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A Calcium-43 NMR Study of Calcium Binding to an Acidic Proline-Rich Phosphoprotein from Human Saliva†

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ABSTRACT: The ^{43}Ca NMR line width measured for Ca^{2+} bound to protein A, an acidic proline-rich salivary protein, is 1 order of magnitude narrower than has previously been observed for other proteins of similar molecular weight. The correlation times, quadrupole coupling constants, and chemical shifts estimated for Ca^{2+} ions bound to the intact protein ($M_r \sim 10\,000$) and its 30 amino acid residue long acidic N-terminal TX peptide were indistinguishable within experimental error. These results—as well as the outcome of ^1H NMR relaxation rate measurements—are indicative of extensive motions for the protein residues, which in turn give rise to a high degree of flexibility for the protein-bound Ca^{2+}. Ca^{2+} titration and pH-dependent measurements on protein A, the TX peptide, and the dephosphorylated TX peptide established the importance of the two phosphoserine residues in the binding of Ca^{2+}. Moreover, a comparison of the ^{43}Ca NMR parameters with those obtained for other Ca^{2+}-binding proteins suggests the presence of Ca^{2+}-binding sites of similar symmetry in all these proteins. No evidence was found for a proposed interaction between the highly acidic N-terminal and the weakly basic C-terminal regions of protein A. In contrast, the high pH inflection that was observed in the pH titration curve for the intact protein was also found for the phospho and dephospho TX peptides, thus suggesting that basic moieties in the N-terminal region rather than those in the C-terminal region may be responsible for this observation.

Proline-rich proteins make up roughly 70% of the total proteinaceous material in human saliva. They constitute a unique superfamily of proteins (Wong & Bennick, 1981), which are also present in salivary secretions from other species (Muenzer et al., 1979; Oppenheim et al., 1979) and in small amounts in tracheobronchial tissue (Warner & Azen, 1984). Approximately 30% of the salivary proteins are "acidic" proline-rich proteins, the major ones being proteins A and C. The amino acid sequence of these two proteins has been determined (Wong et al., 1979; Wong & Bennick, 1981). As is shown in Table I, the N-terminal 30 residues of protein A, which is known as the TX peptide and is obtained by tryptic cleavage, contains 13 of the protein's 15 negatively charged amino acid side chains. This includes the two phosphoserine residues that are present. Only one proline residue is found in this region. The remainder of the protein (residues 31–106) consists almost entirely of glutamine, glycine, and proline

Table I: A Comparison of the Amino Acid Composition of Protein A with That of the TX Peptide

residue type	residue	no. of residues: TX peptide	no. of residues: protein A
acidic	Asp	6	8
	Glu	5	5
	P-Ser	2	2
basic	Lys	0	1
	Arg	1	4
	His	0	2
uncharged polar	Asn	1	3
	Gln	2	23
	Glu[a]	1	1
	Gly	2	20
	Ser	1	3
nonpolar	Ala	0	1
	Val	3	3
	Leu	2	3
	Ile	2	2
	Pro	1	24
	Phe	1	1

[a] The N-terminal residue is pyroglutamate.

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residues. Protein C encompasses the 106 residues of protein A plus a 44-residue extension on the C-terminus. Protein A is possibly the product of the proteolytic cleavage of protein C by kallikrein, an enzyme present in the salivary ducts (Wong et al., 1983).

The physiological role of the acidic proline-rich proteins appears to be associated with their ability to bind Ca^{2+}. Equilibrium dialysis measurements (Bennick, 1976, 1977; Bennick et al., 1981) suggested the presence of several Ca^{2+}-binding sites with binding constants in a range that is optimal to allow these proteins to function as a calcium buffer that maintains a constant concentration of free calcium in the saliva. Furthermore, the observation that proteins A and C inhibit the growth of hydroxylapatite (Moreno et al., 1979) suggests that one important function of these proteins may be to prevent the spontaneous growth of crystal onto the teeth. This is a rather important function since teeth are permanently surrounded by saliva, which is usually supersaturated with respect to calcium and phosphate ions. Previous work has demonstrated that the Ca^{2+}-binding properties of protein A are localized on the highly negatively charged N-terminal 30 residues (Bennick et al., 1981).

