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$n \rightarrow \pi^*$ Interactions in the Molecules of Life

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Introduction

Noncovalent interactions modulate the structure, function, and dynamics of the molecules of life [1]. We have discovered a noncovalent interaction in proteins and nucleic acids, termed the $n \rightarrow \pi^*$ interaction, in which the lone pair (n) of a donor group (typically a carbonyl oxygen) overlaps with the antibonding orbital (π^*) of an acceptor group (typically a carbonyl group) (Figures 1A and 1B) [2]. The $n \rightarrow \pi^*$ interaction is reminiscent of the approach of a nucleophile to an electrophilic carbon along the Bürgi–Dunitz trajectory [2a] and analogous to a hydrogen bond, which likewise involves the delocalization of a lone pair of an acceptor over an antibonding orbital (σ^*) of a donor [3]. The stereoelectronic constraints necessary for an energetically meaningful $n \rightarrow \pi^*$ interaction are met in several fundamental protein secondary structures, such as α-, 3_{10}-, and polyproline II helices, and twisted β-sheets. A signature of the $n \rightarrow \pi^*$ interaction in proteins is a short $O_{i-1} \cdots C'_i$ contact [2b, 2d]. It has been 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 nature of this interaction. Regardless of the origin 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=O···C=O interaction.

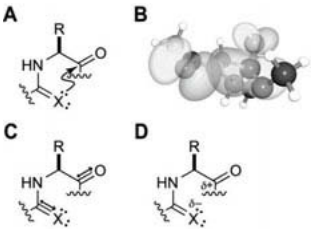

Fig. 1. Plausible C=X···C=O interactions [2d].

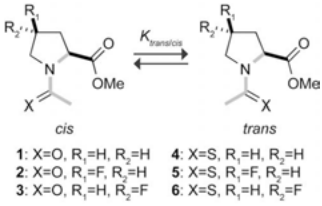

Fig. 2. Compounds used to examine the C=X···C=O interaction [2d].

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 [2d]. 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. The degree of pyramidalization, like $K_{trans/cis}$, should increase with the strength of the $n \rightarrow \pi^*$ interaction. We employed a subtle means to alter the strength of the $n \rightarrow \pi^*$ interaction [6]. In accord with a potent $n \rightarrow \pi^*$ interaction, a positive correlation is observed between the degree of acceptor carbonyl pyramidalization and the value of $K_{trans/cis}$.

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 substitution with an amide bond isostere, the fluoroalkene isostere, leads to reversal of the conformational preference from *trans* to *cis*. Such reversal of the conformational preference cannot be explained by classical electrostatic models. It is plausible that this conformational reversal stems from closed shell repulsion between the lone pair of the donor (O_{i-1}) and the π-orbital of the fluoroalkene isostere. Such closed shell repulsions are countered by an $n \rightarrow \pi^*$ interaction in amides, which are absent in their fluoroalkene isostere.

Our computational studies indicated significant $n \rightarrow \pi^*$ interaction in certain regions of the Ramachandran plot [8]. This expectation was validated by a statistical analysis of a

large, non-redundant subset of protein structures determined to high resolution (Figure 3). Moreover, these studies indicated that $n \rightarrow \pi^*$ interactions are abundant and especially prevalent in common secondary structures such as α-, 3_{10}-, and polyproline II helices, and twisted β-sheets. 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. Other signatures of the $n \rightarrow \pi^*$ interaction such as pyramidalization of the acceptor carbonyl carbon [9], considerable carbonyl bond lengthening [10], and polarization of its π-electron cloud [10] have been observed in the α-helices of high-resolution protein structures. Occasionally, β-strands have a bulge - an amplified right-handed twist - resulting in local disruption of the β-sheet structure. Such β-bulges are involved in the dimerization of immunoglobulin domains and can assist in enclosing the active sites of proteins. Two common types of β-bulges, the G1 and wide types, adopt φ and ψ dihedral angles indicative of considerable $n \rightarrow \pi^*$ interactions. The conformational stability of the collagen triple helix has already been attributed in part to the $n \rightarrow \pi^*$ interaction [11]. Interestingly, the absence of 4*S* diastereomer of hydroxyproline from collagen has been attributed in part to a strong $n \rightarrow \pi^*$ interaction that provides it with unusual conformational features [12].

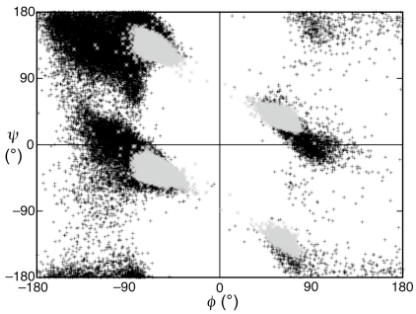

Fig. 3. Ramachandran map of $n \rightarrow \pi^*$ interactions (gray) in proteins [8].

In addition to its widespread occurrence in proteins, the $n \rightarrow \pi^*$ interaction has been postulated to play an important role in the origin of life [13] as well as modulating the conformational and physical properties of aspirin [9]. Finally, we note that $n \rightarrow \pi^*$ electronic delocalization likely plays a role in many protein–ligand interactions and catalytic processes.

Acknowledgments

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