Anion modulation of the $^1\text{H}/^2\text{H}$ exchange rates in backbone amide protons monitored by NMR spectroscopy

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Abstract

The ability of three anionic cosolutes (sulfate, thiocyanate, and chloride) in modulating the $^1\text{H}/^2\text{H}$ exchange rates for backbone amide protons has been investigated using nuclear magnetic resonance (NMR) for two different proteins: the IgG-binding domain of protein L (ProtL) and the glucose–galactose-binding protein (GGBP). Our results show that moderate anion concentrations (0.2 M–1 M) regulate the exchange rate following the Hofmeister series: Addition of thiocyanate increases the exchange rates for both proteins, while sulfate and chloride (to a less extent) slow down the exchange reaction. In the presence of the salt, no alteration of the protein structure and minimal variations in the number of measurable peaks are observed. Experiments with model compounds revealed that the unfolded state is modulated in an equivalent way by these cosolutes. For ProtL, the estimated values for the local free energy change upon salt addition ($m_{3,\Delta G}$) are consistent with the previously reported free energy contribution from the cosolute's preferential interaction/exclusion term indicating that non-specific weak interactions between the anion and the amide groups constitute the dominant mechanism for the exchange-rate modulation. The same trend is also found for GGBP in the presence of thiocyanate, underlining the generality of the exchange-rate modulation mechanism, complementary to more investigated effects like the electrostatic interactions or specific anion binding to protein sites.

Keywords: amide proton exchange; Hofmeister series; sulfate; thiocyanate; cosolute; anion

Supplemental material: see www.proteinscience.org

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Abbreviations: ΔG_{hydr} , free energy of hydration; NMR, nuclear magnetic resonance; HSQC, heteronuclear single quantum correlation; ProtL, IgG-binding domain of *peptostreptococcal* protein; GGBP, glucose–galactose-binding protein; k_{ex} , experimental exchange rate; $m_{3,ex}$, slope for the variation of the exchange rate with cosolute concentration; k_{cl} , closing rate; k_{op} , opening rate; k_{rc} , intrinsic exchange rate in the unfolded state; ΔG_{ex} , local free energy change; $m_{3,\Delta G}$, slope for the variation of the local free energy with cosolute concentration; ΔG_{ex}^0 , local free energy change in the absence of cosolute; $\Delta G_{\Sigma K'}$, global variation of the free energy due to the preferential interaction term; BPTI, bovine pancreatic trypsin inhibitor.

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Solvent composition can significantly alter the constitutive properties of the biomolecules and, in particular, their biological activity (Record Jr. et al. 1998; Timasheff 1998; Fayos et al. 2005; Ellis and Minton 2006; Holthausen and Bolen 2007). Early works of Hofmeister demonstrated that the addition of moderate amounts of inorganic salts to the media (up to 1 M) efficiently modulates protein stability and solubility, dividing these ions between “kosmotropic” (well-solvated cosolutes with a large negative ΔG_{hydr} value) and “chaotropic” (poorly solvated cosolutes) (Hofmeister 1888). Although there is no simple model to explain such effects, different mechanisms seem to be directly related, including a reduction in the translational

entropy (excluded volume effect) (Davis-Searles et al. 2001), the nonspecific weak binding (or exclusion) of the ions to the protein surface (Timasheff 2002a), and dispersion forces (Zhang and Cremer 2006). The influence of salts is not limited to protein stability (Baldwin 1996), and many posterior studies have reported a plethora of properties that are affected by the presence of these cosolutes (i.e., changes in chromatography retention times or deviations in the measured pH when using an electrode) (Collins and Washabaugh 1985).

$^1\text{H}/^2\text{H}$ amide proton exchange rates have a well-documented relationship with the strength of the participating hydrogen bond (Loh et al. 1993; Dempsey 2001) and provide valuable restraints for solution protein structure elucidation (Main et al. 2005; Wilson et al. 2007). Moreover, the rate variability can also be interpreted to identify segmental motions (Kossiakoff 1982) and to characterize protein–protein (Sari et al. 2007) and protein–ligand (Pascal et al. 1995) interactions. Finally, exchange in $^1\text{H}_2\text{O}$ is required for reconstituting the amide proton in protein samples obtained from perdeuterated cell growths, used in the nuclear magnetic resonance spectroscopy (NMR) characterization of large proteins (Gardner and Kay 1998).