^{43}Ca NMR[1] represents a unique and completely ion-specific means of obtaining both qualitative and quantitative information about Ca^{2+} binding to proteins [for reviews, see Vogel et al. (1983), Braunlin et al. (1985), and Vogel & Forsén (1986)]. This method has primarily been applied to study the relatively tight binding of Ca^{2+} to intracellular calcium-binding proteins such as calmodulin, troponin C, and related proteins (Andersson et al., 1982; Drakenberg et al., 1983; Vogel et al., 1984, 1985; Vogel & Braunlin, 1985). However, also the weaker binding of $^{43}Ca^{2+}$ to prothrombin and to factor XIII (Marsh et al., 1979; Sarasua et al., 1982) as well as to phospholipase A_2 (Drakenberg et al., 1984) has been probed. Here, we report ^{43}Ca NMR measurements of Ca^{2+} binding to protein A and to the TX peptide. Also included here are some ^{1}H NMR selective and nonselective relaxation time measurements, which provide information relevant to the interpretation of the ^{43}Ca results.

Materials and Methods

Salivary protein A and the TX fragment of protein A were prepared as previously described (Bennick, 1975; Wong et al., 1979). The dephosphorylated form of the TX fragment was prepared by digestion with calf intestinal alkaline phosphatase, attached to beaded agarose (Sigma Chemical Co.). A total of 8 mg of the TX fragment was digested for 24 h at 37 °C in the presence of 25 units of phosphatase. Completion of the phosphomonoester cleavage was checked by comparing ^{31}P NMR spectra recorded before and after the incubation with phosphatase (Vogel & Bridger, 1982). The dephosphorylated fragment was then separated from inorganic phosphate and other small ions by gel exclusion chromatography on a Sephadex G-25 fine column (2.8 × 34 cm, distilled H_2O).

Isotopically enriched ^{43}CaO (60%) was obtained from Matreco (Södertälje, Sweden). All other chemicals used were of analytical grade.

^{43}Ca NMR measurements were performed at 24.3 MHz on a Nicolet WB-360 spectrometer equipped with a horizontal solenoid probe (Neurohr et al., 1983). Sample tubes were glass-blown cylindrical cells of approximately 3 mL. All samples were made up in ^2H_2O (99.8% from Ciba-Geigy), which was used to lock the spectrometer. All measurements were performed at ambient temperatures with a 90° pulse width of 40 μs, a spectral width of ±4000 Hz, and an acquisition time that varied between 0.128 and 0.512 s, depending on the line width. A spectrometer dead time of 200 μs was used, which resulted in a flat base line. The Ca^{2+} concentration in all NMR samples was determined by atomic absorption.

^{1}H NMR measurements were carried out at 361 MHz on a Nicolet WB-360 spectrometer, using standard 5-mm sample tubes (Wilmad). Samples were prepared in ^2H_2O, which was used for locking the spectrometer. A 90° pulse width of 11 μs was used, together with a spectral width of ±2500 Hz and an aquisition time of 0.819 s. For the nonselective T_1 measurements, the proton decoupler was used for HDO suppression. For the determination of selective T_1's, alternate delay accumulation (Roth et al., 1980) was employed to decrease the HDO signal, which allowed us to use the decoupler to supply the selective low-power pulse (for which we obtained a pulse width of 20 ms).

For both ^{1}H and ^{43}Ca NMR, T_1's were determined from three-parameter fits to inversion–recovery data (Nicolet 1280 software). ^{43}Ca line widths were obtained from fits to Lorentzian curves. The line widths reported here are corrected for magnetic field inhomogeneity contributions. ^{43}Ca NMR and ^{1}H NMR chemical shifts are referenced to 10 mM $CaCl_2$ and DSS, respectively. pH readings were not corrected for the ^{2}H isotope effect. All experiments were performed at 23 °C, pH 7.2, unless otherwise indicated.

