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The Role of Aromatic Residues in Stabilizing the Secondary and Tertiary Structure of Avian Pancreatic Polypeptide

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Abstract

Avian Pancreatic Polypeptide is a 36 residue protein that exhibits a tertiary fold. Results of previous experimental and computational studies indicate that the structure of aPP is stabilized more by non-bonded interactions than by the hydrophobic effect. Aromatic residues are known to participate in a variety of long range non-bonded interactions, with both backbone atoms and the atoms of other side-chains, which could be responsible, in part, for the stability of both the local secondary structure and the tertiary fold. The effect of these aromatic interactions on the stability of aPP was calculated using BHandHLYP/cc-pVTZ. Aromatic residues were shown to participate in multiple hydrogen bonded and weakly polar interactions in the secondary structure. The energies of the weakly polar interactions are comparable with those of hydrogen bonds. Aromatic residues were also shown to participate in multiple weakly polar interactions across the tertiary fold, again with energies similar to those of hydrogen bonds.

Keywords

Aromatic residues; Avian Pancreatic Polypeptide; Density functional theory; Tertiary fold; Weakly polar interactions

Introduction

Avian Pancreatic Polypeptide (aPP), is a short protein with a tertiary fold formed by the packing of a polyproline-II helix (PPII) on an α-helix [1] (Figure 1). Its relatively small size makes it ideal for computational study and several investigators have used it as a model to study protein folding and for prediction of structure [2-6]. Vibrational and electronic circular dichroism spectroscopies demonstrated that, when the fragments of aPP representing the PPII helix and the α-helix, aPP(1-11)-NH_2 and Ac-aPP(12-36), respectively, were placed in solution in equal concentrations, they refolded into the conformation of the full structure [7].

A molecular dynamics (MD) simulation of the conformation of aPP by Zhang and associates [8] showed that the structure of aPP appears to be stabilized more by electrostatic interactions than by the hydrophobic effect. These stabilizing interactions are most likely to be weakly polar non-bonded interactions [9]. Aromatic side-chains, in particular, are known to participate in a variety of weakly polar interactions including those with other side-chains, such as those of Pro, Lys, Arg and aliphatic residues [10-13]. Aromatic sides-chains can also participate in weakly polar interactions with the peptide backbone [14].

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The goal of this investigation was to determine the effect of aromatic residues on the structural stability of aPP by calculating the strength of the non-bonded interactions in which they participate, using DFT methods.

Methods

Initial structure

The structure of aPP (code 2BF9) used in this study was obtained from the Protein Data Bank [15].

Determination of non-bonded interactions

A "weakly polar" interaction was assigned when any backbone H^{α}, H, or O atoms, or any side chain atoms were within 6.0 Å of the centroid of an aromatic ring [16]. A mixed interaction was assigned when the criteria for a weakly polar interaction are satisfied and an atom of the aromatic residue participated in at least one hydrogen bond. A hydrogen bonded only interaction was assigned when the aromatic residue had an atom participating in at least one hydrogen bond but the ring was not close enough for a weakly polar interaction with another atom. Further classification of interactions was based on whether residues stabilized either local and/or secondary structure or the interaction between the α-helix and the PPII helix.

Interaction energy calculations

Intramolecular interaction energy calculations were performed at the BHandHLYP/cc-pVTZ level of theory because it closely approximates high-level results for weakly polar interactions [14,17]. The BHandHLYP functional used in this study was expressed as in ref. 14:

$$0.5 \cdot E_X^{HF} + 0.5 \cdot E_X^{S} + 1.0 \cdot E_C^{LYP} \quad (1)$$

where E_X^{HF} and E^S_X are the exact HF and local Slater exchange, respectively, and E_C^{LYP} is the Lee-Yang-Parr correlation functional.

