β-turn region and the N-terminal end of the α-helix (residues 11, 12, 15, and 16), and a positive-charge resides in the C-terminal end of the α-helix (residues 33 and 35). In the following we address the question of whether this strong conservation of charge clusters is important for the stability of the PP-fold tertiary structure and thereby for the biological activity of this family of peptides.

Molecular Modeling. Three-dimensional models of human NPY, PYY, and PP and of lamprey PMY were constructed based on their sequence homology with avian PP. The

Table I: RMS Deviation between the Starting and the Minimized Structures and Dipole Moments for the Minimized Structures of Avian PP, Human PP, Human PYY, Human NPY, and Lamprey PMY

	RMS deviation		dipole moment coordinates[a]	total dipole moment[a] (D)
	heavy atoms (Å)	backbone atoms (Å)	(X, Y, Z)	
avian PP	0.8	0.8	(377, 97, 182)	430
human PP	0.9	0.8	(260, 168, 102)	326
human NPY	0.9	0.7	(352, 192, 118)	418
human PYY	0.9	0.7	(377, 209, 123)	449
lamprey PMY	0.9	0.8	(338, 158, 34)	374

[a] Dipole moments were calculated in a coordinate system with the origin at the center of mass of the molecules: $\vec{\mu} = \sum_i q_i \vec{r}_i$, where i denotes the individual atoms in the molecule. The dipole moment of the α-helix alone was calculated to be 41 D with coordinates of (−40, −11, −1).

structural changes caused by minimization are evaluated as root mean square (RMS) deviations between all heavy atoms and main-chain atoms for the starting structure and the minimized structure, respectively. The RMS differences are reported in Table I. No major changes are observed in the overall structure for any of the minimized structures. The structural changes introduced by minimizing the avian PP crystal structure are comparable to the changes of the other modeled members of the PP-fold family. This indicates that the residue substitutions from avian PP to the other four members of the PP-fold family do not introduce any severe steric interactions. The RMS differences are larger for all the heavy atoms relative to the backbone atoms only, reflecting the rigidity of the overall PP-fold and the larger flexibility of the side chains of the structures.

Electrostatics. The electrostatic potential map as calculated by DelPhi for the five structures including solvent screening are shown as isoenergetic contours of ±1 kT in Figure 3. For simplicity, the protein structures are represented as ribbons and only the charged residues are explicitly displayed. The overall appearance of the potential is similar for all members of the family. A strong dipole moment associated with the structures is observed as a large positive potential in the receptor-binding region of the molecule and a negative potential in the N-terminal end of the α-helix. The potentials are "squeezed" at the middle of the structure where mostly uncharged residues are located (Figure 3). The potentials also differ qualitatively between the peptides in this region because of the different charge distribution especially in positions 4, 22, 23, 25, and 26. However, the positive potential in the receptor-binding region is very similar among all the peptides due to the conserved arginines (Arg33 and Arg35) and the N-terminal amino group.

The results of the dipole-moment calculations are given in Table I and in Figure 4a as vectors reflecting the relative size and direction of the dipole moment for the different members of the PP-fold family. These quantitative data support the presence of a strongly conserved dipole moment for the PP-fold peptides in agreement with the electrostatic potential maps and the sequence alignment (Figures 2 and 3). The dipole moments of human NPY and human PYY are almost identical in both direction and size, whereas the other three peptides have dipole moments which differ from the two previously mentioned in both direction and size (Table I and Figure 4a).

The negatively charged part of the PP-fold in NPY can be exchanged by an artificial spacer without major loss of biological effect on the Y_2 receptor (Beck-Sickinger et al., 1990a,b; Schwartz et al., 1990). Thus, the NPY analogue NPY(1–4-Ahx-25–36)peptide, in which most of the PP-fold

motif (residues 5–24) has been replaced by a single aminohexanoic acid spacer (Ahx), is equally as potent as NPY (Beck-Sickinger et al., 1990a,b). As shown in Figure 3, this analogue has a similar electrostatic potential in the receptor-binding region of the molecule. Since this analogue, which is a monopole, is fully biologically active in certain systems, the dipole moment does not seem to be important for receptor binding per se. It is more likely that the dipole moment is important for the structural stability of the PP-fold and thereby indirectly for the biological activity of the intact PP-fold molecule. This notion, which is discussed in more detail below, is supported by studies on avian PP itself performed by Tonan et al. (1990), who have shown that elimination of the charge clusters in each end of the PP-fold motif caused the PP-fold to be unstable in solution.

