# p$K_a$ Shift Effects on Backbone Amide Base-Catalyzed Hydrogen Exchange Rates in Peptides

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**Abstract:** Amide proton exchange (HX) rates are known to depend on protein primary structure as well as local and global protein structure and dynamics. Measurement of HX rates gives information on the local exposure of amide protons to solvent and on local rates of structural openings. It has long been recognized that the amide p$K_a$ directly influences the HX rate. Using the finite-difference solution of the Poisson−Boltzmann equation, we investigated the electrostatic effects on HX rates, via calculated shifts in the amide p$K_a$ for model compounds (*N*-methylacetamide, dipeptides entailing almost all amino acid side chains, and a tripeptide). Rather than shifts in the same compound with varying environmental conditions, we address shifts in the HX rates of different compounds relative to each other. The results for selected model compounds which resemble Ala and Gly residues, with a standard choice of parameters, agree to a high degree of accuracy with experimentally determined rates. Application of the same methodology to naturally occurring amino acids is promising but requires refinement to take into account flexibility and inductive effects.

Amide proton exchange (HX) rates are known to depend on protein primary structure as well as local and global protein structure and dynamics. Measurement of HX rates gives information on the local exposure of amide protons to solvent and on local rates of structural openings. A satisfactory theoretical description of the effect of molecular structure on HX rates, which would be highly desirable to shed light on partially unfolded conformations, is still missing, although electrostatic and inductive effects have been repeatedly pointed out. It has long been recognized that the amide p$K_a$ directly influences the HX rate.[1] Since the first recognition of electrostatic effects on HX rates, refined tools to treat electrostatics for molecules in solution have been developed.[2] In particular, calculations based on the Poisson−Boltzmann equation have provided a reasonably accurate quantitative explanation for the observed p$K_a$ shifts of ionizable groups in proteins.[3] Using the finite-difference solution of the Poisson−Boltzmann equation, we investigated the electrostatic effects on the HX rates, via calculated shifts in the amide p$K_a$ for model compounds resembling Ala and Gly residues (*N*-methylacetamide, dipeptides entailing almost all amino acid side chains, and a tripeptide). It is shown that this methodology is accurate for small model compounds. All compounds studied have blocked termini so the only net charges in the molecules come from the ionized amide backbone (imidate anion) or side chain.

The basic model which relates the experimental observations to protein structural physics was proposed 40 years ago by Linderstrøm-Lang and co-workers and has been critically reviewed.[1b,c]

To understand the details of the HX process, small model compounds have been thoroughly studied. Analysis of the experimental data allowed the identification of different effects on HX rates[4] and, later, the delineation of different catalytic mechanisms.[5] In particular, for base catalysis, deprotonation of the amide by hydroxide anion appears to be the first step of the reaction, followed by the fast replacement of the proton by water. For the acid-catalyzed exchange, protonation of the carbonyl group, followed by imidization of the amide and subsequent replacement of the hydrogen from water, appears to be the dominant process in peptides and proteins.[5,6]

Very little is known about the "open" states of proteins[1c] or about the influence that the residual side chain conformation or secondary and tertiary structure exerts on the "intrinsic" HX rates[1c] when compared to small model compounds. The subject is somewhat obscure because, according to the widely accepted picture that the exchange process requires local unfolding or at least some structural opening, it is not possible to firmly establish to what extent rigid structural elements are present when hydrogen exchange takes place and how different such residual structures are from the calibrant ones.

We investigated structural effects due to electrostatics on base-catalyzed HX rates by computing the electrostatic shifts

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(1) (a) Eigen, M. *Angew. Chem., Intl. Ed. Engl.* **1964**, *3*, 1−19. (b) Hvidt, A.; Nielsen, S. O. *Adv. Protein Chem.* **1966**, *21*, 287−386. (c) Englander, S. W.; Kallenbach, N. R. *Q. Rev. Biophys.* **1984**, *6*, 521−655.

