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Measurement of the exchange rates of rapidly exchanging amide protons: Application to the study of calmodulin and its complex with a myosin light chain kinase fragment

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SUMMARY

A technique is described for measuring the approximate exchange rates of the more labile amide protons in a protein. The technique relies on a comparison of the intensities in ^{1}H-^{15}N correlation spectra recorded with and without presaturation of the water resonance. To distinguish resonance attenuation caused by hydrogen exchange from attenuation caused by cross relaxation, the experiment is repeated at several different pH values and the difference in attenuation of any particular amide resonance upon presaturation is used for calculating its exchange rate. The technique is demonstrated for calmodulin and for calmodulin complexed with its binding domain of skeletal muscle myosin light chain kinase. Upon complexation, increased amide exchange rates are observed for residues Lys^{75} through Thr^{79} located in the 'central helix' of calmodulin, and for the C-terminal residues Ser^{147} and Lys^{148}. In contrast, a decrease in amide exchange rate is observed at the C-terminal end of the F helix, from residues Thr^{110} through Glu^{114}.

INTRODUCTION

The rates with which amide backbone protons in a protein exchange with water typically vary over many orders of magnitude. Exchange rates have been used to obtain information about solvent accessibility and the hydrogen bond stability of amide protons. For a detailed review about hydrogen exchange in proteins, see Englander and Kallenbach (1984). The slowly exchanging amide protons in a protein are commonly defined as the amide hydrogens that can be observed

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after the protein has been dissolved in D_2O. Because the amide region of the one-dimensional ^1H NMR spectrum of a protein typically shows extensive overlap, a two-dimensional scheme is usually used to resolve these proton resonances (Wagner and Wüthrich, 1982; Marion et al., 1989). This puts a lower limit of at least several minutes on the exchange time for an amide proton to be observable and to be classified as a 'slow exchanger'.

An elegant indirect method for measurement of hydrogen exchange rates observes the coalescence of the ^2H-isotope shifted carbonyl resonance and the unshifted carbonyl resonance, corresponding to a protonated amide, in a 50% H_2O-50% D_2O solvent (Hawkes et al., 1978). In order to accurately measure this coalescence a high-resolution well resolved spectrum of the carbonyl region of the protein is needed which generally requires selective isotopic enrichment of the carbonyl resonance (Kainosho and Tsuji, 1982; Kainosho et al., 1987; Henry et al., 1987).

In the pH 4-9 range, amide hydrogen exchange in proteins is catalyzed by OH^- ions and thus shows a strong pH dependence. For a range of pH where none of the titratable groups in a protein change their net charge one expects a 10-fold increase in the exchange rate for every unit increase in the pH. If the experiments can be performed at a low pH of around 4, most of the amide hydrogens can still be observed five minutes after the protein is dissolved in D_2O. However, for proteins that cannot be handled at such a low pH, exchange rates can only be obtained for a much smaller fraction of the hydrogens. At pH 7, for example, most of the amide protons are not 'slow exchangers' and their exchange rates cannot be measured by dissolving the protein in D_2O. Since most of these more labile amide protons are located at or near the protein surface, they are the primary carriers of structurally important information on protein-protein and protein-ligand interaction.

In the present paper we describe a different approach that allows measurement of the exchange rates of the rapidly exchanging protons and use this method to determine the exchange rates of calmodulin (CaM) and its complex with a 26 amino acid fragment known as M13, which comprises the binding site (residues 577-602) of rabbit skeletal muscle myosin light chain kinase. The binding between CaM and M13 is very tight, with a dissociation constant of $\sim$ 1 nM (Blumenthal et al., 1985).

EXPERIMENTAL APPROACH

In small peptides the exchange rates of rapidly exchanging amides can be measured from the effect of presaturation on simple one-dimensional spectra (Waelder and Redfield, 1977; Rosevaer et al., 1985). This requires the recording of two 1D spectra on the peptide dissolved in H_2O. The first experiment uses no presaturation of the H_2O resonance and employs an observe pulse that does not excite the H_2O resonance. The second spectrum is recorded under exactly identical conditions with the only difference that presaturation of the H_2O resonance is employed during the delay between scans. In the absence of cross relaxation, attenuation of the amide resonances in the experiment with presaturation depends on the amide hydrogen exchange rate, k, in the following fashion (Krishna et al., 1979):