An α-helix comprises an overall dipole arising from the alignment of peptide dipoles parallel to the α-helix axis (Wada, 1976). The presence of negatively charged residues in the N-terminal end of the α-helix and positively charged residues in the C-terminal end stabilizes the helix by charge–dipole interactions (Serrano & Fersht, 1989; Fairman et al., 1989; Richardson & Richardson, 1988; Shoemaker et al., 1987). For the PP-fold family the α-helix dipole moment is calculated to be 41 D (cf. Table I). One to two negatively charged residues are present in the N-terminal end of the helix (residues 15 and 16), in agreement with the helix stabilization described above. Apart from this, two negatively charged and two positively charged residues are present outside the α-helix in each end (residues 10 and 11, and 33 and 35, respectively). These residues result in the strong overall dipole for the entire structure, described above, which is approximately antiparallel to, and significantly larger than, the dipole moment of the α-helix (Figure 4b). Qualitatively, it is clear that this antiparallel arrangement of the two dipoles is energetically favorable.

In order to calculate this stabilization energy quantitatively, we have performed a *formal* separation of the partial charges assigned to each atom in the molecule: Point charges which are part of a residue side chain carrying a net charge are assigned Q_i (full-charge approximation; see Experimental Procedures), and the rest of the partial charges in the molecule, including the backbone point charges from the charged residues, are assigned q_i. The index i denotes the individual atoms in the molecule.

This formal division results in three terms for the total Coulomb energy. We denote these three contributions as

$$E_{\text{total}} = E_{q_i,q_j} + E_{Q_i,Q_j} + E_{q_i,Q_j} \quad (1)$$

where E_{q_i,q_j} is the net interaction energy between all point charges q_i, E_{Q_i,Q_j} is the similar interaction energy between all point charges Q_i, and E_{q_i,Q_j} is the interaction energy between the two charge distributions involving q_i and Q_j, respectively. Since the first term involves interactions between locally neutral parts of the molecule, it has only a short range, and we therefore do not believe that it plays a crucial role for the stabilization of the tertiary structure. The second term accounts for the interactions between the net charges of the charged residues: the repulsion of the charges within the charge clusters situated in each end of the molecule as well as the attraction between these clusters. This energy is not associated with the stabilization energy of the α-helix dipole moment in the electrostatic field, since the α-helix is overall neutral. Both the first and the second term are expected to be positive when we consider that the nearest, and thereby strongest, interactions are between charges of the same sign. However, the steric energy, mostly the binding energy, compensates for this; i.e., the chemical bonds hold the charges together. The last term arises from the interaction between the field created by the charged residues and the rest of the molecule, and hence it is this term which reflects the stabilization energy of the α-helix in the field created by the charged residues.

This electrostatic stabilization energy term, E_{q_i,Q_j}, can be calculated directly by the use of DelPhi. From the electrostatic potential calculations based on the net charges, Q_j, the total energy of interaction between these potentials and the partial charges, q_i, can be calculated by

$$E_{q_i,Q_j} = \sum \phi_j q_i$$

where ϕ_j is the potential arising from Q_j at the site of the charges q_i. The stabilization energies are reported in Table II with and without screening effects. The screening ratio, which is the ratio between the stabilization energies determined with and without screening, is also shown.

The total electrostatic stabilization energy as calculated by DelPhi depends on the particular grid mapping used. This is due to the interpolation of the potential value in a given coordinate of a charge q_i from the values of the surrounding grid points. To evaluate the magnitude of this grid dependence, we have performed an alternative calculation of the electrostatic stabilization energy, E_{q_i,Q_j}(unscreened), using the standard E_{total} calculation performed with Discover. This is done by performing the following calculations: (1) a normal calculation of $E_{\text{total}}(Q_i,q_j)$ and (2) a calculation of E_{total} in which all partial charges within the charged residues are reversed, $E_{\text{total}}(Q_i\rightarrow(-Q_i),q_j)$. The second calculation gives

$$E_{\text{total}}(Q_i\rightarrow(-Q_i),q_j) = E_{q_i,q_j} + E_{Q_i,Q_j} - E_{q_i,Q_j} \quad (2)$$

since E_{q_i,q_j} and E_{Q_i,Q_j} are unchanged and the sign of E_{q_i,Q_j} is reversed. By solving eqs 1 and 2 we get

$$E_{q_i,Q_j} = \frac{E_{\text{total}}(Q_i,q_j) - E_{\text{total}}(Q_i\rightarrow(-Q_i),q_j)}{2} \quad (3)$$

