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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

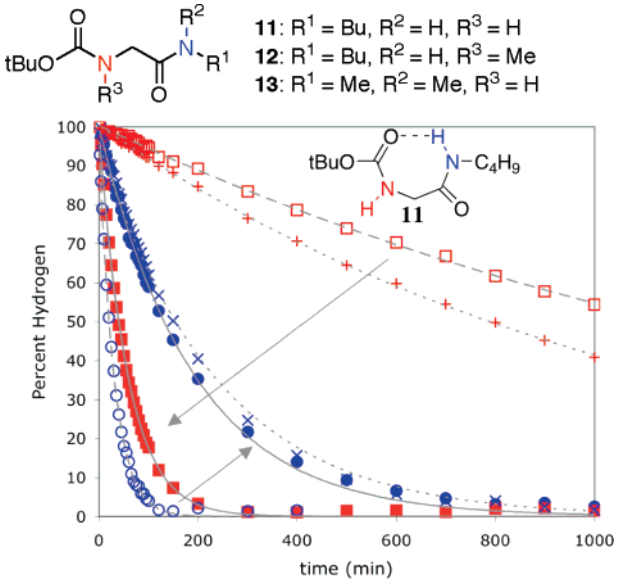

Figure 1. H/D exchange kinetics comparing glycine derivatives **11** (Boc-*NH* = ■, *NH*Bu = ●), **12** (×) and **13** (+) with controls **2** (○), **7** (□). Arrows indicate the differences in rates between non-hydrogen-bonding controls and similar hydrogen-bonding groups.

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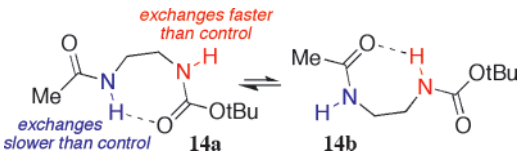
Table 2. Half-lives of H/D Exchange in 10% $CD_3OD/CDCl_3$ for Peptides (**11**−**15**) Capable of Making Intramolecular Hydrogen Bonds

	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.

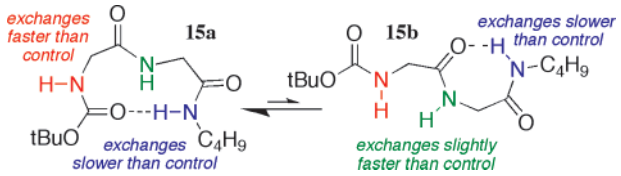
A comparison with other techniques indicates the sensitivity of H/D exchange in illustrating these somewhat weak hydrogen bonding interactions.[7] Both N−H protons of **11** show significant changes in NMR chemical shift (≥2 ppm) upon the addition of a hydrogen bonding solvent,[10] making it difficult 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.



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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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