$$k = (1 - M_{ps}/M_o)/T_{1_{app}} \tag{1}$$

where M_{ps} is the resonance intensity in the experiment with presaturation, and M_o is its intensity

without presaturation. $T_{1_{app}}$ is the apparent longitudinal relaxation rate as measured in a selective T_1 experiment. $T_{1_{app}}$ includes the effects of both exchange and true relaxation:

$$1/T_{1_{app}} = 1/T_1 + k \tag{2}$$

Equations 1 and 2 can be rewritten as

$$k = (M_o/M_{ps} - 1)/T_1 \tag{3}$$

where T_1 describes the true longitudinal relaxation as measured in a selective T_1 experiment in the absence of chemical exchange. For macromolecules, this rate is dominated by the cross-relaxation rate.

In proteins larger than about 10 kDa, very few amide resonances are sufficiently well resolved to permit measurement of their intensity in a simple 1D spectrum. However, the same approach as described above and previously commonly used for peptides can be applied to proteins if a 2D experiment is used to remove overlap in the amide region. The heteronuclear multiple quantum coherence (HMQC) pulse scheme (Bendall et al., 1983; Bax et al., 1983) is a convenient method for correlating ^1H and ^{15}N chemical shifts with and without the use of presaturation, provided the ^1H pulses are substituted by jump-and-return pulses (Roy et al., 1984). The pulse sequence used in the present experiments is:

$$\begin{array}{lll} ^1H\text{: (presat)} - 90_x - \tau - 90_{-x} - \Delta - & -t_1/2 - 90_\psi - 2\tau - 90_{-\psi} - t_1/2 - & -\Delta - \text{ Acquire} \\ ^{15}N\text{:} \qquad\qquad\qquad\qquad 90_\varphi & \qquad\qquad\qquad\qquad 90_x & \text{Decouple} \end{array}$$

where the ^1H carrier is set on the H_2O resonance and τ is set to $1/(4\delta)$ where δ is the frequency difference between the center of the amide protons and the H_2O resonance. The delay Δ is set slightly shorter than $1/(2\,J_{NH})$, 4.5 ms in practice. The phase cycling used is as follows: $\psi = x,y,-x,-y$; $\varphi = 4(x),4(-x)$; Acq. $= x,-x,x,-x,-x,x,-x,x$. Quadrature in the t_1 dimension is most easily obtained using the States procedure (States et al., 1984): after the above phase cycle is completed, the phase φ is incremented by 90° and data are stored separately. To obtain a flat F_1 baseline (Bax et al., 1990) it is recommended that the programmed first t_1 duration, $t_1(0)$, is set to:

$$t_1(0) = \Delta t_1/2 - (4 \times 90(^{15}N)/\pi + 2\tau + 2 \times 90(^1H)) \tag{4}$$

where $90(^1H)$ and $90(^{15}N)$ are the ^1H and ^{15}N 90° pulse widths, and Δt_1 is the t_1 increment. When using these parameters, a 180° linear phase correction is required in the F_1 dimension, and resonances that are possibly folded will have a phase that is exactly opposite to resonances that have not been folded. Note that no scaling of the first data point in the t_1 dimension should be used in this case prior to Fourier transformation.

For small peptides, cross relaxation is typically much smaller than the intrinsic longitudinal relaxation rate and therefore may be safely neglected. For proteins, however, the k value as determined from Eq. 3 may include a substantial contribution from magnetization exchange caused by cross relaxation. This magnetization exchange can occur via different pathways: First, Hα

protons that resonate very close to the water resonance are also saturated by the H_2O presaturation and through spin diffusion this may attenuate nearby HN resonances. Second, for cases where there is a direct NOE between water and protein, attenuation of the amide resonances can occur either via a direct NOE effect, or via spin diffusion. Finally, amide resonances that are in the vicinity of labile hydroxyl protons may be attenuated via spin diffusion if the hydroxyl proton is saturated via exchange with bulk water. As will be shown later, this effect is frequently observed for the HN resonances of Thr and Ser residues. Note that none of these NOE-mediated magnetization exchange mechanisms is expected to show a strong pH dependence. Thus, the observed magnetization transfer rate, k, can be decomposed in a pH-independent component, k_{NOE}, and the true hydrogen exchange rate, k_{HX}:

$$k = k_{NOE} + k_{HX} \tag{5}$$

In this equation, k_{NOE} includes the fact that the neighboring proton is only partly saturated:

$$k_{NOE} = \sum_i k_i(1 - M_i/M_o) \tag{6}$$

where k_i is the cross-relaxation rate with proton i, and $1\text{-}M_i/M_o$ is the degree of saturation of proton i under steady state conditions when water presaturation is used. Note that because M_i/M_o may be pH-dependent, k_{NOE} also can be pH-dependent. Judging by the measured pH dependence of k for residues that are known to have slowly exchanging amides, weak pH dependence of k_{NOE} sometimes can be observed. However, this pH dependence of k_{NOE} is much too small to have a significant impact on the k_{HX} values determined for amides with rapid hydrogen exchange.

The hydrogen exchange rate, k_{HX}, depends exponentially on pH, and can be written as:

$$k_{HX} = k_{HXo} \times 10^{pH - pHo} \tag{7}$$

where k_{HXo} is the exchange rate at a pH value pHo. By repeating the experiment at several different pH values, the effect of chemical exchange can be separated from the effect of NOE-mediated magnetization exchange. Using Eq. 3, it is necessary to know what the value of the selective longitudinal relaxation time is in the absence of hydrogen exchange. Although in principle 2D methods can be devised that measure these rates for each individual amide site separately, in practice this would be a very tedious and time-consuming task. Measuring the selective T_1 value for nine of the resonances that are resolved in the 1D spectrum of calmodulin and that correspond to slowly exchanging amide resonances indicates that the selective T_1 values are quite uniform, ranging from 106 ms to 163 ms for free calmodulin, and from 98 to 144 ms in the complex. Therefore, in our determination of k_{HX} we assume a single average T_1 value of 125 ms. It should be realized that errors on the order of 25% may be introduced by this assumption, and possibly more for residues with a small order parameter which may have a T_1 value significantly longer than 125 ms. For calmodulin, ^{15}N relaxation studies indicate that the order parameter for residues Leu^4 through Ser^{147} is quite uniform ($0.6 < S^2 < 0.9$) (Barbato et al., unpublished) and no dramatic variation in the amide proton T_1 is therefore expected.

EXPERIMENTAL

Sample Preparation

Drosophila CaM was over-expressed using the pAS vector in *E. coli* (strain AR58) and purified as reported previously (Ikura et al., 1990). Samples used contained uniformly ^{15}N-labeled (~95%) CaM in 95% H_2O/5% D_2O solution. Chemically synthesized HPLC-purified peptide M13 (KRRWKKNFIAVSAANRFKKISSSGAL) (Peptide Technologies Corp., Washington, D.C.) was used without further purification. The complex of CaM with M13 was prepared according to the procedure outlined by Seeholzer and Wand (1989): 12 mg of decalcified CaM was dissolved in 4.5 ml of H_2O solution containing 0.01M KCl and 0.68 mM $CaCl_2$; the pH was adjusted to 6.8. The concentration of CaM (0.16 mM) in this solution was determined by UV spectroscopy using an extinction coefficient ($\varepsilon^{1\%}_{276nm}$) of 0.945 in the presence of Ca^{2+}. M13 stock solution (0.18 mM) was added slowly to the CaM solution until the concentration ratio of M13 and CaM reached 1:1. No precipitation was observed under these conditions. The solution was concentrated without freezing using a speed vacuum concentrator to a total volume of 0.43 ml after which 0.02 ml D_2O was added for a deuterium lock. Spectra were recorded at three different pH values: 6.55, 6.9 and 7.8. pH values were measured inside the 5 mm NMR sample tube at 25°C, using an Ingold 6030-5 electrode. Experiments on M13-free CaM were also carried out at three different pH values: 6.35, 6.75 and 8.0. For both the M13-free CaM sample and the sample of the CaM-M13 complex the protein concentration was 1.5 mM.