(2) (a) Warwicker, J.; Watson, H. C. *J. Mol. Biol.* **1982**, *157*, 671−679. (b) Gilson, M. K.; Sharp, K. A.; Honig, B. *J. Comput. Chem.* **1987,** *9*, 327−335. (c) Sharp, K. A.; Honig, B. *J. Phys. Chem.* **1990**, *94*, 7684−7692. (d) Davis, M. E.; McCammon J. A. *Chem. Rev.* **1990**, *90*, 509−521. (e) Honig, B.; Nicholls A. *Science* **1995**, *268*, 1144−1149.

(3) Antosiewicz, J.; McCammon J. A.; Gilson M. K. *J. Mol. Biol.* **1994**, *218*, 415−436.

(4) (a) Sheinblatt, M, *J. Am. Chem. Soc.* **1970**, *92*, 2505−2509. (b) Klotz, I. M.; Frank, B. H. *J. Am. Chem. Soc.* **1965**, *87*, 2721−2728. (c) Molday, R., S.; Englander, S. W.; Kallen, R. G. *Biochemistry* **1972**, *11*, 150−158.

(5) Perrin, C. L. *Acc. Chem. Res.* **1989**, *22*, 268−275.

(6) (a) Tüchsen, E.; Woodward, C. *J. Mol. Biol.* **1985**, *185*, 405−419. (b) Tüchsen, E.; Woodward, C. *J. Mol. Biol.* **1985**, *185*, 421−430.

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in the p$K_a$ values of amide protons using a method developed by Antosiewicz et al.[3] The acid-catalyzed exchange appears somewhat more difficult to model in this framework and so will not be considered here. The underlying concept for the calculations is the following: the base-catalyzed exchange rate is the product of a diffusion-limited encounter rate and the equilibrium fraction of collisions that are likely to be successful due to the difference in the p$K_a$ between the amide and the exchange catalyst. Compared to the work of Antosiewicz et al.[3] we can neglect multiply ionized states, since we expect backbone amide ionization is a rare and short-lived event. However, the ionization state of the side chain can have a profound effect on that of the backbone, so the neutral and ionized forms of all ionizable amino acid side chains (except Ser, Thr, and Cys) were considered.

While previous work involving electrostatics focused on variations in the HX rate for a given compound due to pH or salt effects,[1b,6b,7] the present calculations address absolute deviations of HX rates in different compounds (and different conformers of these compounds) with respect to a model one, due to electrostatics. The present work is an example of how Poisson−Boltzmann free energy calculations may be applied to the determination of HX rates.

## Methods

Here we briefly summarize the theory of HX exchange following the work of Eigen,[1a] Hvidt and Nielsen,[1b] and Englander and Kallenbach.[1c]

The process of hydrogen exchange may be schematized as follows:

$$\mathrm{N} \underset{k_{cl}}{\overset{k_{op}}{\rightleftarrows}} \mathrm{I} \xrightarrow{k_{ex}} \text{exchange}$$

where N indicates the backbone amide proton in the native state of the protein, while I indicates the intermediate state which, through partial unfolding, exposes the same proton to the solvent. The kinetic constants $k_{op}$, $k_{cl}$, and $k_{ex}$ refer to the opening, closing, and exchange processes, respectively.

The rate at which a proton in the intermediate state I is exchanged with the solvent may be expressed in turn as a sum of contributions from different catalysts (namely, $OH^-$, $H_3O^+$, and $H_2O$):

$$k_{ex} = \sum_i k_{ch}^i [\mathrm{Cat}]_i$$

where $k_{ch}^i$ is the chemical exchange rate for the catalyst, $i$, with concentration $[\mathrm{Cat}]_i$.

