# Energetics of hydrogen bonds in peptides

Sheh-Yi Sheu\*<sup>†‡</sup>, Dah-Yen Yang<sup>†‡§</sup>, H. L. Selzle<sup>11</sup>, and E. W. Schlag<sup>†‡11</sup>

\*Department of Life Science, National Yang-Ming University, Taipei 112, Taiwan; <sup>§</sup>Institute of Atomic and Molecular Science, Academia Sinica, Taipei 106, Taiwan; and <sup>¶</sup>Institut für Physikalische und Theoretische Chemie, Technische Universität Muenchen, Lichtenbergstrasse 4, 85748 Garching, Germany

Communicated by A. Welford Castleman, Jr., Pennsylvania State University, University Park, PA, June 3, 2003 (received for review December 8, 2002)

Hydrogen bonds and their relative strengths in proteins are of importance for understanding protein structure and protein motions. The correct strength of such hydrogen bonds is experimentally known to vary greatly from  $\approx$ 5–6 kcal/mol for the isolated bond to  $\approx$ 0.5–1.5 kcal/mol for proteins in solution. To estimate these bond strengths, here we suggest a direct novel kinetic procedure. This analyzes the timing of the trajectories of a properly averaged dynamic ensemble. Here we study the observed rupture of these hydrogen bonds in a molecular dynamics calculation as an alternative to using thermodynamics. This calculation is performed for the isolated system and contrasted with results for water. We find that the activation energy for the rupture of the hydrogen bond in a  $\beta$ -sheet under isolated conditions is 4.76 kcal/mol, and the activation energy is 1.58 kcal/mol for the same  $\beta$ -sheet in water. These results are in excellent agreement with observations and suggest that such a direct calculation can be useful for the prediction of hydrogen bond strengths in various environments of interest.

The strength of the hydrogen bond in the linking of protein structures particular in a water environment is of essential importance to predict the activity of proteins such as enzyme action, protein folding, binding of proteins, and many other processes (1, 2). Although much has been written on protein dynamics in water (3), a detailed energy calculation including the correct water environment has been difficult to put into a computational framework. The energetics of hydrogen bonds within proteins is known to undergo large changes in water. The effect of water is also process dependent, so it is different here from protein signal transport (4). Such environmental changes in a hydrogen bond strength are important to the understanding of protein interactions, including drug design (5, 6). The drugreceptor hydrogen bond is operative in many applications (7).

Hydrogen bonds are one of the major structural determinants, controlling active configurations by connecting protein structure in a fluxional equilibrium. The making and breaking of hydrogen bonds profoundly affects the rates and dynamic equilibria, which are responsible for much of the biological activity of proteins. This behavior is strongly medium dependent, so the action of these hydrogen bonds in isolated systems is quite different from the action in a water environment. The complex environment presented to the hydrogen bond by water is not easy to incorporate in calculations, but it is of major relevance, and results obtained need to be checked against experiments. A huge and complex phase space contributes to the effects of entropy on the hydrogen bond, particularly in water, and thus influences the free energy of these bonds. The general task of assessing the entropic contributions to the dynamic strength of these bonds is a matter of extensive research (8) and is difficult to quantify. Furthermore, it must be recognized that various relevant bonding environments will be affected quite differently by the large entropic network of states. Molecular dynamics calculations have been used extensively to calculate free energy changes caused by hydrogen bond rupture. Here the water environment is often ignored or treated in a global mean field approach. For practical purposes of judging the effect of hydrogen bonds in various protein processes and for drug design, it would be of great interest to have a general method of directly estimating the

change in hydrogen bond strengths upon changing the microscopic environment of proteins.