To calculate intermolecular interaction energy between the PPII - and α-helices, an indirect approach was used. The aromatic side-chains of Phe20 and Tyr27 were removed and it was assumed that the interaction energy between the secondary structural elements is additive and thus, it can be calculated as follows:

$$\Delta E_{int} = \Delta E_{int}(FM) + \sum \Delta E_i(Phe20 \vee Tyr27) \quad (2)$$

where ΔE_i(Phe20∨Tyr27) is the energy of the i^{th} pairwise, tertiary structure-stabilizing interaction which involves Phe20 or Tyr27 (Table 2) and ΔE_{int}(FM) is the interaction energy between the fragmented and mutated structures which were generated as described below. Firstly, aPP was fragmented into Gly-Pro-Ser-Gln-Pro-Thr-Tyr-Pro-Gly-Asp-NHMe and *N*-Ac-Val-Glu-Asp-Leu-Ile-Arg-Phe-Tyr-Asn-Asp-Leu-Gln-Gln-Tyr-Leu-Asn-Val-Val-NHMe. Secondly, residues which had side-chains far from the interacting surfaces of the fragments were replaced with Ala. Finally, Phe20 and Tyr27 were replaced with Gly, resulting in two fragments, Gly-Pro-**Ala**-Gln-Pro-**Ala**-Tyr-Pro-Gly-Asp-NHMe ($PPII_{frag}$) and *N*-Ac-**Ala**-**Ala**-Asp-Leu-**Ala**-**Ala**-**Gly**-**Ala**-**Ala**-Asp-Leu-**Ala**-Gln-**Gly**-Leu-**Ala**-Val-Val-NHMe (α_{frag}) as shown in Figure 2. The positions of the introduced hydrogens were then optimized at the HF/3-21G level of theory and the interaction energies were calculated with the BHandHLYP/6-31+G** method. For interactions involving non-adjacent residues, the Boys and Bernardi basis set superposition error (BSSE) correction was used [18]. The peptide bond (PB) between Pro-Xaa, where Xaa was Ser3, Thr6, or Gly9 was not broken so as to preserve the electronic structure of the backbone. The energies of the interaction of the aromatic side-chains of Phe20 and Tyr27, respectively, with the peptide bonds of Thr6Tyr7 (PB6) and of

Ser3Gln4 (PB3) were counted twice. Therefore the energies of interaction of the side-chains with the overlap structure, represented by CH_3-NH-C(=O)-CH_3, were subtracted from the total interaction energy. For interactions involving adjacent residues, a rotation method previously described [14] was used to correct the BSSE.

Program packages—The Jaguar V5.5 release 11 and V6.0 release 11 (Schrödinger LLC, Portland, OR), and Gaussian 03 (Revision C.01) program packages were used for all calculations. YASARA (http://www.yasara.org) was used for visualization and the preparation of figures.

Results and Discussion

Tyr7, Phe20, Tyr21, Tyr27 and Tyr36 participated in all three categories of non-bonded interactions (Table 1). Residues Phe20, Tyr21 and Tyr27 appear to have a role in stabilizing the α-helix, whereas Tyr7 and Tyr36 appear to participate in stabilizing local structure.

The energies of all three categories of interaction which stabilize secondary and local structure are of the same order of magnitude. Furthermore, the energies of the weakly polar interactions between Phe20 and Pro5Thr6 and between Tyr27 and Pro2Ser3, were similar to those calculated for similar interactions which ranged from -4.35 to -4.80 kcal · mol^{-1} in a model helix [14]. Previously [19], it was found that the MD simulations estimate of the aromatic-backbone amide interaction energy is between -0.5 and -2.22 kcal · mol^{-1} whereas the quantum chemical calculation here shows that the energy of the interaction between Tyr27 and Pro2Ser3 is -7.43 kcal · mol^{-1}. Thus, while the MD simulations are appropriate for estimation of geometries, the present level of theory is the minimum needed for determination of interaction energies. The weakly polar interactions of the aromatic side-chains of residues Phe20 and Tyr27 stabilize the tertiary fold of aPP.