The proton exchange rate of water is also sensitive to the salt concentration in the media (Hertz et al. 1983), suggesting an equivalent dependence for the amide proton exchange rate. An increase of the solution ionic strength can alter the exchange rate for the amide protons belonging to charged residues (Kim and Baldwin 1982), and sodium chloride can also modulate the exchange rate due to nonspecific weak binding to the protein (Laurents et al. 2005; Brorsson et al. 2006). Nevertheless, the use of other inorganic salts in the proton amide exchange rate has not been investigated in detail, and, in this study, we have explored the influence of low to moderate concentrations of several anions on the exchange rate of the IgG-binding domain of protein L (ProtL; 64 residues) (Kim et al. 2000) and the glucose–galactose-binding protein (GGBP; 309 residues) (Shilton et al. 1996). In addition to sodium chloride, we have used sulfate and thiocyanate sodium salts as two representative examples for kosmotropic and chaotropic reagents, respectively (Tadeo et al. 2007). Our results show that the influence of these osmolytes in modulating the amide proton exchange rate follows the Hofmeister series. The anion effect on the exchange kinetics is explained in terms of the preferential binding or exclusion to the protein.

Results

The anion modulation of the amide proton exchange rate follows the Hofmeister series

To investigate any possible effect of the anions into the protein structure, we have recorded proton–nitrogen cor-

relation spectra (HSQC) for both proteins (ProtL and GGBP) at increasing concentrations (0–1 M) of sulfate and thiocyanate. Linear chemical shift displacements have been found for all the observed peaks, characteristic for a nonspecific binding with a low-affinity constant, and no evidences of conformational changes are observed in the NMR spectra upon osmolyte addition. Moreover, the circular dichroism spectra in the presence of 1 M sodium thiocyanate or 1 M sodium sulfate are identical to the one obtained when no salt is added (data not shown). A small number of residues that do not appear in the HSQC spectrum at low buffer concentration show HSQC peaks in the presence of cosolute, suggesting the existence of an exchange process in the microsecond–millisecond timescale that is altered by the anion. In support of this hypothesis, the same peaks are also observed when the HSQC spectra are recorded at lower temperatures.

For ProtL and in the absence of cosolute, the disappearance of the HSQC signal intensity could be measured for 28 out of 64 peaks belonging to structured regions of the molecule. The times required to reduce the signal intensity to a half spanned from 0.6 to 50.2 h (the experimental exchange rates, k_{ex} , are listed in the Electronic supplemental material). The decay curves for two representative amide peaks are shown in Figure 1. Figure 2 shows the effect of a moderate cosolute concentration (660 mM) of the three salts under consideration on the ProtL exchange rates, and the average change in the exchange rates are shown in Table 1. Opposite trends are observed depending on the salt used: While thiocyanate

Figure 1. Experimental signal intensity decay for two peaks in ProtL: Ile11 (black signs) and Phe22 (white signs), in the absence of cosolute (circles) and in the presence of 660 mM sodium sulfate (triangles), sodium chloride (squares), and sodium thiocyanate (diamonds). The solid lines represent the best exponential fit to the experimental data.

Figure 2. Exchange rates for the backbone amide protons of ProtL in the absence of cosolute (black bars) and in the presence of 660 mM cosolute (white bars) for (A) sodium thiocyanate, (B) sodium chloride, and (C) sodium sulfate. The gray shaded areas correspond to the secondary structural elements of the protein. Residues marked with an asterisk are not detectable in the absence of cosolute. The error bars correspond to the fitting error (χ^2) of the exponential decay.

anion clearly increases the proton-deuterium exchange rate for all the amide peaks in ProtL (10.8-fold on average), moderate to large decelerations of the reaction are observed when the same molar concentrations of sodium chloride and sodium sulfate are added, respectively. This is in agreement with the Hofmeister series for the anions, complementing other previously reported mechanisms for the effect of inorganic salts on the amide proton exchange rate (Kim and Baldwin 1982; Laurents et al. 2005).