The results of the Coulombic calculations are also reported in Table II. The energies calculated without screening by the use of DelPhi and of Coulomb's law are almost identical, indicating that the grid dependence of the results of the DelPhi calculations is of minor importance. The electrostatic interaction between the α-helix and the intramolecular electrostatic field is favorable, having values from –40 to –60 kcal/mol for the five members of the PP-fold family when screening is excluded. These numbers agree well with a simple model in which the helix dipole is placed midway between two point charges of $\pm 3e$ separated by $\sim$30 Å.[3] The stabilization energy of a dipole of 40 D in this model is about –40 kcal/mol. It must be noted that these stabilization energies are upper estimates, since the screening effect of the solvent has not been included.

When screening is included, the electrostatic stabilization energies are lowered by a factor of 5–14. The resulting energies agree well with the electrostatic stabilization energies calculated by others for α-helix–α-helix and α-helix–β-sheet interactions (Hol et al., 1981; Sheridan et al., 1982). It is remarkable how the screening ratio varies from one peptide structure to the other. Since the side chains of all charged residues in all structures are fully exposed to solvent, the different screening ratios cannot be due to differences in solvation state for the charged residues in the different

[3] The magnitude of the field midway between two point charges is calculated by $|\vec{E}| = 2Q/[4\pi\epsilon_0\epsilon_r(R/2)^2]$, where R is the distance between the point charges. The energy of the dipole in this field is given by $U = -\vec{E}\cdot\vec{\mu}$.