The concept of the hydrogen bond goes back to its discovery by Huggins in 1919 (9, 10). In the gas phase, the strength for a peptide environment has been computed to be ~4.9 kcal/mol (11–13), in close agreement with the general value of 5-8kcal/mol first suggested by Pauling in 1936 (14). Measurement of model compounds by Klotz (15) indicated that the N-H-O=C bond, even in a CCl<sub>4</sub> environment, has an appreciable value of 4.2 kcal/mol. Furthermore, Klotz interestingly showed that this high value is reduced in water to near 0.5 kcal/mol. Similarly, Williams (16) estimates a value of  $\approx 0.5-1.5$ kcal/mol. Experiments of Fersht (17) found values closer to 1.5 kcal/mol (7). This very large reduction in energy from the value for the isolated system in the gas phase mounts up because of the many hydrogen bonds, this reduction being attributed to the various hydrophobic effects of water, essentially involving contributions from the entropy, which almost completely negates the effect of enthalpy observed in the isolated molecule. Such a lowering of the free energy, of course, is required to facilitate the ready folding and unfolding observed for proteins. The gas phase value, if applicable, would make many biological processes quite irreversible; on the contrary, we know that the facile fluxional equilibrium between the folded and unfolded structures is essential to much biological activity.

It would be highly desirable, for many practical purposes, to be able to estimate not only the enthalpies, but also the much lowered values of the free energies in the water or other environment for the hydrogen bonded structures of interest in a given peptide. Quantitative values of such changes in strengths are required to explain many of the biological functions that such hydrogen bonds can undergo. The common procedure is to identify initial and final states and calculate the various thermodynamic contributions to the free energies in these initial and final states. Various extensive techniques have been applied to estimate these values (18). Sample calculations show that it is even difficult to identify a single representative structure for the calculation of the thermodynamic free energy. Although there may be one single final state for any given rupture, the next sample in the calculation will rupture the same bond with quite a different final structure. Thus, an entire ensemble with a wide variety of hydrogen bond angles is observed to be consistent with the rupture of a given hydrogen bond. Hence, any calculation of such thermodynamic free energies must be a proper ensemble average over final states, rather than a single value. Even then, these are free energies of annealed states, including many added contributions such as reorganization energies.

We here take a novel approach to this problem in that we assert that the relevant energetic problem for biological processes is not primarily related to the many thermodynamic equilibrium states of the system, but rather the quantity of interest is the free energy of direct rupture that must be found

<sup>±</sup>To whom correspondence may be addressed. E-mail: sysheu@ym.edu.tw, dyyang@ po.iams.sinica.edu.tw, or schlag@ch.tum.de.

BIOPHYSICS

Abbreviation: MD, molecular dynamics.

<sup>&</sup>lt;sup>+</sup>S.-Y.S., D.-Y.Y., and E.W.S. contributed equally to this work

<sup>© 2003</sup> by The National Academy of Sciences of the USA

to initiate the biological process in question, an activation energy. Such energies are here computed directly from an averaged time taken by the kinetic process along a single one-dimensional reaction coordinate; this is the time required for the distension of the hydrogen bond length to a critical value along a single reaction coordinate. We compute this distension in a molecular dynamics (MD) calculation for an ensemble of systems and thus obtain the time correlation function of the hydrogen bond rupture. Because this employs a complete MD calculation, it contains a proper weighted summation of all forces responsible for this process. It does, however, still share the limitation of all MD calculations in the sense that the potential surface is approximate. A quantum mechanical calculation is, however, not tractable for protein structures, not even approximately; hence, MD calculations remain the state of the art here for the present. It will be of interest, at least in this application, to see how well this MD-based method produces the correct energy changes provided by the aqueous medium.

We observe from the results of the MD calculation that this time scale, interestingly, undergoes an identifiable critical distension (Fig. 1); moreover, this with an observed periodicity. This periodicity is of considerable interest in protein dynamics and represents a time period of  $\approx 40$  ps for the change between free dynamics and structural fixation. This time provides an interesting time window for chemical activity. Because, for such a real ensemble, the passage time is a stochastic process, we expect it to wash out in time as observed. If we consider this as a mean critical motion to reach an activated complex, then the mean first passage time to reach this critical configuration relates to the properly averaged rate constant for this process as a result of all forces present, and no further breakdown into individual contributions affecting the hydrogen bond strength is required. Thus, we focus on time scales rather than thermodynamic energies and this for a single coordinate. We then compute these mean time scales at several temperatures, and from this we obtain energies of activation as the direct relevant quantity appropriate for the biological process: the strength of hydrogen bonds is directly observed as relevant to the experiment. The calculation thus represents the free energy of the activated state and directly measures the energy required for the biological process.