Theory

Under normal conditions, the nuclear magnetic relaxation of ^{43}Ca (nuclear spin $I = {}^7/_2$) is dominated by the quadrupolar relaxation mechanism (Cohen & Reif, 1957). For the measurements reported here, the so-called extreme-narrowing condition always applies for ^{43}Ca NMR (see Results). Under these circumstances, and in the presence of fast exchange of $^{43}Ca^{2+}$ between free and protein-bound environments, the observed relaxation rate (R) is the weighted average of the free (R_f) and bound (R_B) relaxation rates:

$$R = \pi\nu_{1/2} = p_F R_F + p_B R_B$$

where $\nu_{1/2}$ is the observed line width at half-height and p_F and p_B are the fractions of free and bound $^{43}Ca^{2+}$. An analogous equation can be written for the ^{43}Ca chemical shift. For a spin $^7/_2$ nucleus such as ^{43}Ca, under extreme narrowing conditions

$$R_B = \frac{2\pi^2}{49}\chi_B^2\tau_c$$

where χ_B is the quadrupole coupling constant of the bound $^{43}Ca^{2+}$ and τ_c is the correlation time for the motions that modulate the quadrupole interaction. Roughly speaking, χ_B provides a measure of the symmetry of the Ca^{2+}-binding sites, whereas τ_c depends on the internal flexibility of the bound site or, if there is no such flexibility, on the overall rotational mobility of the protein molecule. In order to place reasonable bounds on the correlation time governing ^{43}Ca relaxation, we have performed ^{1}H selective and nonselective T_1 measurements. For the theory and relevant equations, the reader is referred elsewhere (Noggle & Schirmer, 1971). Although spin–diffusion or cross-relaxation in proteins normally precludes the use of ^{1}H NMR relaxation rate measurements for obtaining motional information (Kalk & Berendsen, 1976), a combination of selective and nonselective T_1 measurements allows one to extract the motional characteristics of the protein residues [see, for example, Valensin et al. (1982)].

[1] Abbreviation: NMR, nuclear magnetic resonance.

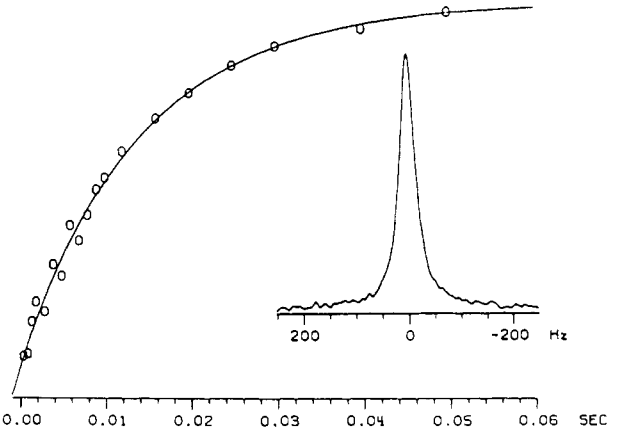

FIGURE 1: ^{43}Ca T_1 measurement for a solution containing 14.3 mg/mL protein A and 3.3 mM Ca^{2+}, pH 7.3, at 24 °C. The y axis of this graph represents peak heights in arbitrary units. A three-parameter least-squares fit gives T_1 = 0.014 s. The inset shows the spectrum obtained for the longest delay time. From the line width of this spectrum upon correcting for ~3-Hz inhomogeneity broadening, we obtain an apparent T_2 = 0.012 s.

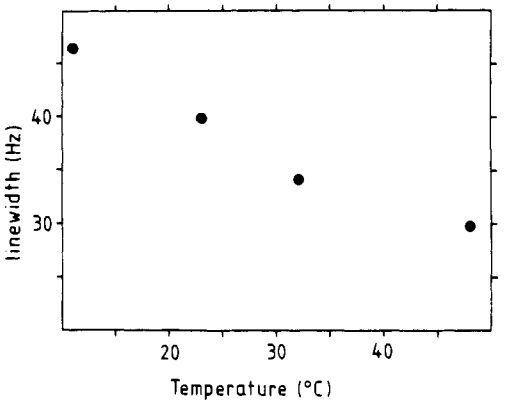

FIGURE 2: Temperature dependence of the ^{43}Ca line width for a solution containing 14.3 mg/mL protein A and 2.2 mM Ca^{2+}, pH 7.3.

