Breaking Away: Proceedings of the 21st American Peptide Symposium
Michal Lebl (Editor)
American Peptide Society, 2009

A Donor–Acceptor Perspective on Carbonyl–Carbonyl Interactions in Proteins

Amit Choudhary[1] and Ronald T. Raines[2]

[1]*Graduate Program in Biophysics, University of Wisconsin–Madison, Madison, WI 53706, U.S.A.,*
[2]*Department of Biochemistry and Chemistry, 433 Babcock Drive, University of Wisconsin–Madison, Madison, WI 53706, U.S.A.*

Introduction

Electronic delocalization, a central concept in organic chemistry, is being invoked increasingly in biological contexts [1–3]. We have discovered a non-covalent interaction in proteins, termed the $n\rightarrow\pi^*$ interaction, in which the lone pair (n) of the oxygen (O_{i-1}) of a peptide bond overlaps with the antibonding orbital (π^*) of the carbonyl group ($C'_i{=}O_i$) of the subsequent peptide bond (Figure 1A, B) [1]. The $n\rightarrow\pi^*$ interaction is reminiscent of the renowned Bürgi–Dunitz trajectory [1c] and analogous to a hydrogen bond, which likewise involves the delocalization of a lone pair of an acceptor atom over the antibonding orbital (σ^*) of a donor [2]. The stereochemical constraints required for an energetically meaningful $n\rightarrow\pi^*$ interaction are met in several fundamental structural elements in proteins, including α-helices, 3_{10} helices, and polyproline II type helices, as well as within the backbone of peptoids. A signature of the $n\rightarrow\pi^*$ interaction is a short $O_{i-1}\cdot\cdot C'_i$ contact [3]. Others have argued that the attractive C=O···C=O interaction is primarily a dipole–dipole (Figure 1C) [4] or a charge–charge interaction (Figure 1D) [5]. We used a peptidic model system (Figure 2) to explore the origin of this interaction. Regardless of the nature of the interaction between the adjacent carbonyl groups, the interaction stabilizes the *trans* conformation preferentially over the *cis* conformation. Thus, the value of $K_{trans/cis}$ reports on the strength of the C=X···C=O interaction.

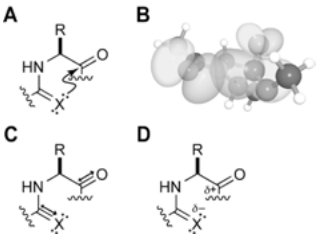

Fig. 1. Possible C=X··C=O interactions.

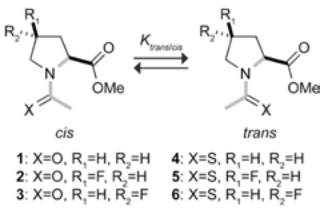

Fig. 2. Compounds used to examine the C=X···C=O interaction.

Results and Discussion

To distinguish between a charge–charge interaction and an $n\rightarrow\pi^*$ interaction, we envisaged the replacement of O_{i-1} with sulfur, S_{i-1}, in this model system. A charge–charge interaction would be attenuated because sulfur is less negatively polarized than oxygen, whereas the $n\rightarrow\pi^*$ interaction would be strengthened because sulfur is a softer base than oxygen. An increase in $K_{trans/cis}$ is observed from this isosteric substitution. Hence, the stabilization of the *trans* conformation cannot be due to a charge–charge interaction. Another signature of the $n\rightarrow\pi^*$ interaction is the pyramidalization of the acceptor carbonyl group. Such pyramidalization should appear in the computationally optimized, gas-phase geometries and the crystal structures. Additionally, the degree of pyramidalization should increase as the distance between the donor and the acceptor atoms is decreased. We employed a subtle means to alter the distance between the donor and acceptor atoms [6]. In accord with a potent $n\rightarrow\pi^*$ interaction, a positive correlation is indeed observed between the C'_i pyramidalization and the $K_{trans/cis}$ value in both the computational and experimental data (Figure 3).

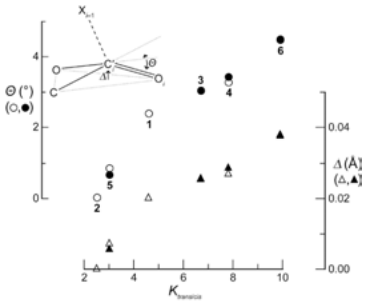

Fig. 3. Relationship between the degree of C'_i pyramidalization and the value of $K_{trans/cis}$. Open symbols, computational; filled symbols, experimental.