NMR Experiments

NMR spectra of M13-free CaM were recorded on a Bruker AM-500 spectrometer and experiments on the CaM-M13 complex were recorded on a Nicolet NT-500 spectrometer. In both cases 2D ^1H-^{15}N correlation maps were recorded with the HMQC pulse scheme described above, with and without presaturation during the 1.2 s delay between scans. The strength of the presaturating field was carefully adjusted to 20 Hz for all experiments employing presaturation. The data matrix size used was (128 complex) × (1024 real) (Bruker) and (128 complex) × (512 complex) (Nicolet). Acquisition times were 51.2 ms (t_2) and 102.4 ms (t_1). Data were processed off-line with the NMR2 software package (NMRi, Syracuse, NY). Resolution enhancement using a shifted sine bell digital filter and zero-filling were used in both dimensions. Peak intensities for resolved resonances were measured as interpolated peak heights and not as integrated peak volumes. For the few cases where partial overlap of two resonances prohibited the measurement of their individual peak intensity, a different approach was employed. By calculating a difference spectrum (no presat) $- p$(presat), the value p was carefully adjusted to give zero for the least attenuated component of the overlapping cluster. The decrease in intensity of the resonance attenuated most could be measured in this manner. Assuming a 'no presat' intensity comparable to other protons in the same ^1H chemical shift range, an attenuation factor, M_{ps}/M_o, was calculated. If the difference spectrum ($p = 1$) of a cluster of overlapping resonances did not show peak intensity higher than 50% of the intensity of a single isolated resonance in the corresponding spectrum without presaturation, this was taken as evidence that for all residues in the cluster $k_{HX} < 1\ s^{-1}$.

RESULTS

Complete backbone assignments of M13-free calmodulin (Ikura et al., 1990) and the M13-CaM complex (Ikura et al., in press) have recently been reported. These assignments form the basis for our present study of hydrogen exchange. For both the M13-free CaM and the CaM-M13 complex, 2D HMQC spectra were recorded with and without presaturation of the H_2O resonance