RESULTS

^{43}Ca NMR of Saliva Protein A. In the presence of protein A, the $^{43}Ca^{2+}$ signal shifts downfield and broadens. The resonance could be readily fitted to a single Lorentzian curve under all experimental conditions examined here. Further, as illustrated in Figure 1, the ^{43}Ca longitudinal relaxation is well characterized by a single-exponential relaxation time, T_1. A comparison of the best fit T_1 with the transverse relaxation time T_2 calculated from the line width demonstrates that the extreme narrowing condition ($T_1 = T_2$) applies. As is shown in Figure 2, the temperature dependence of the ^{43}Ca resonance in protein A solutions implies that fast exchange conditions prevail.

Figure 3 shows the pH dependence of the $^{43}Ca^{2+}$ chemical shift (δ) and line width in the presence of protein A. Note that for both parameters the dependence on pH continues up to at least pH 10. The major pH-dependent transition occurs with a midpoint of pH 6.3, which is in good agreement with the pK_a's determined for the two phosphoserine groups, as measured by ^{31}P NMR (Bennick et al., 1981). The overall shape of the curve in Figure 3 is the same as previously determined for the pH dependence of Ca^{2+} binding (Bennick, 1976).

The results of a titration of protein A with $CaCl_2$ are presented in Figure 4. Upon fitting the chemical shift data to a single binding constant model, we obtain $K_a = 2 \times 10^3$ M^{-1} and a shift for the protein bound Ca^{2+} of δ_B = 8 ppm. From a similar fit of the line widths, we again obtain $K_a = 2 \times 10^3$ M^{-1} and a bound line width of 100 Hz. These best fits are shown as solid curves in the figure.

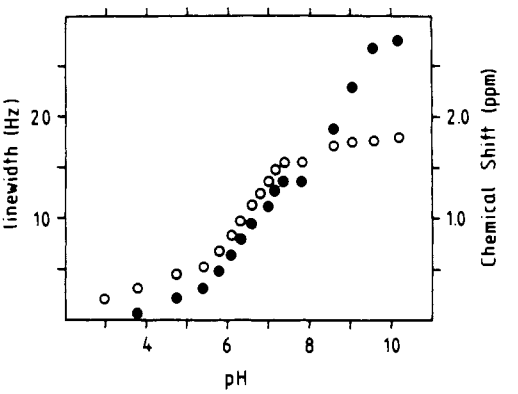

FIGURE 3: Dependence of the ^{43}Ca chemical shift (●) and line width (○) on pH. The solution contained 6 mg/mL protein A and 2.5 mM Ca^{2+}.

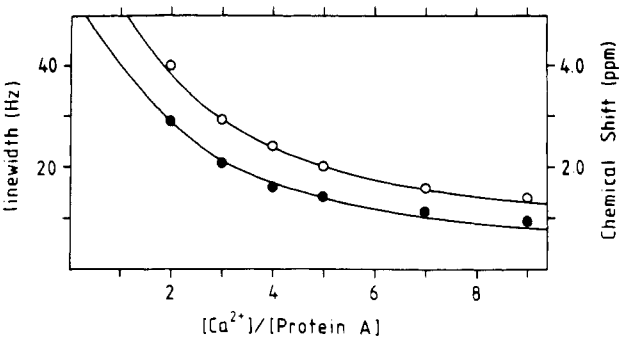

FIGURE 4: Dependence of the ^{43}Ca chemical shift (●) and line width (○) on the molar ratio of Ca^{2+}/protein for a solution containing 14.3 mg/mL (1.4 mM) protein A, pH 7.3.

