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Reference parameters for protein hydrogen exchange rates

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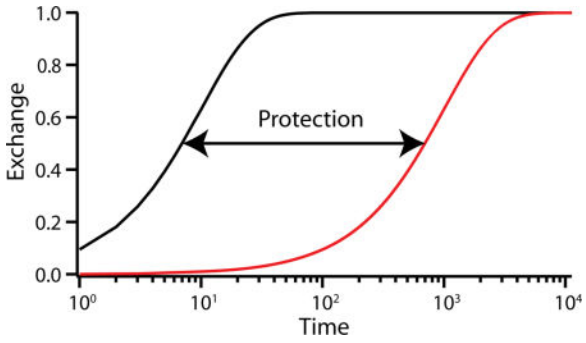
Abstract

The analysis of many hydrogen exchange (HX) experiments depends on knowledge of exchange rates expected for the unstructured protein under the same conditions. We present here some minor adjustments to previously calibrated values and a stringent test of their accuracy.

Graphical abstract



Introduction

Hydrogen exchange (HX) methods can now provide detailed information about protein structure, structure change, interactions, folding, energetics, and dynamics in solution under any chosen conditions resolved to near amino acid levels [1–8]. The evaluation of structure-dependent effects depends on knowing the HX rates expected for the unprotected structure-free situation. Free peptide HX rates have been calibrated as a function of their important determinants - pH, temperature, nearest neighbor effects, isotope effects, and salt concentration [9, 10]. The detailed accuracy of these values has been ambiguous. This paper considers these values, describes minor adjustments, and demonstrates their reliability.

The reference rate

Calculation of the expected HX rate for any individual main chain amide is based on the reference value for an amide between two alanine residues in an unstructured peptide under the existing conditions and the superimposed effects of nearest neighbor side chains. For the Ala-Ala reference rate, our previous calibrations used main chain amide HX rates measured by 1D NMR for poly-D,L-alanine (PDLA). We assume PDLA to adopt a random coil

conformation in solution. (Note: This is not necessarily identical to “disordered” real proteins in native conditions which tend to show a low level of protection against HX [11].

In previous calibrations a surprising anomaly was observed. HX rate varies with the size of the reference molecule. Polymeric PDLA showed 2-fold slower HX rates than a blocked tri-alanine oligomer, perhaps due to their different diffusivities. The present work used a mass spectrometric method (HX MS) [8] to resolve HX rates through a population of PDLA molecules that vary in size from 9 to 44 residues. Fig. 1A shows the exchange curve for Ala_{40}. Interestingly, HX is not single exponential. It can be well fit by either a double exponential or a stretched single exponential ($D = D_0(1-\exp(-(k_{ex}t)^{\beta}))$) with $\beta = 0.74$. Evidently the HX rate of any main chain amide depends on whether adjacent residues have the same or opposite chirality. Fig. 1B shows the stretched exponential rate constant for PDLA peptides as a function of peptide length, which confirms the size effect seen before.

However, the non-single exponential kinetics of PDLA presents a question. What value should be used for the reference rate constant of protein amides? We compared these results with HX MS data measured for a protein, apolipoprotein C3, under conditions where it is wholly unstructured (Fig. 2). In calculating pD we added 0.36 to the pH meter reading rather than 0.40 [12] to account for the 90% D_2O present. An increase of the reference rate previously used by the small factor of 1.35 brings the predicted rate into excellent agreement with the apoC3 data. Except for short outlier peptides that are significantly affected by the histidine residue at position 18, the average deviation is very small, 7%.

Table 1 lists the reference rate parameters from Connelly et al. [10] including this adjustment. Our readjusted HX rate spreadsheets (Online Resource) use this reference value to calculate expected protein amide HX rates and are also updated for the amino acid-level effects described below.

Amino acid side chain effects

Previous work calibrated the effect on the HX rate of any main chain amide due to its nearby amino acid side chains [9, 10]. Effects extend only to the nearest neighbor amide and can be accurately computed as the combined multiplicative effects of the side chains to the right and left.

Additional amino acid-level issues have since become clear. Among many other tests of our calibrations, Mori et al. [13] have accurately evaluated side chain effects in NMR studies of unstructured Δ131Δ staphylococcal nuclease. They found that, at neutral pH where Asp and Glu side chains are deprotonated, our previous reference rate correction factor led to predicted rates that are low (in the context of the Bai et al. alanine base rate) by about 2.5-fold for the amide before the side chain (“Left” in the notation of Bai et al.). In the earlier calibrating work [9, 10] side chain effects were evaluated at low pH where exchange is conveniently slow to allow accurate rate measurements. Calibration for the Asp and Glu effects at higher pH where their side chains are deprotonated used a different less precise NMR method. Mori et al. [13] also decided that the previous effect of a neighboring Gly side chain is too high by a factor of ~1.5 in rate. In the context of the above revised alanine

reference rate, the L effect of deprotonated Glu and Asp should be faster by a factor of 1.85 and Gly slower by a factor of 2.0 compared to the factors from Bai et al. Table 2 reproduces Table 2 from Bai et al. [9] with these changes included.