Under nondenaturing conditions ($k_{op} \ll k_{cl}$) the exchange of a backbone amide hydrogen with solvent follows a single-exponential behavior with the apparent rate constant given by

$$k_{app} = \frac{k_{op} \sum_i k_{ch}^i [\mathrm{Cat}]_i}{k_{cl} + \sum_i k_{ch}^i [\mathrm{Cat}]_i}$$

Two limiting cases may be envisaged: (1) When the catalyst is in large excess ($\sum_i k_{ch}^i [\mathrm{Cat}]_i \gg k_{cl}$) the exchange is a first-order process and the rate constant is given by $k_{app} = k_{op}$. (2) When the closing of the locally opened structure is much faster than the catalyzed exchange process ($k_{cl} \gg \sum_i k_{ch}^i [\mathrm{Cat}]_i$) the exchange is a second-order process and the rate constant is given by $k_{app} = K_{op} \sum_i k_{ch}^i [\mathrm{Cat}]_i$ where $K_{op}$ is the thermodynamic equilibrium constant for the opening process (i.e., $K_{op} = k_{op}/k_{cl}$).

The chemical exchange rate is itself dependent on several factors such as steric accessibility of the site to the catalysts, possible influence of local fields on the rate of diffusion, and the probability that an encounter will be productive. In what follows, we consider only the base-catalyzed process, and replace $k_{ch}^i$ for that process by $k_{ch}$. Since the compounds are very similar to each other, we use a single diffusion constant, $k_D$, for all compounds and differences in steric environment and local fields are not treated, although these might be important, as discussed later in this paper. It therefore proves useful to schematize the process of chemical exchange as follows:

$$\mathrm{solv}(\mathbf{H}) + \mathrm{NH} + \mathrm{Cat} \underset{k_{-D}}{\overset{k_D}{\rightleftarrows}} \mathrm{solv}(\mathbf{H}) + (\mathrm{NH}\cdots\mathrm{Cat} \leftrightarrow \mathrm{N}^-\cdots\mathrm{CatH}) \rightarrow \mathrm{N}\mathbf{H} + \mathrm{Cat} + \mathrm{solv(H)}$$

We assume that in the last step, protonation of the imidate anion by the solvent is very fast. Since within the hydrogen-bonded complex the rearrangement of the proton occurs rapidly, we may consider the two forms within brackets to be in equilibrium with a constant $K_C = 10^{\Delta pK_a}$ determined by their relative p$K_a$ values, where $\Delta pK_a = pK_a^{Cat} - pK_a^N$ is just the difference between the catalyst and amide p$K_a$ values. From the above considerations, $k_{ch}$ may be expressed as $k_{ch} = k_D(K_c/(K_c + 1))$, i.e., the product of the diffusion-limited encounter rate $k_D$ and the fraction of forward reacting encounters $K_c/(K_c + 1)$. It has been shown that in regions of pH well above that resulting in the minimum rate, the catalyst is mainly the hydroxide ion and $K_c/(K_c + 1)$ may be approximated by $K_c$.[1c] According to the equations written above, the electrostatic effect on the intrinsic exchange rates due to the protein structure is contained in the factor $K_c$ and may be calculated by means of Poisson−Boltzmann methods as previously reported by Antosiewicz et al.[3]

For a given native conformation N of the protein, the amide p$K_a$ is shifted with respect to a model conformation M according to the equation

$$pK_a^N = pK_a^M + \Delta(\Delta G^{el})/2.303kT$$

where $\Delta(\Delta G^{el}) = \Delta G^{el,N} - \Delta G^{el,M}$ is the difference between the free energy cost of ionization in native and model conformations. Therefore

$$\Delta \log k_{ch} = \log k_{ch}^N - \log k_{ch}^M = \Delta pK_a^N - \Delta pK_a^M = pK_a^M - pK_a^N = -\Delta(\Delta G^{el})/2.303kT$$