Similar results are obtained with the glucose–galactose-binding protein (GGBP) bound to its natural receptor (glucose). This protein represents a stringent test for $^1\text{H}/^2\text{H}$ exchange experiments because it undergoes a large conformational rearrangement upon ligand binding, resulting in a variation of the protein area accessible to solvent (Shilton et al. 1996). The addition of 660 mM sodium thiocyanate in deuterium oxide increases the GGBP exchange rates 24-fold on average (see Table 1; Electronic supplemental Fig. S1). However, for a small subset of residues (marked with an asterisk in Electronic supplemental Fig. S1), the opposite trend has been observed, indicating that another mechanism may also be active for these amino acids, dominating their exchange-rate modulation. In agreement with ProtL observations, sodium sulfate causes a general decrease in the average exchange rate of GGBP amide protons, but the long experimental times required precluded their quantification. Thus, the similar results obtained for the two proteins tested underline the generality of the Hofmeister effect in the modulation of the $^1\text{H}/^2\text{H}$ amide proton exchange rate.

Changes in the local free energy are related to the anion–protein interactions for ProtL

The local variations of the exchange rate induced by the cosolute suggest the existence of interactions between the thiocyanate anions and ProtL amide groups. At low salt concentrations, specific interactions between sulfate anions and surface residues have been described for ribonuclease A (Ramos and Baldwin 2002). Thiocyanate anion can, in turn, form ionic pairs with lysine or arginine side chains, captured transiently in solution (Sau et al. 2003) and also detected in the crystal state (Vaney et al. 2001). However, inspection of Figure 2 reveals that the effect of thiocyanate is not correlated with residue type. Instead, our results are consistent with a nonspecific, weak anion-binding model, responsible for the modulation of many other properties by these cosolutes (Davis-Searles et al. 2001; Timasheff 2002b).

The exchange rates for ProtL amide groups have been measured at increasing concentrations of sodium

Table 1. Cosolute effect on the backbone amide proton average exchange rate

	No salt	Thiocyanate	Chloride	Sulfate
ProtL				
$\bar{k}_{\text{ex}}$ (h^{-1})	0.089	0.9615	0.036	0.0036
EF ^a	1	10.8	0.4	0.04
GGBP				
$\bar{k}_{\text{ex}}$ (h^{-1})	0.00071	0.017	n.d.	<0.0005
EF ^a	1	24	n.d.	<0.04

^a(EF) Enhancement factor, with respect to the protein in the absence of cosolute.

thiocyanate and sodium sulfate (200, 330 [twice], 660, and 1000 mM). A linear correlation between the natural logarithm of the exchange rate and the cosolute molar concentration has been found for all the measured amide protons in ProtL (two representative examples transformed into the respective ΔG_{ex} values are shown in Fig. 3; see below). The obtained slopes ($m_{3,ex}$) are positive or negative when thiocyanate and sulfate are added, respectively, reflecting the complementary nature of both osmolytes.

Previous studies reported in the literature have shown that ProtL exchanges under the EX2 mechanism regime in the absence of salt and at pH 6.0 (Yi et al. 1997). The EX2 condition will be even more fulfilled in the presence of stabilizing agents, like sulfate or chloride, that are to increase the folding rate (related to k_{cl} ; see Materials and Methods) (Khorasanizadeh et al. 1996; Scalley et al. 1997). On the other hand, a chaotrope like thiocyanate should reflect the opposite trend, but ProtL remains folded in the presence of up to 4 M sodium thiocyanate (data not shown), indicating that the k_{cl}/k_{op} ratio is still large, and it seems plausible to assume that the EX2 condition applies for the thiocyanate concentration range used in the study (0–1 M). The linearity in the plots between the exchange rate and the thiocyanate concentration supports this idea.