Some other side chain issues are of interest. It appears that the protonated histidine side chain can modestly catalyze HX of nearby amides beyond its nearest neighbors. We assume that this effect functions through acid catalysis of nearby amides but the size of the effect and its reach are unknown. Results in Fig. 2 for the shorter peptides containing His18 show that the effect is real and detectable.

Previous work showed that, like all other polar side chain protons, the NδH of the Arg side chain will be rapidly labeled in the usual protein experiment done above pH ~5 but it can be slow at pH 2 to 4 where the HX MS fragment analysis is done. Therefore, unlike all other polar side chains, residual Arg side chain label may not be totally lost due to back exchange during the HX MS analysis.

Finally, polymer end effects occur. These are generally not important for whole protein HX but can affect back exchange results for the many much smaller fragments during HX MS analysis. Earlier work showed that hydroxide-catalyzed exchange of an oligopeptide C-terminal amide is slowed by about 40-fold in addition to the standard effect of the neighboring side chain [14]. Similarly, hydroxide-catalysis of the first amide NH (amide of residue #2) in an oligopeptide is faster by about 40-fold at elevated pH under hydroxide catalysis. At pH 2.5 usually used during the HX MS analysis step, the intrinsic back exchange rate at amino acid residue #2 is ~10-fold faster than it would be further from the N-terminal. As a result, its amide NH will experience relatively fast back exchange during the analysis stage of HX MS experimentation. It is often assumed that back exchange will be complete at that position but slowing due to the effect of large apolar side chains at the first two positions, especially I, L, P, V, & W may prevent complete back exchange, as illustrated in Figure 5 of Engen & Wales [3]

Detailed methods for calculating free peptide HX rates using these parameters including temperature effects are given in Bai et al. [9] and Connelly et al. [10]. Excel spreadsheets incorporating these adjustments are available as an online resource and at http://HX2.med.upenn.edu.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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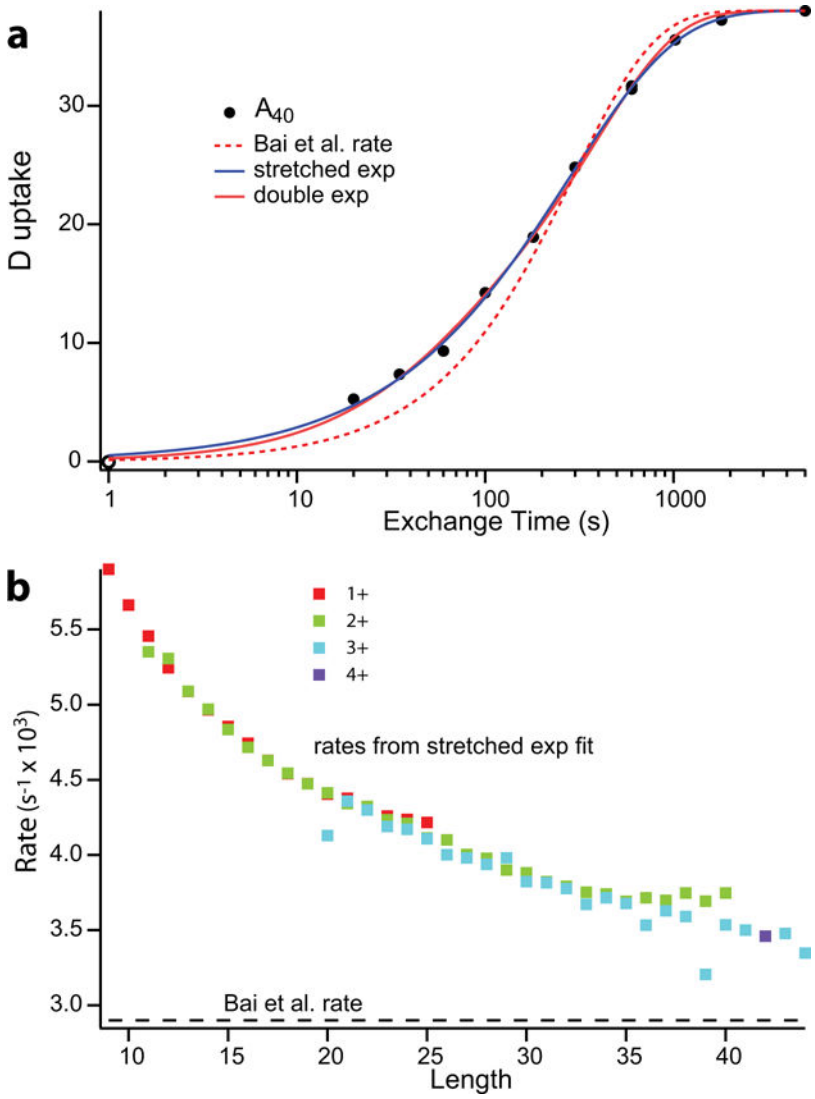