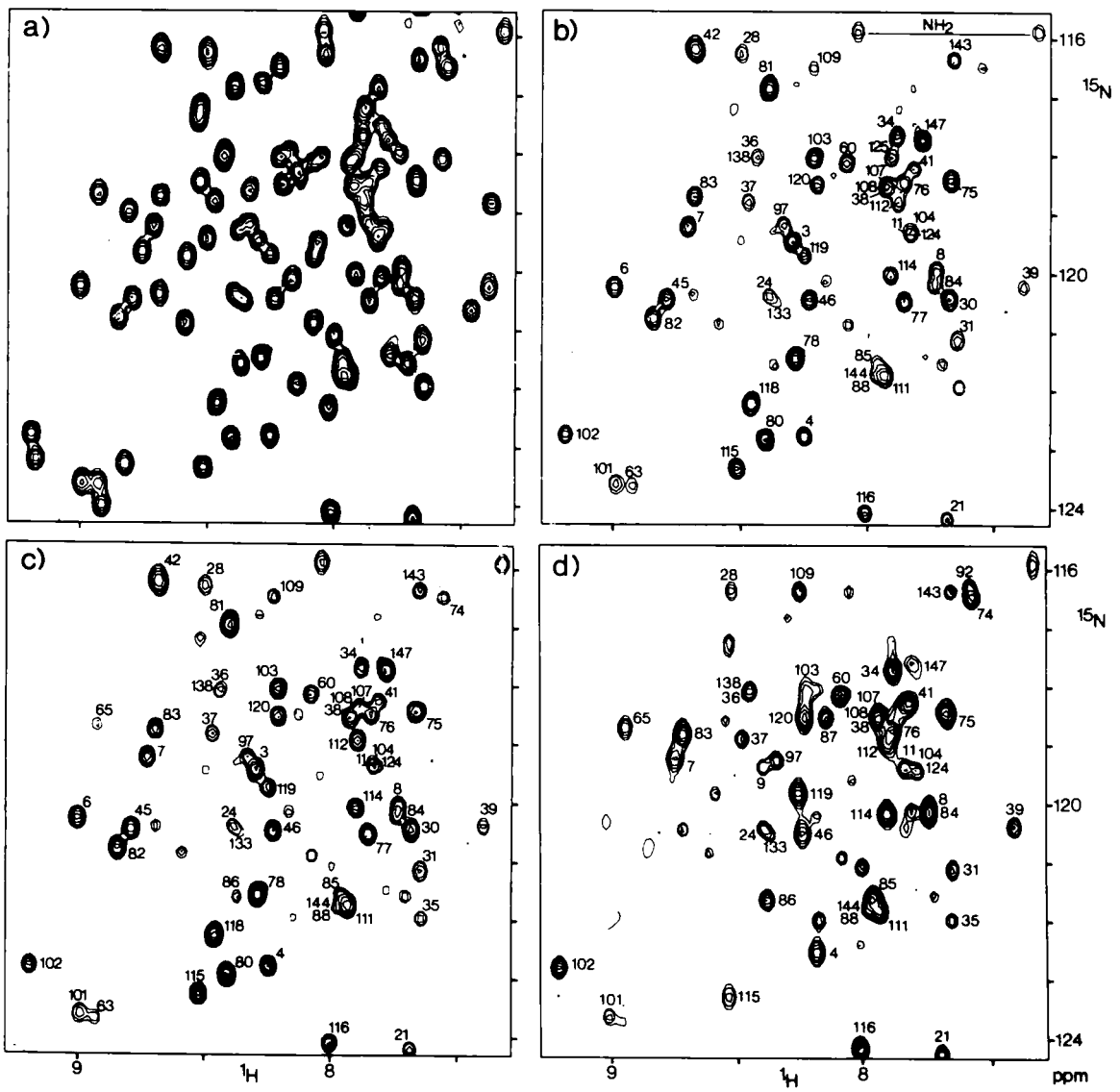

Fig. 1. (a) Region of the ^1H-^{15}N shift correlation spectrum of M13-free Ca^{2+}-ligated calmodulin, recorded with the jump-and-return HMQC experiment, in the absence of presaturation at pH 6.35. (b-d) HMQC difference spectra obtained by subtracting the spectrum with presaturation from the spectrum without presaturation for (b) pH 6.35, (c) pH 6.75 and (d) pH 8.0. Resonances are labeled with the corresponding residue number in the protein. Contours are drawn at levels $A*1.3^N$, N = 0,1,2..., where A is the level of the lowest contour.

at three different pH values. Figure 1a shows a small region of the spectrum recorded for M13-free CaM without presaturation at pH 6.35. At this pH, all backbone amides with the exception of Asp[2] give rise to an observable NH correlation. Figure 1b shows the difference spectrum, ob-

TABLE I

RATIO OF AMIDE INTENSITIES WITH (M_{ps}) AND WITHOUT (M_o) PRESATURATION OF H_2O FOR THREE DIFFERENT pH VALUES, THE APPARENT MAGNETIZATION TRANSFER RATE, k[s^{-1}] AND THE CALCULATED HYDROGEN EXCHANGE RATE AT pH 7 AND 35°C, k_{HX}[s^{-1}], FOR THE FIRST 34 RESIDUES IN M13-FREE CaM

	pH 6.35		pH 6.75		pH 8.0		pH 7.0
	M_{ps}/M_o	k	M_{ps}/M_o	k	M_{ps}/M_o	k	k_{HX}
A1	a		a		a		–
D2	a		a		a		–
Q3	0.203	31.5	a		a		150
L4	0.779	3.8	0.522	7.3	a		11
T5	0.579	5.8	0.533	5.9	0.538	6.9	< 1
E6	0.571	6.0	0.421	11.0	a		16
E7	0.622	4.9	0.502	7.9	a		10
Q8	0.682	3.7	0.679	3.8	0.604	5.2	< 1
I9*	0.859	1.3	0.882	1.1	0.744	2.7	< 1
A10	0.942	0.5	0.928	0.6	0.756	2.6	< 1
E11	b		b		0.892	1.0	< 1
F12	0.870	1.2	0.861	1.3	0.862	1.3	< 1
K13	0.969	0.3	0.968	0.3	0.903	0.8	< 1
E14	0.965	0.3	0.954	0.4	0.796	2.0	< 1
A15*	0.966	0.3	0.964	0.3	1.007	0	< 1
F16*	0.907	0.8	0.933	0.6	1.013	0	< 1
S17*	0.867	1.2	0.859	1.3	0.841	1.5	< 1
L18*	0.914	0.8	0.924	0.7	0.965	0.3	< 1
F19*	0.903	0.9	0.912	0.8	0.959	0.3	< 1
D20*	b		b		0.743	2.8	< 1
K21	0.801	2.0	0.787	2.2	0.491	8.3	< 1
D22	0.790	2.1	0.748	2.7	0.427	10.7	1.0
G23	0.720	3.1	0.731	2.9	0.650	4.3	< 1
D24*	0.896	0.9	0.893	1.0	0.883	1.1	< 1
G25*	0.727	3.0	0.735	2.9	0.82	1.7	< 1
T26*	0.670	3.9	0.681	3.7	0.687	3.6	< 1
I27*	0.756	2.6	0.803	2.0	0.796	2.0	< 1
T28	0.697	3.5	0.687	3.6	0.632	4.7	< 1
T29	0.515	7.5	0.588	11.4	a		12
K30	0.403	9.7	0.291	19.5	a		31
E31	0.664	4.1	0.637	4.6	0.624	4.8	< 1
L32	0.826	1.7	0.834	1.6	0.614	5.0	< 1
G33	0.797	2.0	0.774	2.3	0.486	8.5	< 1
T34	0.608	5.2	0.568	6.1	0.236	25.9	2.2