All the simulations were performed using the UHBD program.[8] All of the models for the investigated compounds were built with InsightII v. 95.0 (MSI, San Diego, CA). The electrostatic calculations employed a grid of 65 × 65 × 65 points with a grid mesh of 0.6 Å. One focusing step was performed, to reach a final grid mesh of 0.3 Å. At this resolution grid effects should be negligible.[9] The Poisson−Boltzmann equation was solved numerically using a finite-difference algorithm as implemented in UHBD.[8] Standard methods to reduce the problems associated with charge and dielectric discretization were employed.[2b,10] After the finite-difference Poisson−Boltzmann equation was solved numerically, free energies were calculated with the use of discretized analogues of the equations reported in Sharp and Honig.[2c] The linearized and the full Poisson−Boltzmann equations gave, for the present compounds and range of ionic strength, virtually identical results so the linearized version was used. The set of charges used for the simulations was obtained from the consistent valence force field (cvff) as supplied in the software package Discover v. 95.0 (MSI, San Diego, CA), since it has been optimized for small molecules. Molecular surfaces were determined by van der Waals exclusion radii (i.e., VDW surface), although similar results were obtained using a probe sphere

(7) (a) Kim, P. S.; Baldwin, R. L. *Biochemistry* **1982**, *21*, 1−5. (b) Matthew, J. B.; Richards, F. M. *J. Biol. Chem.* **1983**, *258*, 3039−3044. (c) Christoffersen, M.; Bolvig, S.; Tüchsen E. *Biochemistry* **1996**, *35*, 2309−2315.

(8) Madura, J. D.; Briggs, J. M.; Wade, R. C.; Davis, M. E.; Luty, B. A.; Ilin, A.; Antosiewicz, J.; Gilson, M. K.; Bagheri, B.; Scott, L. R.; McCammon J. A. *Comput. Phys. Commun.* **1995**, *91*, 57−95.

(9) Mohan, V.; Davis, M. E.; McCammon J. A.; Pettitt, B. M. *J. Phys. Chem.* **1992**, *96*, 6428−6431.

(10) (a) Davis, M. E.; McCammon J. A. *J. Comput. Chem.* **1991**, *12*, 909−912. (b) Luty, B. A.; Davis, M. E.; McCammon J. A. *J. Comput. Chem.* **1992**, *13*, 1114−1118.

of radius 1.4 Å to generate the molecular surface. Test calculations were done for internal dielectric constants of 2.0, 4.0, and 20.0 on the extended conformation. The electrostatic free energy differences scaled approximately linearly with the inverse of the dielectric constant in the region of low dielectric constant. The best agreement, for a set of small model compounds, was achieved by setting the internal dielectric constant to 4.0.

We selected a set of small compounds which contain glycine and alanine residues (experimentally investigated by Molday et al.,[4c] Chart 1) to test the feasibility of our p$K_a$ calculations. These compounds were studied in fully extended, in $\beta$-sheet, and in $\alpha$-helix conformations. In all calculations, the relevant amide group was fixed at the same position on the grid to exclude, by subtraction, unwanted self-energy contributions. The reference $\Delta G^{el,M}$ was that of *N*-methylacetamide (NMA) and was obtained using the linearized Poisson−Boltzmann equation as $\Delta G^{el,M} = \sum_i (1/2) q_i U_i$, where $q_i$ and $U_i$ are the charge and electrostatic potential at the $i$th charged site.

To model the intrinsic flexibility of all the compounds studied, a 70% $\beta$-sheet and 30% $\alpha$-helix conformation was assumed, according to the distribution obtained from long MD simulations on the alanine dipeptide.[11] The theoretical value for $\Delta \log k_{ch}$ (with respect to NMA $\log k_{ch}$, which has the experimental value of $2.51 \times 10^8$ $M^{-1}$ $min^{-1}$ [4c]) was then estimated as the logarithm of a weighted average of $k_{ch}$ over the possible conformers:

$$\Delta \log k_{ch} = \log\left(\frac{\sum_i w_i \times 10^{\Delta \log k^i_{ch}}}{\sum_i w_i}\right)$$

where $w_i$ represents the relative frequency of occurrence of the $i$th conformer.