Figure 1.

PDLA exchange: (a) Deuterium uptake of a 40 residue peptide from random heterogeneously polymerized D,L-alanine. PDLA samples were exposed to H to D exchange (15°C in 90% D_2O at pD_{read} of 4.01) for increasing times, samples quenched at pH 2.4, 0°C to essentially halt exchange, and injected into an online sytem where they were desalted on a small C8 column, and separated by size in a C18 column. The eluant was introduced by electrospray into a Thermo LTQ Orbitrap XL mass spectrometer which further separates peptides by mass and allows time-dependent deuterium uptake of the differently sized PDLA peptides to be measured. Deuterium loss due to back exchange during the analysis was corrected for using an "all D" sample initially fully exchanged in the same buffer. The point shown at 1 sec is a zero time control which was injected immediately after simultaneous addition of quench and D_2O buffer. The dashed line shows the single exponential rate predicted from the Bai et al. parameters for large poly peptides. The blue and red solid lines show single stretched exponential and double exponential fits. (b)

Stretched exponential rates as a function of PDLA polymer length. The dashed line along the bottom indicates the reference rate from Bai et al. (9) for large poly peptides.

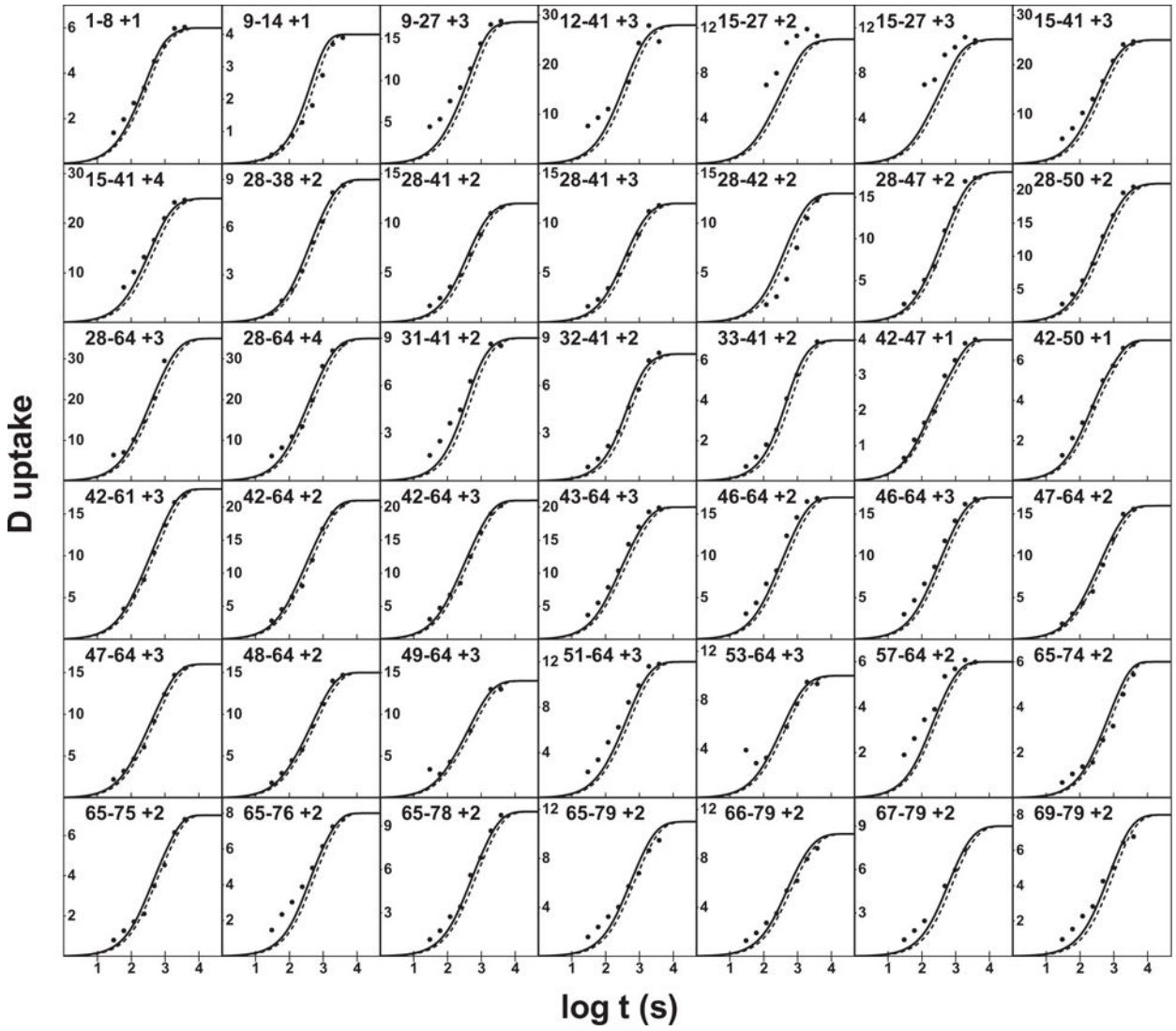

Figure 2.

Exchange curves for peptides obtained by HX MS for unstructured apolipoprotein C3 (90% D_2O, pD_{read} 4.10, 5°C). Samples were analyzed as for PDLA in Fig. 1 with the addition of an online pepsin digest. Peptides are identified by their sequence position in apoC3 and charge state in the MS. The expected free peptide exchange curves calculated with the original Bai et al. & Connelly et al. (9, 10) parameters are shown as a dashed line. The solid line uses the newly recommended value for the reference alanine rate (Table 1) and side chain effects described here (Table 2).