Additionally, a hydrogen bond and three non-canonical CH..O hydrogen bonds [20] between Pro8Gly9 and Ala12 (Figure 3) contribute to the fold stability (Table 2).

The energies of individual interactions across the tertiary fold are comparable with the lower energies of strong hydrogen bonds (-4 to -15 kcal · mol^{-1}) [21]. They are also comparable with the energies, about -5.0 kcal · mol^{-1}, found for other structures including the core of rubredoxin [22]. The interaction energies of the doubly counted peptide bonds, PB3 and PB6, respectively, were -2.22 kcal · mol^{-1} and -3.42 kcal · mol^{-1}. After correction the total energy of the interactions which stabilize the tertiary fold formed by the α-helix and the PPII-helix is -27.33 kcal · mol^{-1}. When Phe20 and Tyr27 were removed from the α-helical fragment, the interaction energy between $PPII_{frag}$ and α_{frag}, ΔE_{int}(FM), was repulsive (20.88 kcal · mol^{-1}). According to eq. 2, the interaction energy that stabilizes the tertiary fold is -6.45 kcal · mol^{-1}.

The functional groups and distances of all interactions are given in Table 3. With few exceptions, they are between 2 and 5 Å, in close agreement with distances observed in a PDB search of proteins with weakly polar interactions [16], though Burley and Petsko [9] stated that distances of weakly polar interactions can be as long as 9 Å. Findings here are also in agreement with the conclusion following a previous clustering analysis [10] that Ar-Pro interactions can constrain local conformations in proteins.

Conclusions

The aromatic residues Phe20 and Tyr27 contribute significantly to the tertiary fold stability of aPP through weakly polar interactions. Aromatic residues can significantly stabilize proteins

through non-bonded interactions. They influence the stability of both secondary and tertiary structure by weakly polar interactions as strong as hydrogen bonds.

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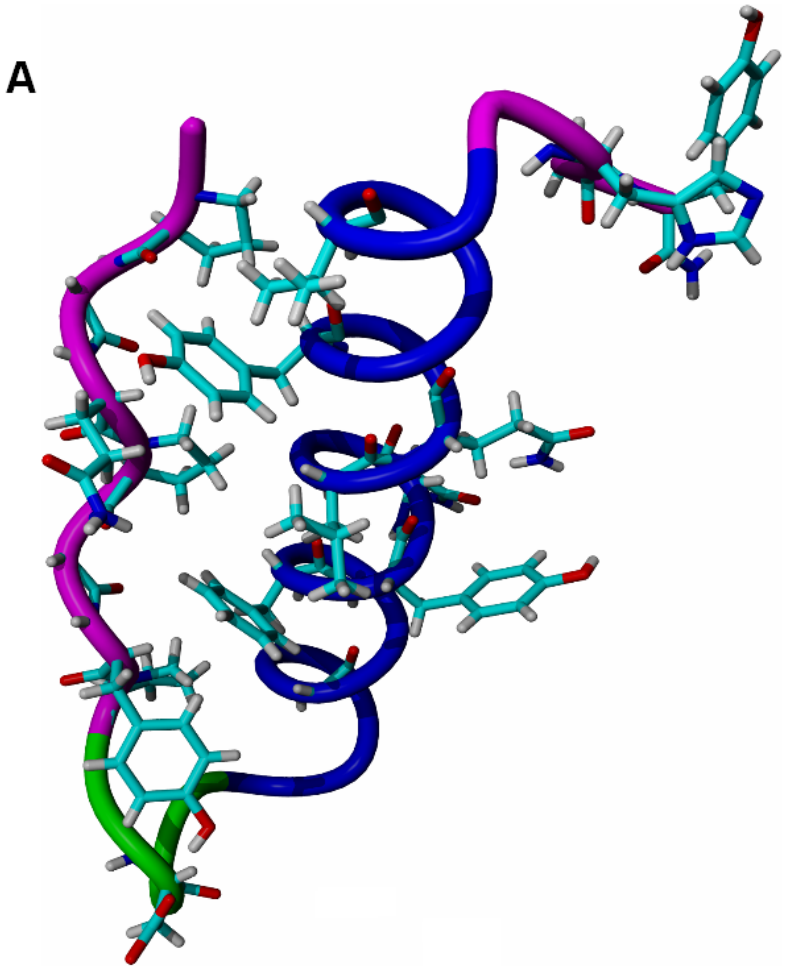