Ionization of the amide group was simulated by adding a −1 charge to the nitrogen. Complete or partial negative charge displacement onto the peptide oxygen resulted in much poorer agreement with experimental data (Table 1).

The same methodology was applied to the amino acids (Chart 2). The well-optimized set of CHARMM all-atom charges for proteins, as obtained from CHARMM software package v. 24b1,[12] was used in the calculations of $\Delta G^{el}$ for the amino acids. The ionic strength was 0.5 M to match experimental conditions.

To compare the results of our calculations with the set of available experimental data for model amino acid compounds,[13] we assumed that the many possible conformers for the side chains followed the statistical distribution for the side chain rotamers found for a set of unrelated proteins, as reported by Abagyan and Totrov.[14] We calculated $\Delta(\Delta G^{el}) = \Delta G^{el,N} - \Delta G^{el,M}$ for all of the rotamers (for $\chi_1$ and $\chi_2$) which had a frequency of occurrence greater than or equal to 10%. The remaining torsional angles were left at the value corresponding to extended conformations found in the residue library supplied by the InsightII v. 95.0 software package (MSI, San Diego, CA).

## Results and Discussion

The results are sensitive to the atomic details of the model, although a different set of all-atom charge parameters gave similar results. A test on the extended conformation of the compounds listed in Chart 1 with the united-atom OPLS parameters for peptides[15] gave poor results. This is probably due to the fact that there are no partial charges on $CH_n$ groups of the side chains and so the local electrostatic environment is not correctly described. However, when reasonable guesses for all-atom charges were used, the results were comparable to the cvff ones. For the amino acids listed in Chart 2 there is a very limited variation in the computed HX rates when the all-atom cvff set of partial charges were used instead of those from CHARMM.

For the set of model compounds (Chart 1), the remarkable agreement between calculated and experimental data is apparent (Figure 1). A linear fit to the data, setting the origin of the plot at the NMA point, gives a slope of 1.08 and an intercept of 0.09. The correlation of the same data is 0.98, and the root-mean-square deviation of the calculated versus experimental $\Delta$

**Chart 1.** Model Compounds Studied[a]

1) CH3-CO-NH-CH3 (reference compound)

*

2) CH3-CO-NH-CH-CO-NH-CH3

| *

CH3

3) CH3-CO-NH-CH2-CO-NH-CH3

* *

4) CH3-CO-NH-CH-CO-NH-CH2-CO-NH-CH3

| * *

CH3

[a] Asterisks indicate the amide for which experimental data were available.[4c]

**Chart 2.** Compounds Used to Test the p$K_a$ Shift Effect on HX Rates[a]

L R

CH3-CO-NH-CH-CO-NH-CH3

* | *

SC

SC = ALA, ASP, ASP-, CYS, GLU, GLU-, PHE, GLY, HIS, HIS+, ILE, LYS+, LEU, MET, ASN, GLN, ARG+, SER, THR, VAL, TRP, TYR

[a] SC is the side chain of the residues mentioned below. R and L designate the right and left amides, respectively, according to the nomenclature of Molday et al.[4c]

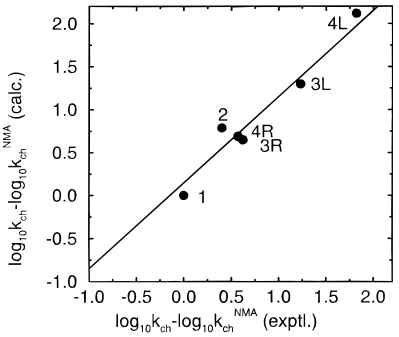


**Figure 1.** Calculated versus experimental[4c] $\log k_{ch} - \log k_{ch}^{NMA}$ for the set of test compounds listed in Chart 1. The letters L and R indicate left and right amide hydrogen according to the nomenclature of Molday et al.[4c] The best fit line with a slope of 1.0 is shown.