[a] Attenuation too large for accurate measurement.

[b] Not determined because of resonance overlap.

* Amide proton remains visible for more than five minutes after the protein is dissolved in D_2O, p^{2}H 6.3, 25°C.

tained by subtracting the spectrum with presaturation from the spectrum without presaturation which indicates that many of the resonances are affected by the presaturation. However, comparison with similar difference spectra recorded at pH 6.75 and 8.0 (Figs. 1c,d) shows that many of these resonances exhibit only a very weak or no pH dependence. For example, all threonine residues give rise to an observable correlation in the presaturation difference spectrum (T28 and T143 at ^{15}N shifts of ~ 116 ppm fall within the window shown, but most resonate more upfield), but only a fraction of them exhibit significant pH dependence. Table 1 illustrates the degree of resonance attenuation upon water presaturation, M_{ps}/M_o, and the derived magnetization transfer rate, k, for residues in the vicinity of the first calcium-binding site in M13-free CaM, for the three different pH values. The last column shows the hydrogen exchange rate at pH 7.0, k_{HX}, calculated from the three k values using Eqs. 5 and 7. For a number of residues, e.g. E11 and D20, overlap in the 2D spectra made accurate measurement of k values impossible. However, if for the overlapping cluster no intense peak is observed in the difference spectrum at any of the three pH values, this indicates that the exchange rate must be smaller than 10 s^{-1} at pH 8, i.e. smaller than 1 s^{-1}

TABLE 2
COMPARISON OF FAST AMIDE EXCHANGE RATES [s^{-1}] IN M13-FREE CaM AND CaM-M13 AT pH 7, 35°C[a]

Residue	CaM	CaM-M13	Residue	CaM	CaM-M13
Q3	150	150	T79	77	130
L4	11	8.1	D80	66	42
E6	16	14	S81	130	130
E7	10	< 1	E82	43	42
I9[b]	< 1	6.0	E83	3.8	2.4
E14[b]	< 1	< 1	E84	< 1	2.9
D22	1.0	< 1	I85	2.4	< 1
T29	12	6.1	A102	1.4	1.0
K30	31	25	A103	17	8.1
T34	2.2	< 1	H107	c	< 1
S38	c	c	V108	c	c
L39	1.0	< 1	T110	2.9	< 1
G40	2.3	2.1	L112	11	< 1
Q41	1.1	c	G113	9.6	< 1
N42	120	150	E114	8.5	< 1
E45	23	11	K115	15	16
A46	11	6.4	L116	1.4	5.3
F65	2.4	< 1	D118	36	21
R74	1.0	1.1	E119	4.7	7.7
K75	4.8	26	T143	< 1	c
M76	16	17	T146	1.4	< 1
K77	26	72	S147	24	65
D78	63	130	K148	1.3	6.2

[a] Rates are only given for residues with $k_{HX} \geq 1\ s^{-1}$, either in M13-free CaM or in the CaM-M13 complex. Rates for A1 and D2 could not be measured.

[b] Residues may be interchanged because of overlap at pH < 7.

[c] $k_{HX} < 1\ s^{-1}$ could not be established because of overlap.

at pH 7.0. For residues that exchange very rapidly, e.g. Q3, accurate measurement of an attenuation factor is only possible at the lowest pH values. In this case, k_{NOE} may safely be neglected, and $k_{HX}=k$ is calculated from Eq. 3 directly. In Table 1, slowly exchanging residues that can be observed five minutes after the protein is dissolved in D_2O at p^2H 6.3, 25°C (Marion et al., 1989) are marked by asterisks. These residues exchange at rates smaller than 0.01 s^{-1}. A small increase in the measured k value with pH is seen even for some of these slowly exchanging residues (for example, residue I9). This increase reflects the weak pH dependence of k_{NOE} discussed above.