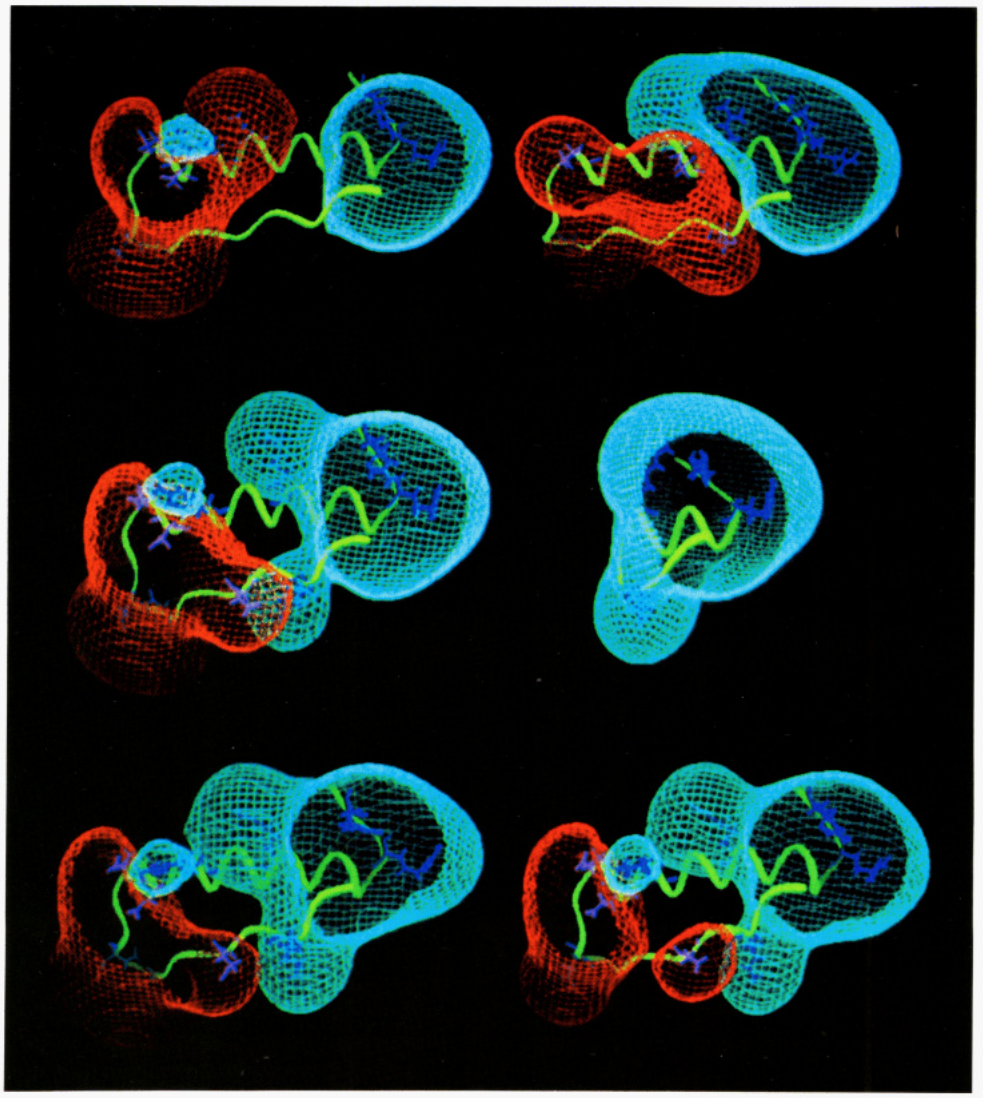

FIGURE 3: Electrostatic potential maps as calculated by DelPhi for avian PP (top left), human PP (top right), human NPY (middle left), NPY (1–4-Ahx-25–36)peptide (middle right), human PYY (bottom left), and lamprey PMY (bottom right). The potentials are contoured at ±1 kT; the negative potentials are red and the positive cyan. The molecules are represented by ribbons, and only the charged residues are shown explicitly. The positive residues (Arg and Lys) are colored blue and the negative residues (Asp and Glu) magenta.

structures. Rather, they are a result of the different positions of the charged residues from one structure to the other. A mean screening ratio of 8 is in good agreement with calculations made by Rogers and Sternberg (1984).

Since it is plausible to assume that the electrostatic stabilization energy of the PP-fold structures is lost upon unfolding, these values should be compared to the thermodynamic enthalpy change upon unfolding of the structure. This enthalpy change is estimated to be 30 kcal/mol for avian PP (Kanazawa & Hamaguchi, 1986). The calculated stabilization energy due to charge–dipole interactions in avian PP is smaller (≈7 kcal/mol), but it is definitely of the same order of magnitude as the enthalpy change for unfolding. Our results therefore strongly indicate that the charge–dipole interactions are of significant importance for the stability of the three-dimentional structure of the PP-fold peptides. Consequently, we propose that an explanation of the surprising stability of the tertiary structure must involve electrostatic considerations along with the well-accepted hydrophobic stabilization.

Fairman et al. (1990) have recently pointed out that a salt bridge in the C-peptide from ribonuclease A (residues 1–13) plays a more significant role than charge–dipole interactions (Shoemaker et al., 1987) in the stabilization of the α-helix of this peptide. Similar effects could provide a stabilization of

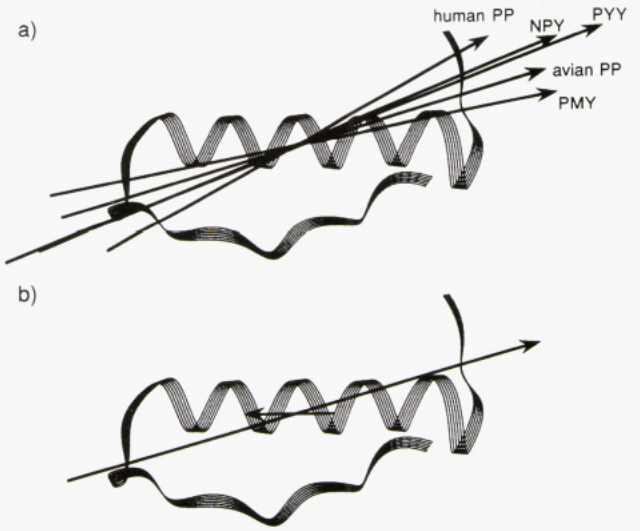

FIGURE 4: (a) Dipole moments for the five members of the PP-fold family displayed relative to each other. (b) Antiparallel arrangement of the overall dipole moment of avian PP (large arrow) and the dipole arising from alignment of the peptide dipoles in the α-helix (small arrow). In both cases, the dipole moments are shown in relation to the three-dimensional structure of the peptide, represented by a ribbon.