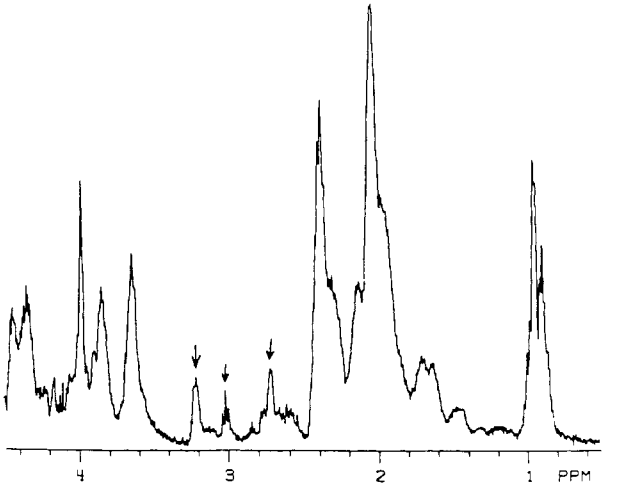

FIGURE 5: ^1H NMR of protein A. The protein A concentration was 9.4 mg/mL (0.9 mM) in solution with 6 mM Ca^{2+} at pH 7.2. Arrows indicate resonances for which T_1 measurements are reported in Table II.

^1H NMR of Protein A. The ^1H NMR spectra of protein A have previously been reported by Bennick and co-workers (Bennick et al., 1981). In Figure 5 we show for reference part of the room temperature ^1H NMR spectrum of protein A. The peaks at 2.7 and 3.2 ppm have been identified as corresponding to some of the H_β Asp and H_δ Arg (four residues) resonances, whereas the triplet at 3.0 ppm corresponds to the ϵ-protons of the single Lys-74 residue. The aromatic part of the spectrum is very simple (not shown), peaks around 7.3 ppm are

Table II: Proton Selective and Nonselective T_1 Measurements and Calculated Correlation Times for Resonances on Protein A

peak	chemical shifts (ppm)	selective T_1 (s)	nonselective T_1 (s)	τ_c (ns)
H-Asp	2.71	0.37	0.32	0.15
H-Lys	3.01	0.60	0.58	0.05
H-Arg	3.20	0.36	0.35	0.1
m-Phe	7.25	0.99	0.93	0.3
o-Phe	7.34	1.45	1.20	0.3

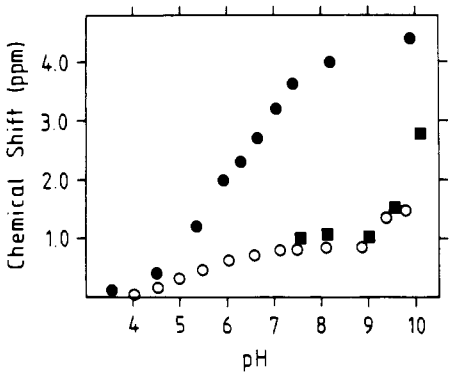

FIGURE 6: pH dependence of the ^{43}Ca chemical shift for solutions of 2.2 mg/mL TX peptide (0.6 mM) and 0.7 mM Ca^{2+} (●) and of 2.0 mg/mL dephosphorylated TX peptide and 4 mM Ca^{2+} with (■) and without (○) 2 mM EDTA.

due to the single Phe-25 residue. These may be partially resolved into a doublet at 7.25 ppm and a triplet at 7.34 ppm, which corresponds to the ortho and meta protons, respectively. Also seen in this region (at neutral pH) are two peaks at around 7.0 ppm and a broad resonance at 7.8 ppm due to the ring protons of the two His residues. Thus, particularly the Phe-25 and the Lys-74 residues provide convenient markers for determining the mobility of the N-terminal part vs. the rest of the protein. When selective and nonselective proton T_1 measurements are performed, the results shown in Table II are obtained. The nonselective inversion–recovery curves were in all cases well fitted by single-exponential relaxation rates, which would not be expected if cross-relaxation effects were significant for this protein. Further support for this notion is that, for the resonances examined, only minor differences are found between selective and nonselective T_1's. Also included in Table II are τ_c values calculated from the relaxation data, assuming the so-called rigid rotor nearest-neighbor model[2] (Jardetzky & Roberts, 1981). Although this model is too simple to characterize the motions of protons in a flexible protein, it does provide a lower bound on the correlation time appropriate for the ^{43}Ca relaxation. This will be discussed in more detail below.