Using the approach illustrated for the N-terminal 34 residues of CaM we have measured the rates for all backbone amides both in M13-free CaM and in the CaM-M13 complex. Table 2 summarizes the results for all residues for which we could not establish unambiguously that k_{HX} is smaller than 1 s^{-1} at pH 7.0, either in M13-free CaM, or in the CaM-M13 complex.

DISCUSSION

Inspection of Table 2 shows that rapidly exchanging amides are clustered together. It is interesting to note, however, that a substantial number of the rapidly exchanging amides are hydrogen bonded in the X-ray crystal structure of calmodulin (Babu et al., 1988) and appear to be hydrogen bonded on the basis of a preliminary investigation of the NMR spectra (Ikura et al., in press). For example, in M13-free CaM both the X-ray and the NMR structure suggest that residues M76 and K77 are in an α-helical arrangement. Nevertheless, both show rapid exchange with solvent. The same is observed for residue T110 near the C-terminal end of helix F*. In the most popular model describing hydrogen exchange, some concerted local unfolding must take place before exchange can occur, i.e. the hydrogen bond must be broken (Englander et al., 1988). According to Englander et al., comparison of the exchange rate with the exchange rate occurring for a short peptide encompassing the same residues gives an indication about the fraction of the time that the hydrogen bond is actually present. Clearly, the small degree of protection observed for residues R74-I85 in the central helix of CaM suggests that this helix is not very stable. If amide exchange rates steadily increase when approaching the end of the helix, as seen at the N-terminus of the protein, this can be interpreted as fraying of the helix. If a cluster of sequential amides all show similarly high exchange rates, this can be interpreted as indicative of local unfolding events. It is interesting to note, however, that not all residues that are protected from amide exchange are necessarily hydrogen bonded. For example, most of the residues in the calcium-binding loops are not expected to be hydrogen bonded-based on the high-resolution X-ray structure of homologous loops in parvalbumin (Moews and Kretsinger, 1975), but nevertheless are more than 100-fold protected from amide exchange compared to the rates predicted for random coil peptides (Molday et al., 1972), indicating that, apart from hydrogen bond breakage, solvent accessibility is an important parameter determining the hydrogen exchange rate.

Complexation between CaM and a 19-residue myosin light chain kinase peptide has been studied previously by Seeholzer and Wand (1988). They observed that a much larger number of amide protons show very slow exchange in the CaM-peptide complex compared to peptide-free

* Regions of secondary structure observed in the crystal structure (Babu et al., 1988) are: helix A, T5-F19; helix B, T29-S38; helix C, E45-V55; helix D, F65-K75; helix E, M76-F92; helix F, A102-N111; helix G, D118-A128; helix H, Y138-K148. Calcium-binding loops span from D20-T28, D56-D64, D93-S101, and N129-N137.

CaM. In their study, hydrogens are defined as slowly exchanging if they remain for at least several hours after the protein is dissolved in D_2O. Their study indicates that slowly exchanging amide protons exchange even more slowly upon complexation of CaM with target peptide, i.e. complexation inhibits local unfolding. Our present study focuses on rapidly exchanging protons, mostly located at or near the surface of the protein and shows that, on average, there is little difference in the number of protons that exchange rapidly with water, on a time scale of seconds, when CaM is complexed with M13. However, several clear differences between CaM and CaM-M13 are also observed. For a number of residues in the C-terminal half of the 'central helix' the amide exchange rate increases upon complexation (K75 through T79). Other NMR evidence also strongly suggests that the 'central helix' is non-helical in the CaM-M13 complex between residues K75 and S81 (Ikura et al., in press). A pronounced decrease in the exchange rate for residues T110 and L112-E114 upon M13 complexation is indicative of stabilization of the C-terminal end of the F-helix which these residues are part of. In a model for the CaM-M13 complex proposed by Persechini and Kretsinger (1988) these residues come in close proximity to the M13 peptide and also close to the N-terminal half of CaM. This model also suggests that the end of the C-terminal helix of CaM gets distorted upon complexation with M13. Some support for this model may be found in increased amide exchange rates observed for the last two residues of this helix. The slow exchange rate frequently observed for the C-terminal residue in a protein may be ascribed to the negative charge on the carboxy-terminus, decreasing OH^- access to the adjacent amide (Rosevaer et al., 1985). It may therefore be speculated that the increase observed in k_{HX} of residue K148 is caused by an electrostatic interaction between the carboxy-terminus and a positively charged residue either of the target peptide or of the N-terminal domain of CaM.