Fig. 1.

A. Backbone structure of aPP with interacting side chains and backbone atoms displayed. The N-terminal PPII helix is magenta, the turn structure is green and the α-helix is dark blue. **B.** The primary structure of aPP, interacting residues are in boldface.

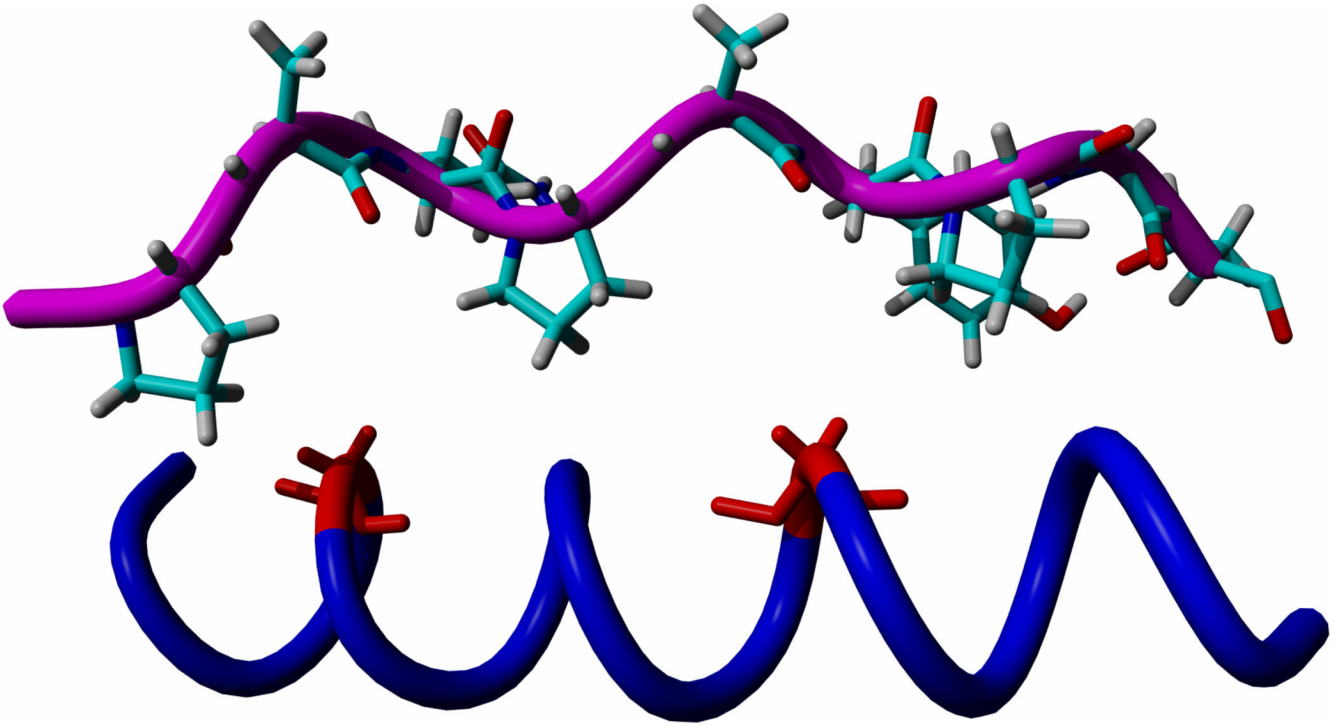

Fig. 2.