We describe a practical approach to directly assess the hydrogen bond strength and test this by going between two extreme prototypical environments, from the isolated gas phase to water. We do this not by thermodynamics, but by directly determining the mean first passage time for the ensemble of a onedimensional dynamic process in various environments. This direct technique for hydrogen bonds in proteins represents a novel approach for determining the energy of such bonds.

#### Calculations

The CHARMM package version 27 has been used in all of our MD simulations (19). The  $\alpha$ -helix with a 13-mer, i.e., Ace-SDELAKLLRLHAG-NH<sub>2</sub> with Ace = -COCH<sub>3</sub>, is minimized first in vacuum and is the starting configuration for the subsequent MD simulation. The solvated system is constructed after minimization of the initial protein structure by wrapping with water molecules. The  $\beta$ -hairpin 12-mer, i.e., Ace-V5PGV5-NH<sub>2</sub>, has been simulated in the same way. We pick up the N—O distance vs. time as plotted in Fig. 1b. In counting the first passage time, the N—O distance has to be started from point D in Fig. 1a and after passing through the equilibrium point D<sub>eq</sub>, the N—O distance returns to point D again. Here, point D<sub>eq</sub> has to be included to guarantee that this process traces out the activation energy.

The typical outcomes of the N—O distance vs. time figure contain a large-scale protein backbone undulation of  $\approx 0.5$  ns. On top of that large-scale undulation curve, we observe a smaller



Fig. 1. (a) Scheme of hydrogen bond rupture process. In our MD simulation, we calculate the N-O distance change starting from its dissociated position D, which is defined for the upper limit of the hydrogen bond length. A stochastic motion follows the association process, and the N-O distance reaches its minimum point,  $\mathsf{D}_{\mathsf{eq}}$ , and then returns to point D again. Here point D is defined near 3.5 Å. This guarantees that the whole activation process has been visited by the system. (b) A typical trajectory for hydrogen bond length vs. time. This  $\beta$ -sheet is dissolved in 485 water molecules. Here the 10NH—40 bond length variation with time is shown. Note that the notation mNH-nO means NH atoms from residue m and O atom from residue n. This figure shows a clear patterned structure for the rupture process and indicates a stable configuration of the  $\beta$ -sheet in water. The zero point energy differences from typical quantum mechanical computation are not expected to be significant here. Therefore, in our MD simulation the N-O distance variation has the same pattern as H—O distance variation. This is because we actually measure the backbone vibration.

scale modulation of sharp peaks. These sharp peaks represent the making and breaking of hydrogen bonds. This proceeds on an  $\approx$ 29-ps time scale. The apparent noise in the spectrum represents the high-frequency vibrations, and the hydrogen bond excursions are five times the level of that noise. In water, we observe  $\approx$ 0.5-ns amplitude modulations in addition to this "noise" We pick up those sharp peaks that have an amplitude nearly five times larger than this noise, which is typically >3.4 Å, to reach the position of point D.

#### **Kinetic Model**

For the purpose of modeling we choose a model system, and for our purposes we start with the  $\beta$ -sheet hairpin structure of  $\approx 12$ aa and  $\alpha$ -helix with 13 aa (see above). Here we had observed that the isolated  $\beta$ -sheet molecule calculation typically produces six hydrogen bonds (4). Alternatively, if the calculation of this structure is performed in the presence of 485 water molecules as a solvation environment, the typical number of hydrogen bonds is reduced to just a single bond, clearly demonstrating the weakening of hydrogen bonding caused by the added presence of the solvent. For another case, the  $\alpha$ -helix has 11 hydrogen bonds in the gas phase and eight in water. Here, the external hydrogen bonds between the side chains and water molecules are seen. When we simulate the  $\alpha$ -helix in gas phase, those side chains fold back to form internal hydrogen bonds, leading to an increase in the number of hydrogen bonds. These results indicate that among the functions of water, apparently, is the softening of these hydrogen bonds, as well as the realization of new hydrogen bonds. Nevertheless, structures are only indirect indicators.