Our present study shows that exchange rates of quite labile amides can be measured quantitatively in a very straightforward manner provided that ^{15}N labeled protein is available. These rates reflect structurally significant information on protein-ligand interaction that cannot be obtained by other NMR techniques such as the elegant and powerful pH-jump methods (Roder et al., 1988; Paterson et al., 1990; Hughson et al., 1990).

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REFERENCES

Babu, Y. S., Bugg, C. E. and Cook, W. J. (1988) *J. Mol. Biol.*, **204**, 191-204.

Bax, A., Griffey, R.H. and Hawkins, B.L. (1983) *J. Magn. Reson.*, **55**, 301-315.

Bax, A., Ikura, M., Kay, L.E. and Zhu, G. (1990) *J. Magn. Reson.*, **90**, in press.

Bendall, M.R., Pegg, D.T. and Doddrell, D.M. (1983) *J. Magn. Reson.*, **52**, 81-117.

Blumenthal, D. K., Takio, K., Edelman, A. M., Charbonneau, H., Titani, K., Walsh, K. A. and Krebs, E. G. (1985) *Proc. Natl. Acad. Sci. USA*, **82**, 3187-3191.

Englander, S.W. and Kallenbach, N.R. (1984) *Quart. Rev. Biophys.*, **16**, 521-625.

Englander, J.J., Englander, S.W., Louie, G., Roder, H., Tran, T. and Wand, A.J. (1988) In *Structure and Expression: From Proteins to Ribosomes*, (Eds, Sarma, R.H. and Sarma, M.H.) Adenine Press.
Henry, G.D., Weiner, J.H. and Sykes, B.D. (1987) *Biochemistry*, **26**, 3626-3634.
Hughson, F.M., Wright, P.E. and Baldwin, R.L. (1990) *Science* (Washington DC), **249**, 1544-1548.
Ikura, M., Kay, L. E. and Bax, A. (1990a) *Biochemistry*, **29**, 4659-4667.
Ikura, M., Kay, L. E., Krinks, M. and Bax, A., *Biochemistry*, in press.
Kainosho, M., Nagao, H. and Tsuji, T. (1987) *Biochemistry*, **26**, 1068-1075.
Kainosho, M. and Tsuji, T. (1982) *Biochemistry*, **21**, 6273-6279.
Krishna, N.R., Huang, D.H., Glickson, J.D., Rowan, R. and Walter, R. (1979) *Biophys. J.*, **26**, 345-366.
Marion, D., Ikura, M., Tschudin, R. and Bax, A. (1989) *J. Magn. Reson.*, **85**, 393-399.
Moews, P.C. and Kretsinger, R.H. (1975) *J. Mol. Biol.*, **91**, 201-228.
Molday, R.S., Englander, S.W. and Kallen, R.G. (1972) *Biochemistry*, **11**, 150-158.
Paterson, Y., Englander, S.W. and Roder, H. (1990) *Science* (Washington DC), **249**, 755-759.
Persechini, A. and Kretsinger, R. H. (1988) *J. Cardiovascular Pharmacology (Suppl.5)*, **12**, 1-12.
Roder, H., Elove, G.A. and Englander, S.W. (1988) *Nature*, **335**, 700-704.
Rosevaer, P.R., Fry, D.C and Mildvan, A.S. (1985) *J. Magn. Reson.*, **61**, 102-115.
Roy, S., Papastavros, Z., Sanchez, V. and Redfield, A. G. (1984) *Biochemistry*, **23**, 4395-4400.
Seeholzer, S. H. and Wand, A. J. (1989) *Biochemistry*, **28**, 4011-4020.
States, D.J., Haberkorn, R.A. and Ruben, D.J. (1982) *J. Magn. Reson.*, **48**, 286-292.
Waelder, S. and Redfield, A.G. (1977) *Biopolymers*, **16**, 623-629.
Wagner, G. and Wüthrich, K. (1982) *J. Mol. Biol.*, **169**, 325-344.