^{43}Ca NMR of the TX Peptide. Under equivalent conditions, the chemical shifts and line broadening of the $^{43}Ca^{2+}$ resonance induced by the TX peptide are comparable to those obtained for the intact protein. Also, as is shown in Figure 6, the pH dependence of the $^{43}Ca^{2+}$ chemical shift is qualitatively similar to that previously seen for the entire protein (compare Figure 3). Note in particular the continued increase in δ at higher pH and the major transition at around 6.3. Interestingly, as is also shown in Figure 6, a high pH transition is seen for dephosphorylated TX as well. For the latter, following a steep decrease in going from pH 10 to pH 8, a distinct leveling off is observed prior to the final transition, which occurs with a midpoint of around pH 5. Figure 7 shows the results of $CaCl_2$ titrations of TX and dephosphorylated TX. The solid curve shows the best fit to the TX chemical shift for $K_a = 1.4 \times 10^3$ M^{-1} and $\delta_B = 10$ ppm. The line-width data give $K_a = 1.0 \times 10^3$ M^{-1} and $\nu_\beta = 95$ Hz. For the dephosphorylated TX peptide, the binding of Ca^{2+} is much weaker, and it was not possible to perform measurements at low enough Ca^{2+} concentrations to accurately determine binding parameters. Assuming a similar δ_B for the phosphorylated and dephosphorylated protein, one can estimate by using the data of Figure 7 that $K_a < 100$ M^{-1}.

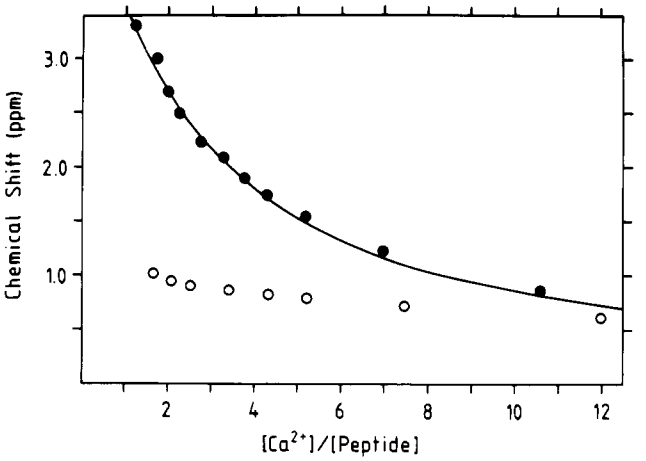

FIGURE 7: Dependence of the ^{43}Ca chemical shift on the molar ratio of Ca^{2+}/peptide for 2.2 mg/mL phosphorylated (●) and 2.0 mg/mL dephosphorylated (○) TX peptide, pH 7.2 in both cases.

Discussion

Ca^{2+}-Binding Behavior. From the pH dependence of the ^{43}Ca spectral parameters, and in particular from a comparison of the results for the phosphorylated and dephosphorylated TX peptide, it is readily apparent that the two phosphoserine residues play a major role in the binding process. The Ca^{2+}-binding data obtained here are in good agreement with those previously obtained by equilibrium dialysis (Bennick, 1976; Bennick et al., 1981). These earlier measurements indicated the existence of between five and eight Ca^{2+}-binding sites per protein (or peptide). These sites could further be divided into one to three stronger sites ($K_a \sim 3 \times 10^4$ M^{-1}) and four to five weaker sites ($K_a \sim 10^3$ M^{-1}). The measurements reported here, though best fitted assuming a single type of binding site ($K_a = 2 \times 10^3$, $\chi^2 = 0.018$),[3] are also well fitted to a model of one strong ($K_a = 3 \times 10^4$ M^{-1}) and five weak ($K_a = 10^3$ M^{-1}) binding sites ($\chi^2 = 0.021$) However, the data are poorly fitted under the assumption of several independent and identical binding sites ($\chi^2 = 1.73$).[4]

By equilibrium dialysis it was found that a larger amount of Ca^{2+} binds to the TX peptide than to protein A. This suggested a significant interaction between the N-terminal Ca^{2+}-binding region of protein A and the C-terminal region (which cannot by itself bind Ca^{2+}). It is tempting to speculate

[2] In performing these calculations, we assumed a distance of 1.78 Å between protons of CH_2 groups, 2.47 Å between Phe ring protons, and 2.5 Å between two protons on neighboring carbons in an aliphatic chain. Note that since according to this model the nonselective T_1 passes through a minimum with increasing τ_c, the observed nonselective T_1 alone is not sufficient to define τ_c.

