Anomalous Amide Proton Chemical Shifts as Signatures of Hydrogen Bonding to Aromatic Sidechains

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General Comments

The authors have searched the Biological Magnetic Resonance Bank (BMRB) for unusual amide proton chemical shifts and amide-aromatic nuclear Overhauser effects of proteins. For each amide proton, they checked at the same time the Protein Data Bank (PDB) for the distances to the nearest aromatic protons of sidechain residues, i.e. tyrosine (TYR), phenylalanine (PHE), tryptophane (TPR) and histidine (HIS). They prepared a huge dataset consisting of 363686 amide protons from 467070 entries. The data show that the proximity of an amide proton to an aromatic ring can lead to a low-field or an upfield or to no signal discplacement with respect to the normal amid chemical shift of the residue of interest. When the azimuthal angle θ between the NH distance vector and the axis perpendicular to the aromatic plane is smaller than the magic angle, high-field shifts and at larger angles low-field shifts result.

Firstly, the distance effects are discussed. Surprisingly, Figure 3 indicates that aromatic induced shifts decay to 1/e only at distances around 30 Å. The shortest distances to the aromatic rings observed are surprisingly small, i.e. around 2.2 Å for TYR. For TYR, PHE and TPR the upfield shifts are more pronounced than the low-field shifts. By contrast, for amide NH interacting with HIS there is a low-field shifts preponderence. The azimuthal angles consistent with the chemical shifts are discussed in detail, as well as NOE distance constraints of amide NH groups to arometic rings. The authors interpret their data in terms of hydrogen bonds of the amide NH groups to the aromatic π electrons, dominating in the case of TYR, PHE and TRP, whereas they discuss amide NH hydrogen bonds to the non-protonated nitrogen atoms of neutral histidine residues.

Finally, two examples are discussed, and force-field calculation are used to discuss the energetics of the interactions.

In summary, the article contains interesting insights into the interactions of amino NH groups with the arometic residues of side chains. I have the following comments, which I hope help the authors to improve the manuscript.

Specific Comments

(1) I had problems with the approach of the authors. As the amide NH chemical shifts probably refer to aqueous solution and the distances to crystal structures taken from the PDB which also contains NMR structures, I would have expected two Figures 3, one with the distances derived from NMR structures and the other from crystals. Or did I overlook something? Weak interactions might change between the liquid and the solid state. A discussion of the origin of the distances will be helpful.

(2) My main scientific concern is the use of the term "hydrogen bonding". The IUPAC definition (doi:10.1351/PAC-REC-10-01-02) is "*The hydrogen bond is an attractive interaction between a hydrogen atom from a molecule or a molecular fragment X–H in which X is more electronegative than H, and an atom or a group of atoms in the same or a different molecule, in which there is evidence of bond formation*." According to the force-field calculations of an alanine-phenylalanine pair presented in Figure 9, a NH…π interaction is found exhibiting an energy minimum at a N-ring distance of 3.3 Å. For the benzene-NH_3 complex in the gas phase a distance of 3.59 Å (Rodham et al. Nature **1993**, *362*, 735) and 3.54 Å (Mons et al. PCCP, **2002**, *4*, 571–576 was found which is in good agreement. That leaves for the ring-H distance values between 2.3 and 2.6 Å. But then only a small part of the data in Figure 3 can refer to NH…π H-hydrogen bonds. That means that - with some exceptions - other forces may be responsible for most of the findings of the authors. However, the approch of the authors provides a diagnostic tool of for NH…π formation: observation of a

small NH-ring distance is not a sufficient criterium for such a bond, but a positive z-value is needed in order to exclude for example, a normal H-bond to a histidine nitrogen. It would be interesting, if the authors could "disentangle" the plots in Figure 3, and associate different interaction types to the different parts. Perhaps they should use a term like "extended NH…π H-bond".

(3) I have problems to believe in a normal interation according to Figure 1 in the distance range above 10 Å extending to 30 Å and more between the interacting rings and NH groups. There is matter in between, perhaps water, the PDB will show that, I doubt that the magnetic interaction will go unaffected through that matter. Figure 1 refers in my opinion to the vacuum. Thus, the magnetic susceptibility needs to be taken into account, and that quantity is heterogeneous.

(4) The Z-core of amino groups is defined in eq 1. That quantity is plotted in the ordinate of Figure 3. In order to access rapidly the ordinate values the authors should discuss eq 1, give a feeling for the values of δ_{res}, $<\delta_{res}>$ and σ_{res} of amino acids. Perhaps a Table will be helpful.

(5) In the examples of Figures 7 and 8 the distance and azimuthal angles should be mentioned in the graph or the caption. In addition, not only the Z values, but also the values of δ_{res} (labeled differently in the caption as δ_H), $<\delta_{res}>$ and σ_{res} should be included.

(6) I had difficulties with the coordinate systems of Figures 3 and 4. The dimensions in the x-axis are lacking. The white help lines in the background are broad that it is almost impossible to determine the distance of shift of a given data point by increasing the size of the graph.