tively.[17] The very low temperature required for these acid-catalyzed migrations contrasts dramatically with those necessary for purely thermal [1,3]sigmatropic shifts. We are presently investigating the stereochemistry of these rearrangements.

Acknowledgments. We thank the donors of the Petroleum Research Fund, administered by the American Chemical Society, for a grant in support of this research.

(17) The term "concerted" is used here in an operational sense, implying the absence of intermediates, such as normal carbenium ions or radicals, which can be intercepted. The possibility of a shallow minimum in the reaction surface (a π-complex, for instance) is not excluded.

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Carbon Magnetic Resonance Study of the Conformational Changes in Carp Muscle Calcium Binding Parvalbumin

Sir:

The subtle conformational changes associated with the release of a calcium ion from the parvalbumin protein of carp have been investigated by carbon-13 nuclear magnetic resonance spectroscopy. This communication discusses the structural changes which occur in that part of the protein molecule made up of aromatic residues as monitored by measurement of chemical shift changes as well as nuclear Overhauser enhancement (NOE) contributions to signal intensity and spin–lattice relaxation times (T_1). Parvalbumin function is usually discussed in terms of control processes affected by calcium ion concentration such as muscle contraction[1,2] and glycogenolysis.[3] These proteins are low molecular weight (12,000), calcium binding ($2Ca^{2+}$/protein) monomeric species with unusual amino acid composition (10% phenylalanine, 20% alanine) found in the white muscle of vertebrates.[1,4]

The natural abundance carbon-13 nuclear magnetic resonance spectrum of parvalbumin component 3 with two bound calcium ions is presented in Figure 1a.[5] Many resonances are resolved in this spectrum including an unusually downfield shifted carboxyl carbon (184.6 ppm), two electron shielded carbonyl carbons (168.9 and 170.9 ppm), the guanido carbon of the single arginine residue (158.5 ppm), as well as some individual resonances arising from the Cγ carbons of the ten phenylalanine residues (134–139 ppm) and the δ-methyl carbons from the five isoleucine residues (10–13 ppm). Individual resonances were identified as arising from single carbons by comparison of integrated intensities to a known one carbon signal (*i.e.*, Arg guanido) in a spectrum without NOE contributions to signal intensities.[6]

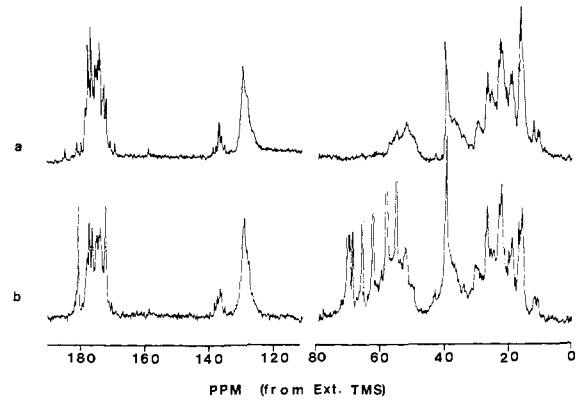

Figure 1. Proton decoupled natural abundance carbon-13 nuclear magnetic resonance spectra of parvalbumin component 3 (pH 7.8), obtained at 25.2 MHz with a Varian XL-100 spectrometer equipped with a Nicolet Technology Corp. pulse unit and data system. Free induction decays were collected following 90° rf pulses at 1.245-sec intervals. Data points (16K) were sampled, with an additional 16K points of zero added. The entire data table was multiplied by an exponential corresponding to 2.0 Hz line broadening followed by Fourier transformation: (a) parvalbumin (20 m*M*) with two bound calcium ions, 50,000 transients collected; (b) parvalbumin (15 m*M*) after the removal of the solvent accessible calcium by addition of EGTA. The additional large peaks in the spectrum are due to the added chelating agent. Transients (75,000) were collected.

The crystal structure[7] shows that the calcium ion which can be first removed from the protein by EGTA[8,9] addition is exposed to the solvent. It is coordinated through the carboxyl groups of aspartic acid residues 90, 92, and 94 and glutamic acid residue 101 as well as the carbonyl oxygen atom of lysine 96. Removal of this calcium gives rise to many spectroscopic changes (Figure 1b), most obviously the loss of the previously noted downfield carboxyl and the most upfield shifted carbonyl. The large changes in the chemical shift of the carboxyl carbon from 184.6 ppm to the overall carbonyl envelope is most likely due to the removal of the side chain of glu-81 from an internal salt bridge to Arg-75, a change previously postulated on mechanistic grounds.[7,10] This change represents a specific rearrangement of residues located more than 20 Å from the calcium binding site. The loss of the upfield carbonyl signal can tentatively be attributed to the removal of the carbonyl group of Lys-96 from the coordination sphere of the calcium ion. Such coordination through a carbonyl function to an alkaline earth metal is predicted to decrease the polarity of the carbon–oxygen bond, which increases the electronic shielding at the carbonyl carbon atom.[11] Additional structural changes, transmitted over large distances relative to the Ca^{2+} binding site, are reflected in the aliphatic region, most notably in the alanine methyl carbon region (15–18 ppm) and in the isoleucine δ-methyl carbon region.