(11) Smart, J. L.; Marrone, T. J.; McCammon, J. A. *J. Comput. Chem.*, in press.

(12) MacKerell Jr., A. D.; Bashford, D.; Bellott, M.; Dunbrack Jr., R. L.; Field, M. J.; Fischer, S.; Gao, J.; Guo, H.; Ha, S.; Joseph, D.; Kuchnir, L.; Kuczera, K.; Lau, F. T. K.; Mattos, C.; Michnick, S.; Ngo, T.; Nguyen, D. T.; Prodhom, B.; Roux, B.; Schlenkrich, M.; Smith., J. C.; Stote, R.; Straub, J.; Wiorkiewicz-Kuczera, J.; Karplus, M. *FASEB J.* **1992**, *6*, A143.

(13) Bai, Y.; Mine, J. S.; Mayne, L.; Englander, S. W. *Proteins* **1993**, *17*, 75−86.

(14) Abagyan, R.; Totrov, M. *J. Mol. Biol.* **1994**, *235*, 983−1002.

(15) Jorgensen, W. L.; Swenson, C. J. *J. Am. Chem. Soc.* **1985**, *107*, 569−576.

**Table 1.** Calculated Shifts in log $k_{ch}$ for the Model Compounds of Chart 1, in Extended, $\beta$-Sheet, and α-Helix Geometries[a]

| | | extended | | | $\beta$-sheet | | | α-helix | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | exptl | I | II | III | I | II | III | I | II | III |
| **1** | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| **2** | 0.40 | 0.81 | 0.78 | 0.61 | 0.93 | 0.78 | 0.58 | −0.20 | 0.30 | 0.66 |
| **3L** | 1.23 | 1.17 | 0.79 | 0.30 | 1.35 | 0.88 | 0.29 | 1.13 | 0.54 | −0.24 |
| **3R** | 0.62 | 0.98 | 0.78 | 0.49 | 0.79 | 0.73 | 0.66 | −0.17 | 0.27 | 0.60 |
| **4L** | 1.82 | 1.99 | 1.57 | 0.88 | 2.27 | 1.64 | 0.85 | 0.69 | 0.69 | 0.32 |
| **4R** | 0.57 | 1.02 | 0.96 | 0.56 | 0.84 | 0.76 | 0.66 | −0.47 | 0.14 | 0.59 |

[a] The experimental values are taken from Molday et al.[4c] In column I deprotonation was modeled via a negative charge placed on the amide nitrogen, in column II the negative charge was equally distributed between the peptide nitrogen and oxygen, and in column III the negative charge was placed on the peptide oxygen. $k_{ch}$ for NMA (the reference compound) under basic conditions is $2.51 \times 10^8$ $M^{-1}$ $min^{-1}$.[4c]

log $k_{ch}$ is 0.16, when data are fitted with a straight line of slope equal to 1.0.

The positive shifts in HX rates for all of the compounds may be attributed to the combined effect of solute charge−charge and solute−solvent interactions. The direct Coulombic energies of ionization for the amides listed in Chart 1, in a uniform dielectric medium ($\epsilon = 4.0$), and for the different backbone conformers, correlate well with the free energy cost of ionization computed via the Poisson−Boltzmann equation. In the $\beta$-sheet conformation of compounds **2**, **3**, and **4** (Chart 1), the proximity of the preceding positively charged $C_\alpha$ group, which is neutral in the reference NMA, and the positive charge at the neighboring $C_\alpha$, which is negatively charged in NMA, helps in accommodating the negative charge on the amide in the ionized state, leading to an increase in the HX rate. As a result of both higher population of the $\beta$-sheet conformer and its higher HX rates, the average HX rates are close to the $\beta$-sheet ones.