In our approach, we chose to only calculate the mean first passage times. We extend the concept of the calculation of the mean first passage times to the hydrogen bond problem for the  $\beta$ -sheet hairpin and the  $\alpha$ -helix. Rather than being concerned with the thermodynamic free energies of breaking the hydrogen bond, we wish to determine the energy required to break such a bond kinetically, because this is the relevant energy one must find in the dynamics of the motion of the protein and the surrounding water environment. This would then be the activation energy for the bond rupture process. Such activation energies are free energies of activation and include all relevant entropy contributions, a correction that in typical gas phase two molecule interaction normally only involves a minor change of a few degrees of freedom. But here the ensemble contribution of the heat bath degrees of freedom is of major importance to the rate constants.

Hydrogen bond dissociation process is a unitary reaction. Its rate constant is expressed in terms of Yamamoto's correlation function expression (20–24). In other words, the phenomenological rate constant is correlated to the microscopic average of the fluctuation–dissipation theorem via

$$k(t) = \frac{\langle h_{\rm A}(x(0))\dot{h}_{\rm B}(x(t))\rangle}{\langle h_{\rm A}(x(0))\rangle} \approx k_{\rm A\to B} \exp(-t/t_{\rm rxn})$$
[1]

(see ref. 23). Here  $h_A$  and  $h_B$  are the characteristic functions of the states. k(t) is the time-dependent rate. After a relaxation time t, which is longer than the system characteristic relaxation time scale  $t_{\text{rxn}}$ , this time-dependent rate constant can be expressed in terms of the transition rate constant  $k_{A\rightarrow B}$  times the relaxation factor. x(t) represents the point in phase space.  $\langle \dots \rangle$  denotes the ensemble average of the system.

Our simulation is based on the ideal of Yamamoto's picture. Hence, the rate constant microscopically is an ensemble average of all of the degrees of freedom, and we focus on observing the hydrogen bond length. In other words, the other related degrees of freedom, such as van der Waals interactions, are properly averaged in. Certainly degrees of freedom other than hydrogen bond length interact with the hydrogen bond degrees of freedom, and therefore, the hydrogen bond length is a fluctuation quantity.

To distinguish whether the energy we calculated is a total energy difference, enthalpy, or free energy, we have to consider this in two ways. First, in the preceding paragraph, the rate constant is an ensemble average quantity. To identify the activation energy, which is enthalpy or free energy, we can go

## Table 1. Hydrogen bond energy for $\alpha$ -helix and $\beta$ -sheet in gas phase and water environment

	Vacuum		Water	
	A	Ea	A	Ea
α-Helix 3-Hairpin	$\begin{array}{c} 2.00 \times 10^{14} \\ 9.37 \times 10^{12} \end{array}$	5.57 4.79	$5.49  imes 10^{11}$ $3.53  imes 10^{11}$	1.93 1.58

 $E_{\rm a}$  is given in kcal/mol, and A is given in s<sup>-1</sup>.

back to the statistical mechanics definition. The Gibbs free energy is defined at constant pressure of the simulation system. Our simulation is done at constant pressure. Second, we extract the activation energy according to Kramers theory, and, hence, our activation energy is a Gibbs free energy rather than an enthalpy.

Our activation energy is not a summed energy from MD simulations in which the total energy is obtained as a sum of all types of energies. Such a total energy difference does not directly consider the dynamics of the system, but is rather just a summation of annealed energy and differs from this work.

