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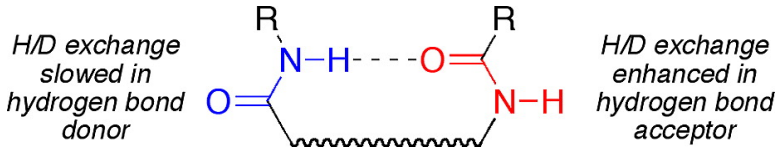
J. Am. Chem. Soc., **2007**, 129 (43), 12956-12957• DOI: 10.1021/ja076185s • Publication Date (Web): 04 October 2007



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Published on Web 10/04/2007

Deuterium Exchange as an Indicator of Hydrogen Bond Donors and Acceptors

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Hydrogen bonding is a fundamental feature in the structured folding of proteins and synthetic foldamers.[1] As interest grows in peptidomimetic design and peptide catalysts,[2] a heightened understanding of the hydrogen bonding properties of these molecules is essential. Herein we demonstrate that hydrogen/deuterium exchange is a valuable tool in determining the relative strengths of hydrogen bonds in relation to controls. Additionally, it provides a means to elucidate the separate roles of both hydrogen bond donors and hydrogen bond acceptors.

Hydrogen/deuterium (H/D) exchange is an analytical technique that has been used to correlate hydrogen bond strength with the rate of chemical exchange of the participating hydrogen to deuterium.[3] While this technique has been widely used to evaluate protein dynamics in water,[4] there has been minimal work involving small molecules in organic solvents.[5] In an effort to demonstrate how this method can be employed to measure intramolecular hydrogen bonding, H/D exchange was applied to a series of model compounds chosen to distinguish the participation of hydrogen bonding from other effects.

Englander has shown that steric and electronic environments affect H/D exchange rates.[6] As a result, any consideration of hydrogen bonding must be in the context of comparison with similar controls that are unlikely to form intramolecular hydrogen bonds. Amide derivatives **1**−**10** (R^1CONHR^2) vary in their amine and carboxyl substituents, and the half-lives of their H/D exchange in 10% $CD_3OD/CDCl_3$ are listed in Table 1.[7] Steric bulk of the carbonyl and nitrogen substituents affects the baseline rate of H/D exchange to differing degrees. A comparison of **1**−**4** shows that changes to the carbonyl substituent (R^1) from methyl through *tert*-butyl produced exchange rates that differed by 1000-fold. Derivatives that differ only in their nitrogen substituent (R^2) showed a lesser difference in their exchange rates. This can be seen by comparing amides **1**, **5**, and **6**, as well as carbamates **7**−**9**. Electronic effects have also been shown to influence H/D exchange rates. Derivatives **6**, **9**, and **10** were all formed from *tert*-butyl amine but contain differing carbonyl functional groups. The electron withdrawal of the trifluoromethyl group accelerated H/D exchange, while the electron donation of the carbamate slowed the rate of exchange. Both the electronic and steric results point to a dissociative mechanism where removal of the proton is rate-determining and inhibited by greater electron density or by increased steric bulk, most appreciably on the carbonyl substituent.[8]

With the establishment of baseline exchange rates for controls, hydrogen/deuterium exchange was measured for fundamental molecules capable of making only a select number of intramolecular hydrogen bonds and with minimal steric differences. Boc-Gly-NHBu **11** was chosen to provide two hydrogen bond donors and two hydrogen bond acceptors, with the possibility of forming hydrogen bonds through either a five-membered or a seven-membered ring. On first inspection, the H/D exchange data for **11** (Figure 1) showed a slower rate of exchange for the amide hydrogen (●) than the carbamate hydrogen (■), consistent with preferential formation of a seven-membered ring γ-turn hydrogen bond shown in Figure 1. More striking is the comparison with control molecules that contain similar functional groups: amide **2** (○) and carbamate **7** (□). The glycine amide shows a significant decrease in the rate of exchange in comparison with the control, consistent with this hydrogen serving as a hydrogen bond donor. Interestingly, the carbamate hydrogen shows a significant acceleration in the rate of H/D exchange. This is indicative of the carbamate serving as a hydrogen bond acceptor. Presumably, the presence of the hydrogen bond serves to accelerate the H/D exchange of the carbamate proton by stabilizing the increased electron density on the carbamate that occurs when the proton is removed.[9]