Under the EX2 regime, the exchange rates are dependent on the protection factor (which can also be quantified as the local free energy change, ΔG_{ex}^0) and on the intrinsic exchange rate of the amide proton (k_{rc}). The k_{rc} values have been estimated from model compounds in the absence of salt. It has been reported that k_{rc} may be sensitive to the ionic strength owing to interactions with charged residues or to the alteration of the residue pK_a (Kim and Baldwin 1982), but site-directed mutagenesis of charged surface residues suggests that electrostatics exert no significant contributions to ProtL stability (Fayos et al. 2005;

data not shown). However, based on the current models available for the cosolute–protein interaction (Timasheff 1998) and on previous experimental results (LeMaster et al. 2006), it is expected for the inorganic salts to affect in a nonspecific way to the unfolded state as well, and we have investigated their effect in the modulation of k_{rc} . We have determined the $^1\text{H}/^2\text{H}$ exchange rates for the two amide protons in the *N*-acetyl-alanine dipeptide, in the absence of cosolute and in the presence of 800 mM sodium thiocyanate and sulfate, respectively. An average rate increase (decrease) of 15- (28-) fold is observed when thiocyanate (sulfate) is added, indicating that the salt modulation follows the Hofmeister series. Thus, the k_{rc} rate modulation induced by the salts follows the Hofmeister series in an equivalent way to the folded conformation.

Under the assumption that k_{rc} varies linearly with the cosolute concentration (see Materials and Methods), the exchange rates at each salt concentration are converted into the changes in the local free energy (ΔG_{ex}). The ΔG_{ex} dependence on the salt is also linear, and it is shown in Figure 3 for Ile11 and Phe22. The slope ($m_{3,\Delta G}$) provides the change in the local free energy induced by the salt ($\delta\Delta G_{ex}/\delta C_3$). Figure 4 shows the variation of $m_{3,\Delta G}$ along the ProtL sequence for both osmolytes. While the thiocyanate anion produces a significant decrease in the local free energy, addition of sodium sulfate stabilizes the folded conformation. According to test statistics, variations in the $m_{3,\Delta G}$ values go beyond the experimental uncertainty with a probability of at least 0.9, indicating that cosolutes can effectively modulate the local equilibrium in a characteristic way for each backbone amide proton. Inspection of Figure 4 shows a certain degree of symmetry between the $m_{3,\Delta G}$ values for sulfate and thiocyanate, suggesting that the preferential interaction and the preferential exclusion are both reporting on the binding site properties.

Figure 3. Local free energy change (ΔG_{ex}) as a function of the cosolute concentration for Ile11 (black symbols) and Phe22 (white symbols): sulfate (diamonds) and thiocyanate (circles). The solid lines correspond to the best linear fits of the experimental data. The error bars correspond to the χ^2 of the exponential fitting as shown in Figure 2.

The local free energies in the absence of cosolute correlate with the salt-induced changes for ProtL

The local free energy in the absence of salt (ΔG_{ex}^0) displays a linear correlation with the cosolute-induced change in free energy ($m_{3,\Delta G}$), shown in Figure 5A for sodium thiocyanate: The more protected the amide protons are, the more prone to become affected by the anion. No equivalent trend has been observed for sodium sulfate (Fig. 5B), probably because of the distinct mechanism of interaction between this anion and the protein backbone, dominated by the preferential exclusion term (Tadeo et al. 2007). Similar correlations have been reported in the literature for the exchange rate in the presence of sucrose (Wang et al. 1995) and for the salt modulation of other properties like thermal stability (Fayos et al. 2005), underlining the generality of the observed effect. The observed correlation reflects the cosolute effect on the folded

Figure 4. Variation of the local free energy ($m_{3,\Delta G}$) induced by thiocyanate and sulfate for the different residues in ProtL. The bars reflect the propagation error from the experimental data. (Inset) Overall contribution of the preferential exclusion (positive value) and preferential interaction (negative value) to the change in free energy induced by the cosolute (calculated from experimental data reported in Tadeo et al. [2007]).

conformation and is complementary to the reported salt dependence of k_{rc} on the denatured state. Together, these results are fully consistent with the well-established concept that weakly interacting cosolutes act on both, the folded and the unfolded states (Record Jr. et al. 1998; Timasheff 1998).