We define the bond rupture process in terms of a onedimensional dynamic variation of the hydrogen bond to a critical rupture position, in the spirit of activated complex theory (25). We only define the one-dimensional motion, so that many angles were found to be consistent with such a rupture. Hence, we do not have a single activated complex, but rather we sample an ensemble. Fig. 1a exhibits the dissociation process of the hydrogen bond. Note that this is not a simple process of motion on a simple surface, but rather is the additive vector of many varying vibrational contributions. In our MD simulation, the beginning time t = 0 is defined at point D, and the hydrogen bond associates to its equilibrium distance (point  $D_{eq}$ ). Then this hydrogen bond dissociates and reaches point D again. Note that this repetition washes out with time because the excitation energy dissipates into the rest of the molecule. The initial peaks required for our method, however, are well defined. This graph interestingly also points out the difficulty of defining a proper final equilibrium state for the thermodynamic determination of the free energy in such a system. The passage time (see Fig. 1b) of interest here is twice the mean first passage of climbing the activation energy barrier. By using the Arrhenius formula,  $k = \tau_{mfp}^{-1} = A \exp\{-E_a/k_BT\}$ , we extract the activation energy  $E_a$  and an attempting frequency(or prefactor A). Here,  $\tau_{mfp}$  is the mean first passage time that is measured in our MD simulation. It should be noted that  $E_{\rm a}$  is an ensemble average of the rupture energy instead of a diatomic dissociation energy.

Typically hydrogen bonds in peptides are  $\approx 2.8-3$  Å in length between N and O atoms (26), so that an extension of the bond to  $\approx 3$  or 3.5 Å should produce a critical configuration on top of the barrier and lead to rupture. We now consider the entire ensemble of hydrogen motions in the MD calculation and consider that the Boltzmann tail of the distribution, which exceeds the activation, or more correctly the critical energy, produces chemical reaction in the typical sense. Now we proceed in the MD simulation and calculate the time required for the passage of the point D. In Table 1, we see that such values can also be calculated for 3.5 Å. This then gives us a mean first passage time for the rupture of the hydrogen bond. Typically we find that, for the solvated hairpin system in water as discussed above, this takes  $\approx$  40 ps for an ensemble at 300 K. It is of interest to note that 40 ps then can be related to the elementary time scale for the fluxional equilibrium of hydrogen bond folded structures. This agrees with experimental results recently found by Fayer (27). If we repeat this calculation for a somewhat higher temperature of 350 K, we obtain a value of 27.4 ps, and thus from the Arrhenius formula we obtain an activation energy of 1.58



**Fig. 2.** (a) Snapshot of the  $\alpha$ -helix in the gas phase. Its structure is slightly bent after 1 ps, and the R side chain interacts with D side chain to form extra internal hydrogen bonds. Hence, there are  $\approx$ 11 hydrogen bonds. (b) Snapshot of a  $\alpha$ -helix in water. The R and D side chains bond to water with a similar secondary structure. The total number of hydrogen bonds in water is approximately eight.

kcal/mol. This is surprisingly near the expected result of 1.5 kcal/mol as found in the literature (17). We now repeat this calculation for the isolated molecule, i.e., the  $\beta$ -sheet hairpin with the six hydrogen bonds in the absence of water, where we obtain a energy of activation of ≈4.79 kcal/mol in close agreement with the correct energy for the hydrogen bond in the isolated system of 4.9 kcal/mol (11–13). The  $\alpha$ -helix also has a lower number of hydrogen bonds in water as compared with the gas phase. The hydrogen bond energies by the above methods are  $\approx$ 5.57 kcal/mol in gas phase and 1.93 kcal/mol in water. In comparison with the  $\beta$ -sheet in the same phase, the  $\alpha$ -helix always has a somewhat larger hydrogen bond energy. Thus, we find that these MD calculations not only display a softening of the hydrogen bonds in water, but also quantitatively reproduce the observed lowering of the energy of activation for a peptide hydrogen bond because of the water environment. This selfconsistent calculation points to the interesting conclusion that the entropy effect of the water environment is summarily included here, and thus provides a direct computational method to access a kinetic value of the energy, as directly required for most experiments. The accuracy of these results leads one to expect here to have a general new method to obtain a properly ensemble averaged value of such dynamic hydrogen bond strengths.