Next, we reasoned that the replacement of the C=O acceptor with a C–F bond would retain the dipole–dipole interaction but attenuate the $n\rightarrow\pi^*$ interaction [7]. This isosteric substitution leads to reversal of the conformational preference from *trans* to *cis*. Again, the observed value of $K_{trans/cis}$ again cannot be explained by classical electrostatic models.

A recent Protein Data Bank search has revealed that more than 81% of α-helical residues exhibit a short contact ($d < r_C + r_O$) between neighboring carbonyl groups [8]. Employing an $AcAla_4NHMe$ peptidic model system, we scanned the allowed regions of the Ramachandran map for $n\rightarrow\pi^*$ interactions. We found a widespread prevalence of $n\rightarrow\pi^*$ interaction in the allowed regions [9]. Common protein secondary structures, such as α-helices and 3_{10} helices, show significant stabilization by $n\rightarrow\pi^*$ interactions. As the adjacent carbonyl dipoles repel each other in an α-helix, the $n\rightarrow\pi^*$ interaction likely plays an important role in helix nucleation. Considerable carbonyl bond lengthening [10], polarization of the π-electron cloud [10], and pyramidalization of the carbonyl carbon [9] have been observed in the α-helices of high-resolution protein structures. Our computational analyses also predict significant $n\rightarrow\pi^*$ interactions in the twisted β–sheet region. The conformational stability of the collagen triple helix has already been attributed, in part, to $n\rightarrow\pi^*$ interactions [11].

The resonance character between adjacent carbonyl groups in proteins has important implications. The distorted conformation of a fluoroalkene isostere emphasizes the stabilization afforded by an $n\rightarrow\pi^*$ interaction, which is absent in that system [7]. Short $O_{i-1}\cdots C_i{=}O_i$ contacts are widespread in common protein folds [8]. Yet, closed shell repulsion between the lone-pair of O_{i-1} and the π-orbital of $C_i{=}O_i$ tends to increase the $O_{i-1}\cdots C_i{=}O_i$ distance and thereby compromise the structural integrity of proteins. We propose that the availability of a low-lying π^*-orbital effectively counters the closed shell repulsion and enables polypeptide chains to adopt α-helices, 3_{10} helices, and polyproline II type helices.

Finally, we note that $n\rightarrow\pi^*$ electronic delocalization likely plays a role in many protein–ligand interactions and catalytic processes. Our data suggest that an isosteric substitution of an amide donor with a thioamide could be used to increase the ligand affinity and stabilize unstable intermediates in a catalytic cycle.

Acknowledgments

This work was supported by grant AR044276 (NIH). We thank G. R. Krow, G. J. Bartlett, D. N. Woolfson, C. E. Jackobsche, and S. J. Miller for collaborative interactions, and F. W. Kotch, I. A. Guzei, L. C. Spencer, M. D. Shoulders, M. J. Palte, and C. N. Bradford for contributive discussions.

References

1. (a) DeRider, M.L., et al. *J. Am. Chem. Soc.* **124**, 2497-2505 (2002).(b) Hinderaker, M.P., Raines, R.T. *Protein Sci.* **12**, 1188-1194 (2003).(c) Hodges, J.A., Raines, R.T. *Org. Lett.* **8**, 4695-4697 (2006).
2. (a) Isaacs, E.D., et al. *Phys. Rev. Lett.* **82**, 600-603 (1999).(b) Weinhold, F. *Adv. Protein Chem.* **72**, 121-155 (2005). (c) Head-Gordon, M., et al. *J. Phys. Chem A.* **111**, 8753-8765 (2007).
3. Choudhary, A., et al. *J. Am. Chem. Soc.* **131**, 7244-7246 (2009).
4. (a) Diederich, F., et al. *Angew. Chem. Int. Ed.* **44**, 1788-1805 (2005). (b) Diederich, F., et al. *Proc. Natl. Acad. Sci. U.S.A* .**105**, 17290-17294 (2008).
5. (a) Milner-White, E.J., et al. *J. Mol. Biol.* **248**, 361-373 (1995). (b) Milner-White, E.J., et al. *J. Mol. Biol.* **248**, 374-384 (1995).
6. A 4*R* electron-withdrawing group (EWG) brings the O_{i-1} or S_{i-1} donor and $C_i{=}O_i$ acceptor closer; a 4*S* EWG increases the distance between the donor and acceptor.
7. Jackobsche, C.E., Miller, S.J., unpublished results.
8. Bartlett, G.J., Raines, R.T., Woolfson, D.N., unpublished results.
9. Choudhary, A., Raines, R.T. unpublished results.
10. Lario, P.I., Vrielink, A. *J. Am. Chem. Soc.* **125**, 12787-12794 (2003).
11. Shoudlers, M.D., Raines, R.T. *Annu. Rev. Biochem.* **78**, 929-958 (2009) and references therein.