the α-helix in the PP-fold peptides which would then add to

Table II: Total Electrostatic Stabilization Energies of the PP-fold Tertiary Structure

	E_{q_i,Q_j} (kcal/mol)			screening ratio
	Coulomb approach, unscreened	P–B approach,[a] unscreened	P–B approach,[a] screened	$\frac{E_{q_i,Q_j}(\text{unscreened})}{E_{q_i,Q_j}(\text{screened})}$
avian PP	−56	−56	−7	8
human PP	−55	−56	−4	14
human NPY	−47	−47	−5	9
human PYY	−58	−60	−10	6
lamprey PMY	−40	−41	−9	5

[a] Poisson–Boltzmann approach.

the pure charge–dipole interactions evaluated in this paper.

In order to further investigate our theoretical prediction concerning the electrostatic stabilization of the tertiary structure in the PP-fold family, we are currently studying three synthetic analogues of human NPY: [Arg^{15}]NPY, [Arg^{11}]NPY, and [Lys^{10},Lys^{11}]NPY. In these analogues we have introduced substitutions in the turn region of the PP-fold which are not directly involved in receptor binding. According to our calculations, these analogues have a dipole moment with major differences in both direction and size. These analogues are expected to be less stable in aqueous solution and thus to have a reduced biological activity (Bjørnholm et al., in preparation).

CONCLUSIONS

A characteristic charge pattern is conserved for all members of the PP-fold family of peptides, indicating that the electrostatic properties are of major importance for the stability of the structure and the biological activity of these peptides. Investigations of this special charge pattern using electrostatic potential calculations show that the receptor-binding region of the peptides is characterised by a highly conserved positive potential, whereas a negative potential is present in the β-turn region of the molecules. This charge distribution gives rise to a strong overall dipole moment for the PP-fold structure (325–450 D). The dipole moment is antiparallel to the dipole arising from summation of the aligned peptide dipoles in the α-helix. Calculations of the stabilization energy of this antiparallel arrangement of the two dipoles indicate that charge–dipole interactions in this system play an important role for the stabilization of the PP-fold tertiary structure. An explanation of the surprising stability of the tertiary structure for this family of peptides, in spite of their relatively small size, must therefore involve electrostatic considerations along with stabilization due to the well-known hydrophobic interactions.

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REFERENCES

Abens, J., Undén, A., Andell, S., Tam, J. P., & Bartfai, T. (1989) in *Neuropeptide Y* (Mutt, V., Fuze, K., Hökfelt, T., & Lundberg, J. M., Eds.) pp 137–142, Raven Press, New York.

Allen, J., Novotn'y, J., Martin, J., & Heinrich, G. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 2532–2536.

Beck-Sickinger, A. G., Gaida, W., Schnorrenberg, G., Lang, R., & Jung, G. (1990a) *Int. J. Pept. Protein Res. 36*, 522–530.

Beck-Sickinger, A. G., Jung, G., Gaida, W., Köppen, H., Schnorrenberg, G., & Lang, R. (1990b) *Eur. J. Biochem. 194*, 449–456.

Blundell, T. L., Pitts, J. E., Tickle, I. J., Wood, S. P., & Wu, C.-W. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 4175–4179.

Chance, R. E., Cieczkowski, M., Jaworek, J., Konturek, S. J., Swierzek, J., & Tasler, J. (1981) *J. Physiol. (London) 314*, 1–9.

Clover, I. D., Haneef, I., Pitts, J. E., Wood, S. P., Moss, D. S., Tickle, I. J., & Blundell, T. L. (1983) *Biopolymers 22*, 293–304.

Clover, I. D., Barlow, D. J., Pitts, J. E., Wood, S. P., Tickle, I. J., Blundell, T. L., Tatemoto, K., Kimmel, J. R., Wollmer, A., Strassburger, W., & Zhang, Y. S. (1984) *Eur. J. Biochem. 142*, 379–385.

Conlon, J. M., Bjørnholm, B., Jørgensen, F. S., Youson, J. H., & Schwartz, T. W. (1991) *Eur. J. Biochem. 199*, 293–298.