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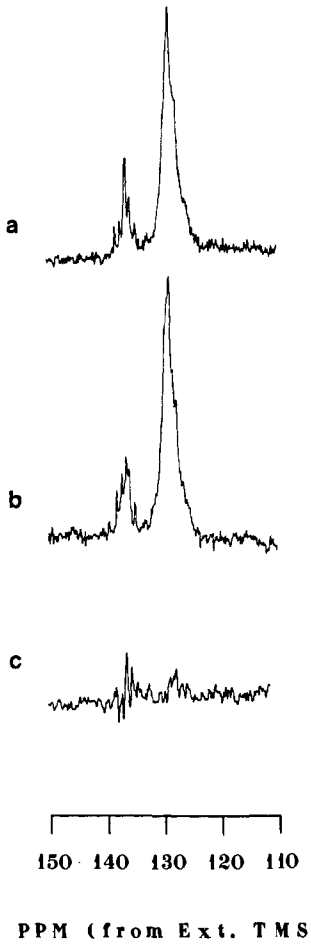

Figure 2. Aromatic region of the carbon-13 spectra from Figure 1. Figure 2a corresponds to the phenylalanine resonance from the parvalbumin protein in the presence of excess Ca^{2+}. Figure 2b shows the region from the parvalbumin with approximately one Ca^{2+} per molecule. Figure 2c illustrates the difference plot, obtained by subtracting the normalized free induction decays from which the spectra in 2a and 2b were obtained.

The spectral changes in the region 100–150 ppm of the difference plot (Figure 2c) can be correlated with changes in the phenylalanine ring carbons. The signals from the C_γ carbons yield a pattern indicative of substantial chemical shift changes while the resonances arising from the other ring carbons show relatively small changes. The most likely explanation for this differential behavior is that the release of calcium disrupts one of the six helical regions of the molecule[10] and this primary change causes secondary structural perturbations. The transmission of these changes through the protein connecting structure is then reflected to a larger extent in the carbons nearest the polypeptide backbone. The carbons further removed from the backbone are, in this case, positioned in a closely packed hydrophobic core of the protein[7] and are undergoing little change in environment.

Table I lists the values of T_1 measured by the inversion-recovery technique[12] and the NOE values determined with the gated decoupler method.[6] Since the measurements are made under conditions of complete proton decoupling, each carbon whose relaxation is dominated by dipolar interactions with a directly attached hydrogen has a well-defined T_1 and NOE which can be calculated by several methods for macromolecules. We utilize the development of Doddrell, *et al.*,[13] where values of relaxation parameters are calculated by assuming an angle and a rate of rotation.

Table I. Nuclear Overhauser Enhancement (NOE) and Spin–Lattice Relaxation Time (T_1) Measurements of the Parvalbumin Aromatic Region

Phenylalanine ring carbon type	$(2Ca^{2+}$/parvalbumin)[a] T_1, msec	NOE	$(Ca^{2+}$/parvalbumin)[b] T_1, msec	NOE
C_γ	350 ± 20	1.0	450 ± 50	1.0–1.1
C_δ, C_ϵ, C_ξ	55 ± 5	1.4	55 ± 5	1.4

[a] Native parvalbumin component 3 in the presence of excess Ca^{2+}. [b] Calcium content per molecule was determined by atomic absorption measurement of total Ca^{2+}, knowledge of total EGTA added, knowledge of protein concentration, and observation of separate nmr signals of measurable intensity of EGTA with and without bound Ca^{2+}.

The overall reorientation time of the protein is 12 nsec.[14] If the phenylalanine residues are rigidly attached to the protein and have the same isotropic correlation time, then the calculated T_1 values are in good agreement with the measured values. The calculated NOE for this isotropic motion is 1.15, less than the measured value of 1.4. This relatively large NOE can be attributed to the phenylalanine rings possessing an additional degree of rotational motion. With an axis of rotation about the C_γ–C_β bond, the data can be accounted for with the internal rotation having a correlation time of about 4 nsec. Rotation about the C_β–C_α bond of aromatic residues of proteins is probably limited by the steric crowding of hydrophobic structural regions.[15,16]

The internal rotation model is consistent with previously suggested functional and structural descriptions of this internal hydrophobic unit,[17] since the motion is constrained in direction. The movement might well be a limited libration at the calculated rate, allowing this part of the protein to function as a major stabilizing force. These data also indicate that while the chemical shift changes show many alterations of environment throughout the protein, there are no large changes in the overall rigidity of the protein structure accompanying the specific translocation of residues associated with calcium release, since the relaxation values are the same for both conformational states.