Discussion

Knowledge of the mechanism for the cosolute interference in the amide proton exchange rate is required for a correct interpretation of the encoded structural and dynamic information. Electrostatic interactions are the most common explanation for the amide exchange-rate modulation by salts, a mechanism that does not depend on the chemical nature of the osmolyte. For example, in a

reported study of the effect of potassium chloride on the exchange rate of BPTI, linear plots between the $\ln(k_{ex})$ and the square root of the ionic strength were found (Debye-Hückel plots), revealing an electrostatic origin for the chloride anion interaction with BPTI (Christoffersen et al. 1996). However, in ProtL, we have observed very different responses depending on the electrolyte used, and, in addition, the $\ln(k_{ex})$ linearly correlates with the molar anion concentration. These two evidences discard an electrostatic origin for the exchange-rate modulation. Differences in the predominant mechanism probably arise from the distinct net charge between the two proteins: +11 (or +6 depending on the experimental conditions) for BPTI versus −3 for ProtL under the present experimental conditions.

Our results agree well with a model in which the Hofmeister effect is ultimately responsible for the observed exchange-rate modulation. ProtL $^1\text{H}/^2\text{H}$ exchange data are fully consistent with previous results where differences in the exchange-rate modulation between sulfate and thiocyanate are explained in terms of the preferential interaction (or preferential exclusion) with the amide proton (Timasheff 2002a). The contribution to the stability originating from the protein–anion interaction ($\Delta G_{\Sigma K'}$) has been estimated from circular dichroism data (Tadeo et al. 2007) and fitted to a previously reported model (Schellman 2003): For sodium sulfate, a minor stabilizing contribution ($+0.6 \text{ kcal}\cdot\text{mol}^{-1}\cdot\text{M}^{-1}$ at 298 K) is produced by the exclusion of the anion from the surface, while a large destabilizing term ($-4.4 \text{ kcal}\cdot\text{mol}^{-1}\cdot\text{M}^{-1}$ at 298 K) is the result of the direct protein–thiocyanate preferential interaction. After the analysis of the exchange data for ProtL, the average value for the cosolute-induced local changes in free energy ($\bar{m}_{3,\Delta G}$) is equal to $+1.9 \text{ kcal}\cdot\text{mol}^{-1}\cdot\text{M}^{-1}$ and $-0.9 \text{ kcal}\cdot\text{mol}^{-1}\cdot\text{M}^{-1}$ for sulfate and thiocyanate, respectively (see Fig. 4). The qualitative agreement between $\bar{m}_{3,\Delta G}$ and $\Delta G_{\Sigma K'}$ suggests that the interaction of the anion with the amide group may be the dominant mechanism for the observed

Figure 5. Correlation between the cosolute-induced variation of the local free energy ($m_{3,\Delta G}$) and the local free energy change in the absence of cosolute (ΔG_{ex}^0) for (A) thiocyanate and (B) sulfate. The bars reflect the errors of fitting the experimental data to an exponential curve (ΔG_{ex}^0) or to a line ($m_{3,\Delta G}$).

modulation in the exchange rate. However, the compared values differ significantly, probably because the exchange rates affect only the amide protons and their local environment, while the $\Delta G_{\Sigma K'}$ term reflects the contribution to the overall protein stability of a larger environment. Different contributions to the folding free energy in the presence of osmolytes arising from backbone and side chains have recently been reported (Auton and Bolen 2005), supporting this hypothesis.

Consistent with previous literature results (Kim and Baldwin 1982), the data presented here show that the assumption of k_{rc} being independent on the buffer concentration does not hold, and its variation with the cosolute concentration requires to be estimated. This is of particular importance in the native hydrogen-exchange experiment, where large concentrations of guanidinium chloride are used.