Structure of the $PPII_{frag}$ and α_{frag}. The color scheme is the same as Figure 1 except that the Gly residues which replace residues Phe20 and Tyr27 are red.

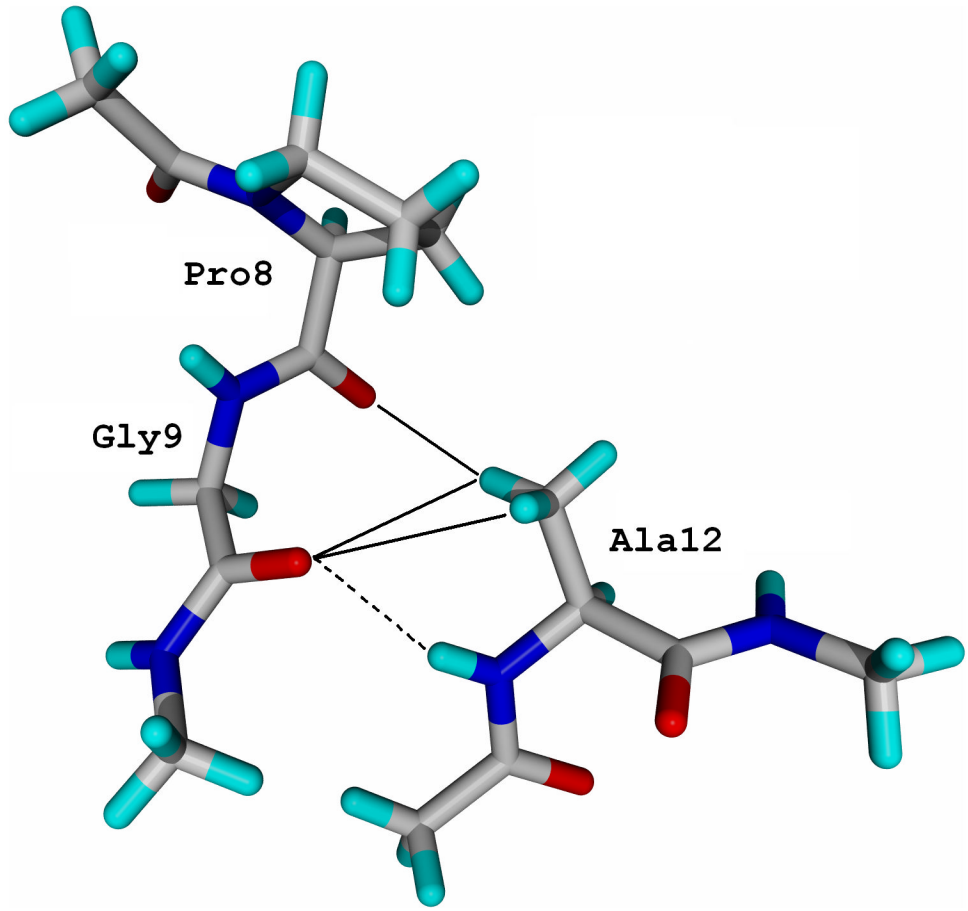

Fig. 3.
Hydrogen bonds of residues Pro8Gly9 and Ala12. The hydrogen bond is indicated with a dashed line, and the three non-canonical CH..O hydrogen bonds are indicated with solid lines.

Table I

Energies (kcal · mol^{-1}) of non-bonded interactions which stabilize secondary and/or local structure.