Although these results show that both  $\alpha$ -helix and  $\beta$ -sheet reduce their H-bond energies more than a factor of four in a water environment, the detailed effect of the rupture process shows a rather different behavior. The  $\alpha$ -helix keeps a similar secondary structure, with the reduced number of hydrogen bonds mainly linked to the water medium (see Fig. 2b). When the  $\alpha$ -helix is in gas phase, its side chains linked instead to neighbors, and after  $\approx 1$  ps, the structure folds back to form additional internal hydrogen bonds (see Fig. 2a). Although this differs



**Fig. 3.** (a) Snapshot of the  $\beta$ -sheet in the gas phase. Its structure is stable in vacuum and almost keeps its structure and number of hydrogen bonds during our simulation. (b). Snapshot of  $\beta$ -sheet in water. This  $\beta$ -sheet is dissolved in water, and its number of hydrogen bonds is reduced from six to one. The side chains of this  $\beta$ -sheet have strong hydrogen bonding with water molecules.

Hence, the peptide is quite flexible.

profoundly from the hydrogen bonds formed in water, it has a similar secondary structure. The  $\beta$ -sheet has a different behavior. Its secondary structure stays always similar to the gas phase (see Fig. 3*a*) with strong hydrogen bonds. However, the  $\beta$ -sheet reduces the number and strength of its hydrogen bonds (see Fig. 3*b*), rather than internally compensating them. This shows that the rupture process and solvation effect differ considerably depending on the protein secondary structure.

The above calculations are thus a direct computational recipe for calculating the properly averaged kinetic free energies for the rupture of hydrogen bonds in peptides in various structures and solvation environments. The very good agreement with the known data for the isolated molecule and the water medium gives encouragement to this view. This method appears to account for the dynamic free energy of the solvent environment avoiding the need for thermodynamic functions. In effect, we proceed to carry out a computer experiment averaged over many initial trajectories, which gives the desired dynamic free energy of breaking the hydrogen bond. The agreement with the accepted values for the free energy of hydrogen bonds in a peptide in water is indeed surprising, and can, no doubt, be further improved by using advanced MD force fields and quantum mechanics/molecular mechanics (QM/MM) corrections to these programs.

### Conclusion

In conclusion, here we provide a novel approach for determining the energies of hydrogen bonds. We do not observe classic thermodynamic energies as derived from partition functions, but rather we observe the time for excursions of trajectories as properly averaged values of a dynamic ensemble of dissociating hydrogen bonds. Classic thermodynamic energies relate to structural equilibrium states. Such final states have many possible final structures that must be identified in the calculation and ensemble averaged. They are typically in an annealed state and as such differ from the transition state calculated in the kinetic approach here.

In this work we suggest a new kinetic approach, as a proper ensemble average of hydrogen bond ruptures, to directly estimate the energy required to break the bond as a critical configuration, the transition state. The approach includes entropic effects and, in this sense, is a free energy in the rate constant, but for essentially different structures and calculated in a way that directly relates to the dynamic issue of interest. It is a practical approach that calculates the time for trajectories of hydrogen bond ruptures in a MD program. In this way, we directly observe the dynamics of hydrogen bonds in an  $\alpha$ -helix and in a  $\beta$ -sheet. We perform the calculations for the secondary structures in the isolated molecule and for the water environment. The results we obtain for our kinetic calculations for the isolated molecule are 4.79 kcal/mol (B-sheet) and 5.57 kcal/mol  $(\alpha$ -helix) and are very close to the well known gas phase experimental data and single molecule *ab initio* calculations. The corresponding value found in the water environment; a thermodynamic system, however, reduces this energy to  $\approx 1.58$  kcal/mol ( $\beta$ -sheet) and 1.93 kcal/mol ( $\alpha$ -helix). This is very close to the generally accepted strength of hydrogen bonds in a water