Although water molecules play an active role in the exchange reaction, no modification of the bulk solvent properties is expected since osmolytes and anions seem to produce negligible effects on water structure (Omta et al. 2003; Batchelor et al. 2004; Zhang and Cremer 2006). Consistent with this idea, recent studies revealed that the poor hydration of thiocyanate (limited to two layers of water molecules) may facilitate the interaction between the cosolute and the protein surface (Mason et al. 2003). On the other hand, the exclusion of sulfate from the surface is the result of the kosmotropic nature of this anion (i.e., favorable free energy of hydration, ΔG_{hydr}) that stabilizes the anion preferentially in the bulk solvent.

The vast majority of residues of GGBP also stand for an increase in the exchange rate upon addition of thiocyanate, underlining the general nature of the modulation mechanism. However, we have detected a noncanonical trend in a small subset of amide groups, suggesting that the anion effect is more complex in large proteins like GGBP, and probably other mechanisms like the binding of the anion to specific protein sites or a Debye screening of the protein surface shall contribute to the exchange-rate modulation for these residues.

Finally, it is worth noting that the determination of the exchange rates suffers from an excessively long experimental time that can take up to years of data collection. Our results show that, for both proteins under study, moderate thiocyanate concentrations result in significant increases in the exchange rates, retaining all the peaks observed in the absence of cosolute. Since sodium thiocyanate is a mild denaturant, in the concentrations used in the present study, the acceleration of the exchange process is achieved with no significant perturbation of the protein structure, resulting in an attractive alternative to more powerful denaturants like guanidinium chloride to exchange amide protons in isotopically labeled protein sample preparations. Moreover, the observed proportionality between $m_{3,\Delta G}$ and ΔG_{ex}^0 [or $m_{3,ex}$ and $\log(k_{exp}^0)$]

reveals that the exchange rate in the presence and in the absence of cosolute results in equivalent structural information for ProtL. Sulfate anion decreases the exchange rate by stabilizing the folded conformation, allowing the experiment to be performed under conditions (e.g., elevated temperature or pH) in which the amide exchange would be otherwise too fast.

In conclusion, the present study underlines the general and nonspecific role that the Hofmeister effects play in the modification of the exchange rates for amide groups in proteins, and it should be accounted for in the analysis of experimental data with samples that contain salts or other cosolutes, often required to increase protein solubility and to avoid aggregation.

Materials and Methods

Protein cloning, expression, and purification

The IgG-binding domain of *peptostreptococcal* protein L (ProtL; 64 residues) and the glucose–galactose-binding protein (GGBP; 309 residues) were expressed in BL21 cells using M9 media and ^{15}N ammonium chloride. ProtL was purified as described previously (Millet et al. 2003). In GGBP, cells were resuspended in 50 mM Tris (pH 9.0), 10 mM CaCl_2 , and 0.05% sodium azide, and after cell lysis, GGBP was purified using ionic exchange chromatography (MonoQ 5/50 GL; Amersham) and size exclusion chromatography (Superdex 75; Amersham). The final sample conditions were: 20 mM phosphate buffer, 0.03% sodium azide (pH 6.0) for ProtL and 20 mM Tris, 1 mM glucose, 0.03% sodium azide (pH 7.1) for GGBP.

NMR spectroscopy

Aliquots of salt-free protein samples (500 μL) were lyophilized and resuspended in an equivalent volume of $^2\text{H}_2\text{O}$ (or $^1\text{H}_2\text{O}$ with the appropriate amount of inorganic salt since it has been reported that freeze-drying of a protein in the presence of thiocyanate enhances unfolding and aggregation) (Allison et al. 1996). All measurements were recorded in Bruker DRX Avance II 800 MHz and 600 MHz spectrometers. For ProtL, experiments were collected at 298 K in the absence of cosolute and in the presence of varying salt concentrations (200, 330 [twice], 660, 1000 mM for sodium thiocyanate and sodium sulfate and 660 mM for sodium chloride), using the FHSQC pulse sequence (Mori et al. 1995) up to a maximum time of 1 wk (160 h). For GGBP, TROSY-HSQC with sensitivity enhancement experiment (Rance et al. 1999), experiments were recorded in the absence of cosolute and in the presence of 660 mM sodium thiocyanate or sodium sulfate at 310 K. For the experiments with *N*-acetyl-alanine dipeptide, a saturated peptide solution in 200 mM acetate buffer (pH 4.0) was diluted 10 times in D_2O , and the decays of the two amide peak intensities were monitored at 278 K using 1D-NMR spectroscopy.