[3] χ^2 is the error square sum.

[4] Normally it is difficult to differentiate between a model with one binding site and a model with several binding sites of identical or similar affinity. However, in this case measurements could be performed at Ca^{2+} levels similar to the protein concentration, and consequently, the shape of the first part of the Ca^{2+} titration curve was extremely dependent on the total amount of sites.

that there might exist an ionic attraction between the C-terminal region, which is on the whole positively charged, and the highly negative charged N-terminal region. This interaction could explain the continuous increase in the ^{43}Ca spectral parameters above pH 7. However, a similar dependence is seen for the TX peptide and even more markedly for the dephosphorylated TX peptide. The data for the dephosphorylated peptide in particular indicate the involvement of a group with a pK_a of 9 or higher. In addition to the N-terminal group, the most reasonable candidate is the only basic residue on the TX peptide, the C-terminal Arg. This Arg may be more mobile in the peptide than in protein A, and the high pH inflection observed for protein A is therefore not necessarily due to Arg-30 but may be caused by an interaction of other Lys or Arg residues in protein A with the N-terminal end. A similar high pH inflection has been reported for another highly negatively charged protein phosvitin (Vogel, 1983). In that case, this observation was explained by assuming either interactions with basic amino acid side chains or the titration of a proton that had a high pK_a because of the high negative charge on the protein.

^{43}Ca Correlation Times, Quadrupole Coupling Constants, and Chemical Shifts. The line width determined in these experiments for $^{43}Ca^{2+}$ bound to saliva protein A ($\sim$100 Hz) is much narrower than any previously observed for Ca^{2+}-binding proteins (Vogel et al., 1983; Braunlin et al., 1985). In particular, it is about 1 order of magnitude narrower than that observed for $^{43}Ca^{2+}$ bound to parvalbumin, a protein of comparable molecular weight. From eq 2 this narrowness of the bound line width implies (1) a small value for χ_B and thus a high degree of symmetry of the Ca^{2+} binding site and/or (2) a short τ_c and thus a high degree of flexibility of the binding site. The measurements reported here are strongly in support of the latter explanation.

In the case of the Ca^{2+}-binding protein parvalbumin, the differences observed between the transverse and longitudinal relaxation rates allowed a calculation of the correlation time dominating ^{43}Ca relaxation (Andersson et al., 1982). In that case, the value obtained (4 ns) agreed well with the rotational correlation times for rigid spherical proteins calculated from the Stokes–Einstein equation. In contrast, for $^{43}Ca^{2+}$ bound to protein A the transverse and longitudinal relaxation rates are equal to within experimental uncertainty. This observation indicates that the relevant τ_c dominating the relaxation of $^{43}Ca^{2+}$ is probably significantly less than the value of 3 ns calculated from the Stokes–Einstein equation for saliva protein A, on the assumption that it would be a spherical protein.

Even stronger evidence for a high degree of mobility of the Ca^{2+}-binding site is that the bound $^{43}Ca^{2+}$ line width obtained for protein A (100 Hz) is roughly the same as that determined for the TX fragment, despite the large difference in molecular weight between these two species. Since the approximate equality of the Ca^{2+}-binding strengths and chemical shifts for the bound ions suggests little difference in the nature of the Ca^{2+}-binding sites, the quadrupole coupling constants for the bound Ca^{2+} ions and as a consequence the relevant correlation times for these two species should likewise be very similar. For the TX peptide we can calculate a "maximal" rotational correlation time of 1 ns assuming a rigid spherical molecule. We conclude that the effective correlation time for $^{43}Ca^{2+}$ relaxation is probably, for both protein A and the TX fragment, less than 1 ns.