In the more compact α-helical conformation the direct Coulombic energy opposes ionization for right amides, contrary to the case of left amides. For left amides however poorer solvation hampers the $\Delta$ log $k_{ch}$ due to direct Coulombic energy by at least a factor of 2.

The results reported in Table 1 suggest that any residual α-helical geometry at the exchanging amide hydrogen should result, by purely electrostatic effects, in a decrease in HX rates by a factor of up to an order of magnitude.

For the set of amino acids, predictions, as judged from the overall deviation between experimental and calculated data, are still unsatisfactory, although a positive correlation (correlation coefficient 0.67, linear regression slope 0.77, and intercept 0.07) was found between calculated and experimental shifts.

However, a closer inspection of the results shows that predictions for the "right" amide HX rate reproduce the experimental data within the same accuracy as for the model compounds (Figure 2). The root-mean-square deviation (RMSD) between calculated and experimental data, when the data were fitted with a line with a slope equal to 1.0, is 0.17, much lower than the average RMSD of the same data (0.32).

A similar analysis on the "left" amide HX rates gives a RMSD between calculated and experimental data of 0.38 comparable to the dispersion of the experimental data (0.44). The theory clearly breaks down for the left amide though the experimental trends are reproduced, with correlation coefficients on single classes of residues comparable to those found for the right amide (0.93 vs 0.46 for hydrophobic residues, 0.93 vs 0.94 for charged residues, 0.65 vs 0.46 for polar residues, and 0.32 vs 0.66 for aromatic residues). For aromatic residues, the observed reduction in HX rates matches the expected steric blocking effect of aromatic rings,[13] which was not taken into account in our models and therefore leads to a systematic error.

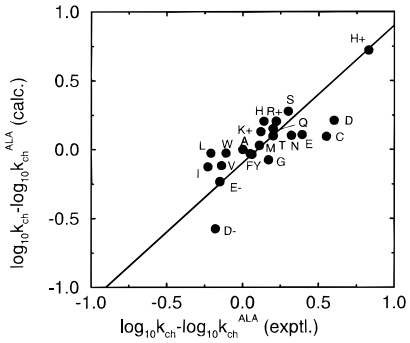


**Figure 2.** Calculated versus experimental[12] log $k_{ch}$ − log $k_{ch}^{ALA}$ for the right amide for the set of compounds listed in Chart 2. Amino acids are indicated with a single letter code. The best fit line with a slope of 1.0 is shown.

The results obtained for the left amide suggest that the deviations from the observed values may be attributed to neglect of both inductive and steric effects, which are indeed expected to affect the left amide more since it is closer to the side chain than the right amide. While steric effects are difficult to treat with static models, inductive effects might be taken into account by properly parametrizing partial charges of the ionized state of the amide.[16] Calculations such as the ones reported by Tannor et al.[16] may be very lengthy in the present case since for each residue the charge distribution should be calculated for two ionization states, for two backbone conformations, and for a number of side chain conformers. For instance, the 22 data points reported in Figure 2 required 260 electrostatic free energy evaluations.

Besides systematic deviations due to steric and inductive effects, for the amino acids, large variations in the HX rate were found for different conformers of both the backbone and side chain of the same residue. This behavior may be compared with the observation that HX rates in the fully unfolded form of BPTI[17] are similar to those in small model compounds, which suggests not only that there are no residual hydrogen bonds[17] but also that extensive conformational averaging should take place in the open state of the protein, since, in general, any residual conformational preference would lead to observable differences between the protein and small compound HX rates.

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(16) Tannor, D. J.; Marten, B.; Murphy, R.; Friesner, R. A.; Sitkoff, D.; Nicholls, A.; Ringnalda, M.; Goddard, W. A. III; Honig, B. *J. Am. Chem. Soc.* **1994**, *116*, 11875−11882.

(17) Roder, H.; Wagner, G.; Wüthrich, K. *Biochemistry* **1985,** *24*, 7407−7411.