- 1. Mattos, C. (2002) Trends Biochem. Sci. 27, 203-208.
- 2. Doruker, P. & Bahar, I. (1997) Biophys. J. 72, 2445-2456.
- 3. Bizarri, A. R. & Cannistraro, S. (2002) J. Phys. Chem. B 106, 6617-6633.
- 4. Sheu, S.-Y., Yang, D.-Y., Selzle, H. L. & Schlag, E. W. (2002) J. Phys. Chem. A 106, 9390–9396.
- 5. Williams, D. H. & Bardsley, B. (1999) Perspect. Drug Discovery Des. 17, 43-59.
- 6. Kalra, P., Reff, T. V. & Jayaram, B. (2001) J. Med. Chem. 44, 4325-4338.
- 7. Davis, A. M. & Teague, S. J. (1999) Angew. Chem. Int. Ed. 38, 736-749.
- Hummer, G., Garde, S., Garcia, A. E. & Pratt, L. R. (2000) Chem. Phys. 258, 349–370.
- 9. Huggins, M. L. (1919) M.S. Dissertation (Univ. of California).
- 10. Huggins, M. L. (1936) J. Org. Chem. 1, 407-456.
- 11. Mitchell, J. B. O. & Price, S. L. (1990) J. Comp. Chem. 11, 1217-1233.
- Avbelj, F., Luo, P. & Baldwin, R. L. (2000) Proc. Natl. Acad. Sci. USA 97, 10786–10791.
- Ben-Tal, N., Sitkoff, D., Topol, I. A., Yang, A.-S., St. Burt, K. & Honig, B. (1997) J. Phys. Chem. B 101, 450–457.
- 14. Mirsky, A. E. & Pauling, L. (1936) Proc. Natl. Acad. Sci. USA 22, 439-447.
- 15. Klotz, I. M. (1993) Protein Sci. 2, 1992-1999.
- Williams, D. H., Searle, M. S., Mackay, J. P., Gerhard, U. & Maplestone, R. A. (1993) Proc. Natl. Acad. Sci. USA 90, 1172–1178.

environment. This very strong reduction in the strength of hydrogen bonding upon going to the aqueous medium is known from experiment, but is obtained from the calculation. This then is a self-consistent direct method of estimating practical hydrogen bond strengths for the  $\alpha$ -helix and  $\beta$ -sheet peptide in water and in the isolated molecule. This method considers a kinetic Ansatz as an averaged time to rupture an ensemble of hydrogen bonds in one-dimensional motion, thus over an ensemble of activated complexes and configurations of water molecules. From this mean first passage time, ensemble averaged, we obtain an activation energy as the relevant quantity of interest. The results of this direct procedure produce an astonishing agreement with experiment. We suggest this as a new recipe for computing properly averaged hydrogen bond energies in peptide environments, a question of some practical interest in understanding and predicting protein behavior.

We thank R. S. Berry and R. A. Marcus for critically reading the manuscript. We thank the Fonol der Chemischen Industrie for support. This work was supported by the Taiwan/Germany program at the National Service Center/Deutscher Akademischer Austauschdienst.

- Fersht, A. R., Shi, J.-P., Knill-Jones, J., Lowe, D. M., Wilkinson, A. J., Blow, D. M., Brick, P., Carter, P., Waye, M. M. Y. & Winter, G. (1985) *Nature* 314, 235–238.
- Kollman, P. A., Massova, I., Reyes, C., Kuhn, B., Huo, S., Chong, L., Lee, M., Lee, T., Duan, Y., Wang, W., et al. (2000) Acc. Chem. Res. 33, 889–897.
- Brooks, B. R., Bruccoleri, R. E., Olafson, B. D., States, D. J., Swaminathan, S. & Karplus, M. (1983) J. Comp. Chem. 4, 187–217.
- 20. Yamamoto, T. (1960) J. Chem. Phys. 33, 281-289.
- 21. Chandler, D. (1978) J. Chem. Phys. 68, 2959-2970.
- Dellago, C., Bolhuis, P. G., Csajka, F. S. & Chandler, D. (1998) J. Chem. Phys. 108, 1964–1977.
- Dellago, C., Bolhuis, P. G. & Chandler, D. (1998) J. Chem. Phys. 108, 9236–9245.
- 24. Bolhuis, P. G., Dellago, C. & Chandler, D. (1998) Faraday Dis. 110, 421-436.
- Glasstone, S., Laidler, K. J. & Eyring, H. (1941) The Theory of Rate Processes (McGraw-Hill, New York).
- 26. Karle, I. L. (1999) J. Mol. Struct. 474, 103-112.
- Gaffney, K. J., Piletic, I. R. & Fayer, M. D. (2002) J. Phys. Chem. A 106, 9428–9435.