Dauper-Osguthorpe, P., Roberts, V. A., Osguthorpe, D. J., Wollf, J., Genest, M., & Hagler, A. T. (1988) *Proteins: Struct., Funct., Genet. 4*, 31–47.

Fairman, R., Shoemaker, K. R., York, E. J., Stewart, J. M., & Baldwin, R. L. (1989) *Proteins: Struct., Funct., Genet. 5*, 1–7.

Fairman, R., Shoemaker, K. R., York, E. J., Stewart, J. M., & Baldwin, R. L. (1990) *Biophys. Chem. 37*, 107–119.

Forest, M., Martel, J.-C., St-Pierre, S., Quirion, R., & Fournier, A. (1990) *J. Med. Chem. 33*, 1615–1619.

Fuhlendorff, J., Johansen, N. L., Melberg, S. G., Thøgersen, H., & Schwartz, T. W. (1990) *J. Biol. Chem. 265*, 11706–11712.

Hagler, A. T. (1985) in *The Peptides: Conformation in Biology and Drug Design* (Hruby, V. J., & Meienhofer, J., Eds.) Vol. 7, pp 213–299, Academic Press, Orlando.

Hagler, A. T., & Lifson, S. (1974) *J. Am. Chem. Soc. 96*, 5319–5327.

Hagler, A. T., Dauber, P., & Lifson, S. (1979) *J. Am. Chem. Soc. 101*, 5131–5141.

Hol, W. G. J., Halie, L. M., & Sander, C. (1981) *Nature 294*, 532–536.

Kanazawa, I., & Hamaguchi, K. (1986) *J. Biochem. 100*, 207–212.

Klapper, I., Hagstrom, R., Fine, R., Sharp, K., & Honig, B. (1986) *Proteins: Struct., Funct., Genet. 1*, 47–59.

Krstenansky, J. L., & Buck, S. H. (1987) *Neuropeptides 10*, 77–85.

Krstenansky, J. L., Owen, T. J., Buck, S. H., Hagaman, K. A., & McLean, L. R. (1989) *Proc. Natl. Acad. Sci. U.S.A. 86*, 4377–4381.

Li, Y., Sutcliffe, M. J., Schwartz, T. W., & Dobson, C. M. (1992) *J. Biol. Chem. 267*, 9–12.

MacKerell, A. D., Jr. (1988) *J. Comput.-Aided Mol. Des. 2*, 53–63.

McLean, L. R., Buck, S. H., & Krstenansky, J. L. (1990) *Biochemistry 29*, 2016–2022.

Richardson, J. S., & Richardson, D. C. (1988) *Science 240*, 1648–1652.

Rogers, N. K., & Sternberg, J. E. (1984) *J. Mol. Biol. 174*, 527–542.

Schwartz, T. W., Fuhlendorff, J., Langeland, N., Thøgersen, H., Jørgensen, J. H., & Sheikh, S. P. (1989) in *Neuropeptide Y* (Mutt, V., Fuxe, K., Hökfelt, T., & Lundberg, J. M., Eds.) pp 534–541, Raven Press, New York.

Schwartz, T. W., Fuhlendorff, J., Kjems, L. L., Kristensen, M. S., Vervelde, M., O'Hare, M., Krstenansky, J. L., & Bjørnholm, B. (1990) *Ann. N.Y. Acad. Sci. 611*, 35–47.

Serrano, L., & Fersht, A. R. (1989) *Nature 342*, 296–299.

Sheridan, R. P., Levy, R. M., & Salemme, F. R. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 4545–4549.

Shoemaker, K. R., Kim, P. S., York, E. J., Stewart, J. M., & Baldwin, R. L. (1987) *Nature 326*, 563–567.

Tonan, K., Kawata, Y., & Hamaguchi, K. (1990) *Biochemistry 29*, 4424–4429.

Wada, A. (1976) *Adv. Biophys. 9*, 1–63.

Wahlestedt, C., Yanaihara, N., & Håkanson, R. (1986) *Regul. Pept. 13*, 307–318.

Wood, S. P., Pitts, J. E., Blundell, T. L., Tickle, I. J., & Jenkins, J. A. (1977) *Eur. J. Biochem. 78*, 119–126.