Weakly polar interactions		*Mixed interactions*		*H-bonded interactions*	
Interacting residues	**ΔE_{int}**	**Interacting residues**	**ΔE_{int}**	**Interacting residues**	**ΔE_{int}**
Tyr7-Pro8Gly9	-14.17	Tyr7-Asp10	-24.76	Phe20-Leu17	-18.16
Tyr21-Asp22	-12.74	Phe20-Leu24	-13.00		
Tyr36-His34Arg35	-2.33	Tyr21-Gln25	-13.11		
		Tyr27-Val31	-13.07		

Table II

Energies (kcal · mol^{-1}) of non-bonded interactions which stabilize tertiary structure.

Interactions	ΔE_{int}
Pro8Gly9-Ala12	-8.71
Phe20-Pro5Thr6	-6.05
Phe20-Tyr7Pro8	-5.25
Tyr27-Pro2Ser3	-7.43
Tyr27-Gln4Pro5	-5.53
Phe20-PB6[a]	3.42
Tyr27-PB3[a]	2.22
Total	-27.33

[a]For doubly counted peptide bonds (PB), see methods.

Table III

Distances (Å) of non-bonded interactions[a].

Interacting residues	Functional group 1	Functional group 2	Distance	Type
	Interactions which stabilize local and/or secondary structure			
Tyr7-Pro8Gly9	Ar	H^{δ} of Pro8	3.58	*Weakly Polar*
Tyr7-Pro8Gly9	Ar	N-H of Gly9	2.99	*Weakly Polar*
Tyr7-Asp10	Ar-OH	O^{δ}	1.87	*Mixed*
Tyr7-Asp10	Ar	PB9[b]	4.38	*Mixed*
Phe20-Leu17	N-H of Tyr21	C=O of Leu17	2.05	*H-bonded*
Phe20-Leu17	N-H of Phe20	C=O of Asp16	2.19	*H-bonded*
Phe20-Leu24	Ar	H^{β}	3.67	*Mixed*
Phe20-Leu24	C=O	N-H	1.95	*Mixed*
Tyr21-Asp22	Ar	PB21	4.06	*Weakly Polar*
Tyr21-Gln25	Ar	H^{β}	4.56	*Mixed*
Tyr21-Gln25	C=O	N-H	2.11	*Mixed*
Tyr27-Val31	Ar	H^{β}	5.03	*Mixed*
Tyr27-Val31	C=O	N-H	2.10	*Mixed*
Tyr36-His34Arg35	Ar	Ring of His34	4.85	*Weakly Polar*
Tyr36-His34Arg35	Ar	PB34	5.00	*Weakly Polar*
	Interactions which stabilize the tertiary structure			
Pro8Gly9-Ala12	C=O of Pro8	H^{β}	2.78	*Mixed*
Pro8Gly9-Ala12	C=O of Gly9	H^{β}	2.98	*Mixed*
Pro8Gly9-Ala12	C=O of Gly9	$H^{\beta 2}$	2.99	*Mixed*
Pro8Gly9-Ala12	C=O of Gly9	N-H	2.35	*Mixed*
Phe20-Pro5Thr6	Ar	H^{β} of Pro5	4.03	*Weakly Polar*
Phe20-Pro5Thr6	Ar	PB6	5.65	*Weakly Polar*
Phe20-Tyr7Pro8	Ar	Ar-H^{δ}	3.21	*Weakly Polar*
Phe20-Tyr7Pro8	Ar	H^{δ}	3.59	*Weakly Polar*
Tyr27-Pro2Ser3	Ar-H^{δ}	Prolyl ring	4.11	*Weakly Polar*
Tyr27-Pro2Ser3	Ar	C=O of Pro2	4.68	*Weakly Polar*
Tyr27-Pro2Ser3	Ar	PB3	4.72	*Weakly Polar*
Tyr27-Gln4Pro5	Ar	H^{α} of Gln4	3.15	*Weakly Polar*
Tyr27-Gln4Pro5	Ar	H^{δ} of Pro5	3.71	*Weakly Polar*

[a] Functional groups 1 and 2, respectively, refer to left and right interacting residues, unless otherwise stated.

[b] PB, peptide bond.