Our proton T_1 measurements provide further evidence for significant mobility of the amino acid residues both in the N-terminal Ca^{2+}-binding region and in the remainder of the molecule. Thus, for the ^1H resonances examined, selective as well as nonselective relaxation measurements are well fitted to single-exponential curves. Moreover, they are about equal. Since the proton relaxation includes the effects of motions not contributing to the ^{43}Ca relaxation, the effective correlation times listed in Table II provide a lower bound on the effective correlation time for ^{43}Ca relaxation. Taking the average of the values given in Table II, this implies that, for ^{43}Ca relaxation, $\tau_c \geq 0.2$ ns.

From these bounds on the ^{43}Ca correlation time ($0.2 \text{ ns} < \tau_c < 1$ ns), using eq 2 we can likewise place bounds on the quadrupole coupling constant χ of $2.0 \geq \chi_B \geq 0.9$ MHz assuming a single Ca^{2+} site per protein. If we assume the presence of one strong and five weak protein Ca^{2+}-binding sites (a total of six sites) as indicated by the equilibrium dialysis data, then the bounds become $1.4 \geq \chi_B \geq 0.6$ (under the assumption that all six sites have the same χ_B). For model compounds, $^{43}Ca^{2+}$ coupling constants have been found in the range $3 \geq \chi_B \geq 0.5$ (Drakenberg & Forsen, 1983). Thus, the bounds we obtain for τ_c also provide reasonable bounds on χ_B, so that it is not necessary to assume an unusually low χ_B to explain the narrowness of the bound ^{43}Ca line width.

The chemical shifts for Ca^{2+} bound to protein A and the TX peptide were virtually identical (8 and 10 ppm). Since similar chemical shifts are found for Ca^{2+} bound to most other proteins (Vogel et al., 1983), this suggests that the surroundings provided by protein for the bound Ca^{2+} ions is not very different in these proteins. Likewise, since the quadrupole coupling constants measured here are very comparable to those measured for other proteins (Drakenberg & Vogel, 1983), it is clear that human saliva protein A does not contain Ca^{2+}-binding sites of unusual symmetry.

Conclusions

A comparison of the ^{43}Ca NMR results obtained for protein A and the TX peptide, combined with proton NMR longitudinal relaxation time measurements, all indicate that the acidic proline-rich protein A is a very flexible protein. Our measurements have also helped in characterizing the groups that are directly involved in Ca^{2+} binding. In particular, we have confirmed the importance of the two phosphoserine residues, and we have discussed the probable involvement of Arg-30. We have not been able to find any evidence for interactions between the N-terminal and C-terminal regions of protein A.

Though ^{43}Ca NMR has proven its utility in the study of Ca^{2+}-binding proteins with structured well-defined Ca^{2+}-binding sites (Vogel et al., 1983; Braunlin et al., 1985; Vogel & Forsén, 1986), very little is known about the molecular details of the interactions between metal ions and the highly charged surfaces of polyelectrolyte proteins such as phosvitin (Vogel, 1983; Braunlin et al., 1984), rat incisor dentine protein (Cookson et al., 1981), and the F1 fragment of the blood-clotting protein prothrombin (Marsh et al., 1979) and human factor XIII (Sarusua et al., 1982). The interactions of Ca^{2+} with such proteins play crucial roles in important biological processes as diverse as blood clotting (Stenflo & Suttie, 1977), bone and tooth mineralization (Linde, 1982), and Ca^{2+} transport to the egg during oogenesis (Wallace, 1970). Despite their relevance, only a few detailed NMR studies have been reported to date. Significantly, phosphoserine or carboxyglutamate residues appear to play a role in Ca^{2+} binding of these proteins. In addition, a high-pH (9–10) inflection has been found in some instances, suggesting the existence of interactions between these negatively charged Ca^{2+}-binding and other positively charged parts of the protein. It has been suggested that the metal ions can diffuse over the surface of

these proteins (Cookson et al., 1981), which would lead to long-lived metal ion–protein complexes; however, we have found fast exchange conditions in the case of human saliva protein A. As is exemplified by the present work, ^{43}Ca is not only a useful tool for the study of Ca^{2+} binding to proteins such as calmodulin or troponin C, but it can also be profitably applied to study the weaker and less defined binding of Ca^{2+} to polyelectrolyte proteins.

Registry No. ^{43}Ca, 14333-06-3; Ca, 7440-70-2.

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