Table 1. Half-lives of H/D Exchange in 10% $CD_3OD/CDCl_3$ for R^1CONHR^2 Derivatives (**1**−**10**)

	R^1	R^2	$t_{1/2}$ (min)		R^1	R^2	$t_{1/2}$ (min)
1	Me	*n*-Bu	14	**6**	Me	*t*-Bu	110
2	Et	*n*-Bu	20	**7**	tBuO	*n*-Bu	1200
3	*i*-Pr	*n*-Bu	42	**8**	tBuO	*i*-Pr	1220
4	*t*-Bu	*n*-Bu	14500	**9**	tBuO	*t*-Bu	1450
5	Me	*i*-Pr	14	**10**	CF_3	*t*-Bu	24

Two additional N-methylated glycine derivatives (**12**, **13**) were created to further probe the role of hydrogen bonding and gauge possible electronic and field effects. The H/D exchange kinetics of these derivatives are also shown in Figure 1, and the corresponding rate data are listed in Table 2. Boc-N-Me-Gly-NHBu **12** (×) exhibited a rate of exchange that was similar to the non-methylated derivative, suggesting comparable hydrogen bonding in both

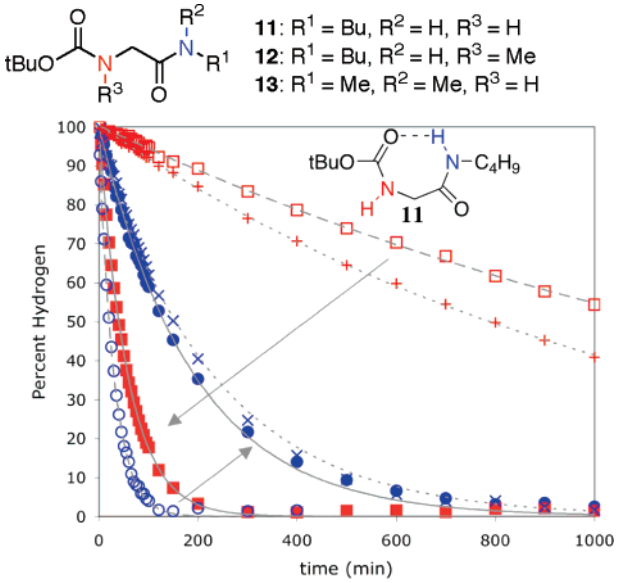


Figure 1. H/D exchange kinetics comparing glycine derivatives **11** (Boc-*NH* = ■, *NH*Bu = ●), **12** (×) and **13** (+) with controls **2** (○), **7** (□). Arrows indicate the differences in rates between non-hydrogen-bonding controls and similar hydrogen-bonding groups.

10.1021/ja076185s CCC: $37.00

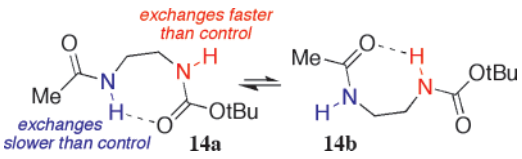
Table 2. Half-lives of H/D Exchange in 10% $CD_3OD/CDCl_3$ for Peptides (**11**−**15**) Capable of Making Intramolecular Hydrogen Bonds

	exchanging NH	$t_{1/2}$ (min)
11	Boc*NH*CH_2CONHBu	40
11	BocNHCH_2CO*NH*Bu	132
12	BocNMeCH_2CO*NH*Bu	150
13	Boc*NH*CH_2CONMe_2	800
14	Boc*NH*CH_2CH_2NHAc	570
14	BocNHCH_2CH_2*NH*Ac	33
15	Boc*NH*CH_2CONHCH_2CONHBu	63
15	BocNHCH_2CO*NH*CH_2CONHBu	13
15	BocNHCH_2CONHCH_2CO*NH*Bu	180

derivatives. The exchange kinetics of Boc-Gly-NMe_2 **13** (+) showed a rate of exchange that was similar to that of the carbamate control, suggesting that when the γ-turn was unavailable there was no significant hydrogen bonding present in this derivative.