Acknowledgment. The support of this study by the National Science Foundation (Grant GB-32025) and

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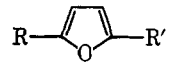
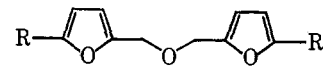
The Furanyl Unit in Host Compounds[1]

Sir:

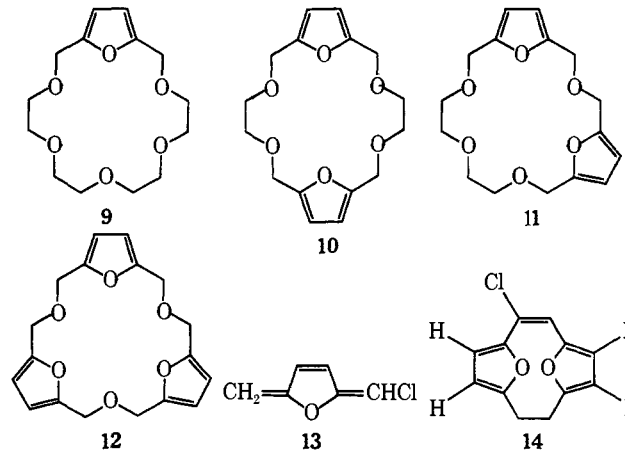
The 2,5-dimethylyltetrahydrofuranyl unit is commonly encountered in antibiotics which complex cations and affect their permeability to natural and synthetic membranes.[2] Most of these antibiotics, generally isolated from various *Streptomyces* strains, uncouple oxidative phosphorylation in rat liver mitochondria. This paper reports the first synthesis of a series of 18-crown-6[3] compounds containing furanyl units spaced in many possible ways as part of the multiheteromacrocycles. These compounds are themselves hosts for binding organic and inorganic cations. More importantly, they serve as starting materials for preparing host compounds whose periphery is lined with a variety of binding and shaping units (*e.g.*, tetrahydrofuranyl).

1, R = CHO, R′ = CH_2OH
2, R = R′ = CH_2OH
3, R = R′ = CH_2Cl
4, R = CHO; R′ = CH_2Cl
5, R = CHO; R′ = $CH_2OCH_2CH_2Cl$
6, R = CH_2OH; R′ = $CH_2OCH_2CH_2Cl$

7, R = CHO
8, R = CH_2OH

Key starting material, **1**, was prepared (41%) from sucrose[4] and in some cases was distilled (62%, mp 28–32°)[5] just before use.[6b,c] Reduction of crude **1** with sodium borohydride gave (92%) **2**,[6b,c,7] mp 75–76.5°. Dropwise addition with stirring of a solution of **2** in 2,6-lutidine to a stirred solution of thionyl chloride in ethyl acetate at −20° gave a frozen solid that was slowly warmed to 25° and stirred for 1 hr. Water and pentane were added, and the unstable **3**[6b,c,8] was isolated at low temperature without distillation and used immediately. Chloroaldehyde **4**[6b,c,9] was also prepared[4] (50%) from sucrose. Treatment of **4** with 2-chloroethanol and barium carbonate at 70° for 16 hr gave after distillation (bp 117° at 0.4 mm) **5**[6] (87%). Reduction of **5** in ethanol with sodium borohydride gave (97%) **6**[6], bp 104–105° at 0.2 mm. Water was azeotropically distilled (4 hr) from crude **1** in toluene containing 0.2% *p*-toluenesulfonic acid to give after chromatography, **7**,[6,10] mp 112–114° (44%). Reduction with sodium borohydride in ethanol of **7** gave (98%) of **8**,[6] mp 92–93°.



Multiheteromacrocycle **9** was prepared by treatment of tetraethyleneglycol ditosylate with diol **2** in tetrahydrofuran–potassium *tert*-butoxide (12 hr at 25°, 12 hr at reflux under nitrogen). The crude product was chromatographed on alumina (dichloromethane–ether) to give **9**[6] (36%), mp ~0°. Treatment of a dimethylformamide solution of chloroalcohol **6** with sodium hydride in portions gave a mixture which was stirred for 48 hr at 25°. Cycle **10**[6] was isolated by extraction and chromatography (11%), mp 109–111°. Cycle **11**[6] was prepared from diol **8** and diethylene glycol ditosylate (see preparation of **9**) to give product (35%), mp 69–70°. Dropwise addition of a solution of **3** in tetrahydrofuran to a stirred tetrahydrofuran solution of **8** and potasssium *tert*-butoxide gave after 48 hr reaction time at 25° a mixture of materials. These were separated by chromatography (dichloromethane–pentane on alumina) to give 70% recovered **8**,[6c] 10% cycle **12**,[6] mp 124–126°, and **14**[6b,c] (29%), which required gel permeation chromatography for purification (oil). Above 52°, in the pmr spectrum of **14** ($CDCl_3$, 100 MHz, δ), the methylenes are a sharp singlet (2.60), which at 0° become an AB quartet, V_A = 2.03, V_B = 3.23 (J_{AB} = 10 Hz), the coalescence temperature being *ca.* 30°. Apparently the members of each pair of vicinal protons have the same chemical shifts, but the geminal protons do not for conformational reasons. The ring-system constraints inhibit equilibration of the geminal protons at lower temperatures. Cycle **14** probably arose by ring closure of a diradical formed by head-to-head dimerization of **13** (formed from **3**)[11] followed by elimination of 1 mol of hydrochloric acid from the product.

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