Data analysis

The spectra were processed using nmrPipe (Delaglio et al. 1995) and in-house build scripts. The time courses of the amide peak intensities were fitted to exponential decays with fitting errors

reported in the Electronic supplemental material, adjacent to the exchange rate for each amide peak. Concentration duplicates have been considered as independent points in the error estimation for the $m_{3,ex}$ slopes. The exchange rates for the unfolded conformation (k_{rc} ; see below) have been estimated from unfolded peptides (Bai et al. 1993), corrected to pH* 6.0 and 298 K as described previously (Connelly et al. 1993) and considered as exact numbers in the error propagation. The value of k_{rc} at each cosolute concentration has been estimated assuming a linear dependence of k_{rc} with the molar anion concentration and using the following data: the reported values of k_{rc} in the absence of salt (Bai et al. 1993) and the measured values of k_{rc} in the presence 800 mM salt (thiocyanate or sulfate). To determine whether the differences in the $m_{3,\Delta G}$ between the different amide protons are significant, test statistics have been used to compare each $m_{3,\Delta G}$ value to the average value ($\bar{m}_{3,\Delta G}$).

Proton–deuterium exchange theory

The most widespread model for the exchange process entails a transition from a closed (cl) to an open (op) conformation, prior to the amide proton exchange (Berger and Linderstrom-Lang 1957; Hvidt and Nielsen 1966). This rearrangement can be produced by either local or global unfolding, and obeys the following scheme:

where k_{op} and k_{cl} are the local rates for opening and closing, respectively, and k_{rc} is the intrinsic exchange rate for the amide proton in the unfolded conformation. In the general case, the experimentally determined rate constant (k_{ex}) is an apparent constant and a complex function of the elementary rates. In folded stable proteins, the inequality $k_{cl} \gg k_{op}$ is largely satisfied and k_{ex} takes the form:

$$k_{ex} = \frac{k_{op} \cdot k_{rc}}{k_{cl} + k_{rc}}$$

Moreover, under most conditions of practical interest, $k_{cl} \gg k_{rc}$ is also true (EX2 mechanism), and k_{ex} can be simplified to

$$k_{ex} = \frac{k_{op}}{k_{cl}} \cdot k_{rc} = K_{eq} \cdot k_{rc} = \frac{1}{PF} \cdot k_{rc}$$

where PF is the protection factor for the amide proton. The random-coil constant (k_{rc}) is also residue dependent, and it can be estimated from the amino acid protein sequence, using reference values obtained from model peptides (Bai et al. 1993; Connelly et al. 1993). The equilibrium constant K_{eq} can be related to the local free energy change (ΔG_{ex}):

$$\Delta G_{ex} = -RT \cdot \ln(K_{eq}) = -RT \cdot \ln\left(\frac{k_{ex}}{k_{rc}}\right)$$

In the presence of a cosolute, we use the Scatchard terminology (Scatchard 1946) that designates [1] to water, [2] to the protein,

and [3] to the added cosolute. It is expected that the apparent exchange rate becomes altered upon addition of [3]. Assuming an exponential variation of k_{ex} with the molar cosolute concentration:

$$\frac{\partial \ln k_{ex}}{\partial C_3} = m_{3,ex}$$

where $m_{3,ex}$ is a constant value (slope). The free energy variation with the cosolute concentration takes the form:

$$\frac{\partial \Delta G_{ex}}{\partial C_3} = -RT \cdot m_{3,ex} = m_{3,\Delta G}$$

Electronic supplemental material

The Electronic supplemental material includes experimental backbone amide exchange rates for ProtL in the absence of cosolute and in the presence of 660 mM NaCl (Supplemental Table S1) and in the presence of increasing concentrations of sodium thiocyanate (Supplemental Table S2) or sodium sulfate (Supplemental Table S3). For a bar plot with the exchange rates for GGBP in the absence and in the presence of 660 mM of sodium thiocyanate, see Supplemental Figure S1.

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