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Experimental Measurement of the Strength of a Cα−H···O Bond in a Lipid Bilayer

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The energy of interaction between two dipoles is strongly dependent on the distance between the dipoles and the polarity of the solvent. As such, interactions that would normally be regarded as weak and insignificant in an aqueous environment may be substantial in the hydrophobic lipid bilayer milieu. One such interaction is the hydrogen bond between the nonpolar Cα hydrogen and a carbonyl oxygen, thought to take place as a stabilization driving force in numerous transmembrane α-helical bundles.[1] As an example, the GxxxG motif, which forms the protein−protein interface in the transmembrane helical dimer of glycophorin A (GPA),[2] is thought to drive dimerization due (in part) to a Gly Cα−H···O bond[1]. Thus, the close apposition of the helices, enabled by the small side chain of the Gly residues, facilitates the formation of a Cα−H···O bond.

In glycophorin A, Engelman and co-workers have pointed to six Cα−H···O bonds which they have categorized as strong (H···O distance < 2.7 Å) and another six potential bonds with a H···O distance shorter than 3.5 Å.[1] Ab initio calculations in the gas phase have resulted in estimations for the energy of such bonds, as high as 2.5−3.0 kcal/mol.[6,7] Thus, six such bonds should be able to contribute substantially toward the stability of the GPA dimer since no other hydrogen-bonding (H-bonding) partners are available in the lipid bilayer milieu. It is clear, therefore, that due to the apparent prevalence of such bonds it is imperative to accurately gauge their contribution to the stability of the glycophorin A dimer, in particular, and other helical bundles, in general. Consequently, in the current study we decided to construct an experimental system to measure the strength of a single Cα−H···O bond in a membrane environment.

A transmembrane peptide encompassing the transmembrane domain of glycophorin A was labeled with a deuterated glycine at position 79. G79 is one of the glycine residues predicted to donate its Cα hydrogen in a Cα−H···O bond.[1] Similarly, a monomeric mutant of glycophorin A containing the mutation G83I,[3] which should therefore be incapable of H-bonding, was labeled in the identical position. Subsequently, infrared spectroscopy should be able to quantitatively detect the frequency shift, due to the apparent bond, upon comparing the isotopically isolated CD_2 stretching mode frequencies in the two peptides. Finally, the measured frequency shift due to H-bonding, can be converted to a bond strength using a simple correlation obtained by Rozenberg and co-workers.[4]

Figure 1 depicts the region of the FTIR spectra encompassing the CD_2 asymmetric stretching mode[5] for two different glycophorin A transmembrane peptides reconstituted in lipid bilayers. The spectra of the wild-type GPA peptide exhibits a peak at 2247, while the spectra of the monomeric GPA peptide resonates at 2253. As expected, no peak is seen in an unlabeled GPA peptide (not shown), proving that the observed peaks are due to the Gly CD_2 label. In addition, the amide I vibrational modes depicted in Figure 1 indicate that both peptides exhibit highly similar secondary structure.

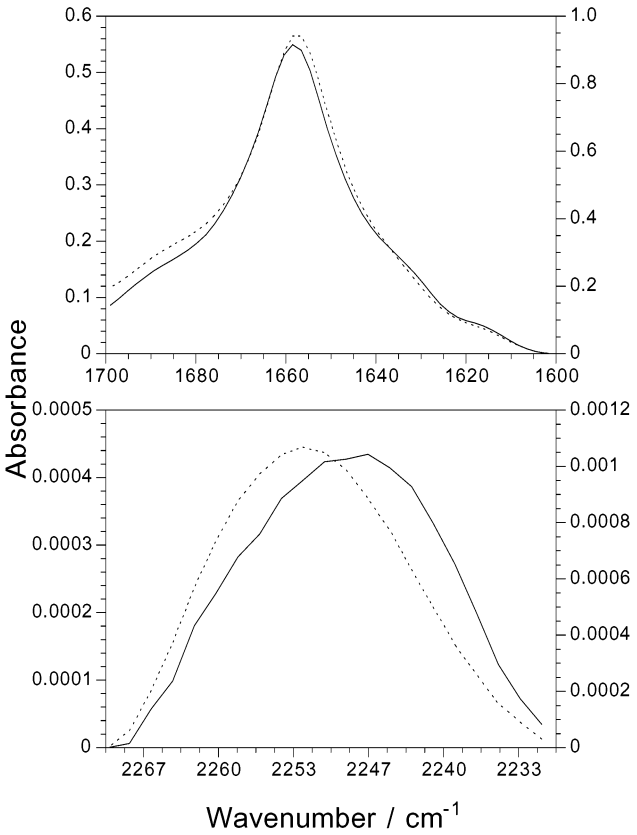

Figure 1. Transmission FTIR spectra of wild-type (solid line) and monomeric G83I[3] (dotted line) GPA transmembrane peptides reconstituted in a lipid bilayer, labeled with Gly79 CD_2. Top panel depicts the amide I modes, while the bottom panel, the asymmetric CD_2 stretching modes. Absorbance units on the left and right sides of the figure correspond to wild-type and G83I GPA peptides, respectively. Glycophorin A transmembrane peptides (70−101) were synthesized using solid-phase f-moc chemistry and purified on reverse phase HPLC; 1 mg of lyophilized, purified peptide was co-dissolved with 10 mg of dimyristoylphosphocholine in 1 mL of 1,1,1,3,3,3-hexafluoro-2-propanol. After evaporating the organic solvent, 1 ml of water at 37° was used to dissolve the lipid peptide mixture for 1 h; 100 μl of sample was deposited onto a Ge infrared window. Bulk water was removed by blowing dry air over the sample. FTIR spectra (1000) were collected, averaged, and baseline-corrected on a Nicolet Magna 550 (Madison, WI).

The asymmetric CD_2 stretching frequency difference measured between the wild-type and G83I monomeric mutant is 6. Since the isotopic frequency shift factor between the CH_2 and CD_2 is 1.36,[5] the observed CD_2 shift is equivalent to an 8.1 shift of a CH_2 mode due to an H-bond.

With the aforementioned shift at hand, it is possible to calculate the Cα−H ···O bond strength using the empirical correlation between the frequency shift and H-bond strength given by Rozen-

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berg and co-workers:[4] $\Delta G = 0.31\sqrt{\Delta\nu}$ kcal/mol. Thus, the 6 CD_2 stretching frequency difference observed between the dimeric and monomeric peptide is indicative of an H-bond energy of $\Delta G =$ 0.88 kcal/mol, a value lower by a factor of 2.5−3.5 than that calculated in the gas phase.[6,7]

What could be the reason for the difference between the strength of the Cα−H···O bond calculated in the gas phase using ab initio methods and that measured experimentally in a lipid bilayer in the current study? One obvious possibility is the difference between the polarity of the gas phase and that of the lipid bilayer.[8] In addition, the geometry of the H-bond measured may deviate from ideality in such a way as to reduce its strength relative to that calculated in the gas phase.

In conclusion, an experimental system was devised capable of measuring the Cα−H···O bond strength between two transmembrane helices. The value obtained of $\Delta G = 0.88$ kcal/mol, is smaller than that calculated theoretically in the gas phase.[6,7] However, if six such H-bonds do exist in GPA1, their overall contribution toward dimerization can account for a significant proportion of the dimerization free energy.[9] This is due to the lack of competition for H-bonding in the hydrophobic lipid bilayer environment. The remaining contributions toward stabilization arise due to other factors, such as maximization of packing interactions.[10]

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