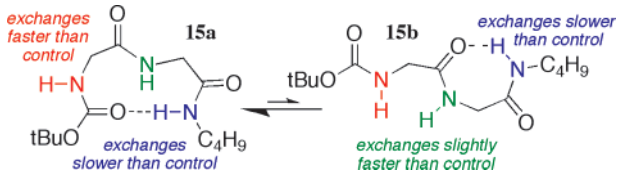
A comparison with other techniques indicates the sensitivity of H/D exchange in illustrating these somewhat weak hydrogen bonding interactions.[7] Both N−H protons of **11** show significant changes in NMR chemical shift (≥2 ppm) upon the addition of a hydrogen bonding solvent,[10] making it difficult to accurately describe the presence of hydrogen bonding. Analysis of the infrared spectra of **11**−**13** does indicate the presence of a hydrogen-bonded amide for **11** and **12**, but even **12** exhibits predominantly a non-hydrogen-bonded amide stretch.[11]

The selectivity of hydrogen bond formation is not purely dependent on preferred ring size; it is also a matter of the functional groups involved. Molecule **14** was designed to permit two possible seven-membered ring hydrogen bonds **14a** and **14b**, each involving an amide and a carbamate functional group. The H/D kinetics (Table 2) exhibited the carbamate exchanging more quickly than control **7**, while the amide exchanged more slowly than control **2**.[7] These results suggest a preference for the amide to be the hydrogen bond donor and the carbamate the hydrogen bond acceptor. In contrast to the glycine derivatives above, these H/D exchange rates differed only slightly from controls. This could indicate a weaker hydrogen bond, but since this molecule can adopt either conformation, the exchange kinetics likely represent the average of the dynamic equilibrium between **14a** and **14b**. This equilibrium would appear to favor conformation **14a**, presumably due the difference in the innate hydrogen bonding ability of the individual functional groups.



Dipeptide Boc-Gly-Gly-NHBu **15** possesses an additional hydrogen bond donor and acceptor, and hydrogen/deuterium exchange is helpful in illuminating the preferred conformations.[12] The kinetic profile[7] showed that, once again, the butyl amide (●) exchanged more slowly than control **2** (○) but also slightly more slowly than the butyl amide in **11** (see Table 2). The carbamate of **15** (■) exchanges at a rate that is much faster than control **7** (□) but not as fast as the carbamate in **11**. The central amide exchanges at a rate slightly faster than control **2**. As a whole, these three rates are most consistent with a β-turn conformation **15a**, in equilibrium with at least one other conformation. The slight acceleration in the exchange of the central amide suggests that it serves to some extent as a hydrogen bond acceptor. The observation that the carbamate in **15** is not accelerated to the same extent as **11** may indicate that its role as a hydrogen bond acceptor is diminished by comparison. Both of these effects point to the presence of conformation **15b**.

These simple molecules demonstrate the usefulness of hydrogen/deuterium exchange in the assessment of hydrogen bonding. Relative rates of H/D exchange can be correlated with the presence of hydrogen bonds, given comparison to controls that account for inherent steric and electronic effects. While a number of existing techniques indicate the role of hydrogen bond donors, this is one of only a few techniques that directly illuminates the participation of individual hydrogen bond acceptors.[13] This approach is currently being employed in ongoing investigations of larger hydrogen bonding molecules.



Acknowledgment. This work was supported by an award from Research Corporation. The authors are grateful to the NSF for financial support (MRI-0116416).

Supporting Information Available: Synthetic details, NMR spectra, and kinetic data for H/D exchange. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA076185S