Table 1

Reference Ala-Ala rate for unfolded protein corrected to 20°C. Newly adjusted rates are in bold.

	$\log_{10}k_A$ M^{-1} min^{-1}	$\log_{10}k_B$ M^{-1} min^{-1}	$\log_{10}k_W$ M^{-1} min^{-1}
NH/D2O	1.62	**10.18**	−1.50
ND/H2O	1.40	**10.00**	−1.60
NH/H2O	1.39	**10.08**	

Table 2

Effect of amino acid side chains on the HX rates of neighboring peptides, given as $Log_{10}k_{ex}(X)$-$Log_{10}k_{ex}(Ala)$. The modified L Base reference rate factors for Gly and deprotonated Asn and Glu are shown in bold. The other values are as in Bai et al. (9). L refers to the effect of that side chain on the exchange rate of the amide to its immediate left, toward the N-terminus. R refers to the effect on the amide toward the C-terminus, separated by an intervening carbonyl.

	Acid		Base	
Side chain	**L**	**R**	**L**	**R**
Ala	0.00	0.00	0.00	0.00
Arg	−0.59	−0.32	0.08	0.22
Asn	−0.58	−0.13	0.49	0.32
Asp(COO^-)	0.90	0.58	**0.10**	−0.18
Asp(COOH)	−0.90	−0.12	0.69	0.60
Cys	−0.54	−0.46	0.62	0.55
Cys2	−0.74	−0.58	0.55	0.46
GlY	−0.22	0.22	**−0.03**	0.17
Gln	−0.47	−0.27	0.06	0.20
Glu(COO^-)	−0.90	0.31	**−0.11**	−0.15
Glu(COOH)	−0.60	−0.27	0.24	0.39
His			−0.10	0.14
His+	−0.80	−0.51	0.80	0.83
Ile	−0.91	−0.59	−0.73	−0.23
Leu	−0.57	−0.13	−0.58	−0.21
Lys	−0.56	−0.29	−0.04	0.12
Met	−0.64	−0.28	−0.01	0.11
Phe	−0.52	−0.43	−0.24	0.06
Pro(trans)		−0.19		−0.24
Pro(cis)		−0.85		0.60
Ser	−0.44	−0.39	0.37	0.30
Thr	−0.79	−0.47	−0.07	0.20
Trp	−0.40	−0.44	−0.41	−0.11
Tyr	−0.41	−0.37	−0.27	0.05
Val	−0.74	−0.30	−0.70	−0.14
N-term (NH_3^+)		−1.32		1.62
C-term (COO^-)	0.96		−1.80	
C-term (COOH)	0.05			