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Physicochemical and Optical Studies on Calcium- and Potassium-Induced Conformational Changes in Bovine Brain S-100b Protein†

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ABSTRACT: The brain-specific S-100 protein is a mixture of two components, S-100a and S-100b, with a subunit composition of $\alpha\beta$ or β_2, respectively. S-100b, isolated by using hydroxylapatite chromatography in its final purification, is homogeneous by the criteria of gel electrophoresis in the absence and presence of sodium dodecyl sulfate ($NaDodSO_4$) and ultracentrifuge studies. Molecular weight studies by both sedimentation equilibrium in 6 M guanidine hydrochloride and 15% $NaDodSO_4$ gels indicated the subunit molecular weight to be 10 500, and since a molecular weight of 21 000 was obtained in native solvents, the protein exists as a dimer in benign medium. The two subunits are held together by noncovalent forces. The S-100b protein undergoes a conformational change upon binding calcium, as revealed by ultraviolet difference spectroscopy and circular dichroism (CD) studies in the aromatic and far-ultraviolet (UV) range. Far-UV CD studies indicated the apparent helical content drops from ~58 to 52% in the presence of Ca^{2+}. The effect of K^+ on the protein was antagonistic to Ca^{2+}, and the protein's affinity for calcium was lowered by the presence of K^+. The conformational state of the protein is very much dependent upon the metal ions (Ca^{2+}, K^+) present, suggesting that changing conformation may be the way S-100 responds to local changes in ionic parameters. Fluorescence studies indicate the presence of an abnormal tyrosine in the protein with the emission maximum centered between 327 and 330 nm when the protein is excited at 280 nm.

The brain-specific S-100 protein, so named because it is soluble in 100% saturated ammonium sulfate at neutral pH and found mainly in the cytoplasm of glial cells (Moore, 1965), is a mixture of two components with a subunit composition of $\alpha\beta$ and β_2 (Isobe & Okuyama, 1981). It represents up to 0.2% of the total soluble brain protein and is also present to a small extent in the nuclei of neurons (Hyden & Lange, 1970; Michetti et al., 1974). Nearly 5% of it is bound to the membrane (Haglid & Stavrou, 1973; Rusca et al., 1972). The two predominant components of this group of proteins are generally referred to as S-100a and S-100b. The exact biological function of S-100 is not known. Studies on rat brain S-100 levels during development suggest that it is related to the maturation and, possibly, differentiation of glial cells (Labourdette & Mandel, 1978). Other workers have implicated S-100 in the control of membrane permeability to rubidium ions (Calissano et al., 1974) and in controlling synapse activation (Hyden, 1974). The large quantities of S-100 present in the brain suggest that it plays a major role in the function of the central nervous system.

The amino acid sequences of S-100 proteins have been determined (Isobe & Okuyama, 1978, 1981). Sequencing and molecular weight studies on S-100b suggested the protein was made up of two identical polypeptide chains (Isobe & Okuyama, 1978). The S-100b protein polypeptide chain (β subunit) consists of 91 amino acid residues and has a molecular weight of 10 507. The sequence shows regions of strong clustering of hydrophobic, basic, and acid amino acids. The acidic cluster is highly homologous to the sequence of one of the calcium binding sites of muscle troponin C. This sequence region is also similar to the E-F loop structure of carp muscle parvalbumin, a characteristic feature of calcium binding proteins, suggesting a possible evolutionary relationship among these proteins. The α subunit consists of 93 amino acid residues with a molecular weight of 10 400. It possesses an extensive sequence homology (58%) with that of the β subunit and also shares an apparent calcium binding site in the C-terminal half of the molecule, implying a close evolutionary relationship between these subunits.

Even though sequence data are available for the S-100 proteins, physical and spectral work has not been carried out on the purified S-100 protein components to date. Earlier work on this system (Calissano et al., 1969, 1976) was carried out on the mixture of S-100 proteins. Hence, the conclusions arrived at have to be taken with due caution. For example, in the earlier work, the Ca^{2+} binding property of this group of proteins was monitored by tryptophan fluorescence. However, the recent sequence data have revealed that only S-100a has a tryptophan, whereas S-100b lacks tryptophan. In addition, the earlier work indicated a value of 7000 as the subunit molecular weight, while the present sequence data suggest a value of 10 500. Such discrepancies in results clearly establish the need to purify the protein components and to look more critically on the purified members, by using physical and optical methods in order to understand their possible structure–function relationship. Such results obtained with the purified system should enable one to compare the calcium binding properties of S-100 proteins with other calcium binding proteins, namely, troponin-C (Tn-C) of muscle and calmodulin, another calcium binding protein present in the brain. The present investigation documents such an attempt with the S-100b protein.

Materials and Methods

The initial extraction procedure used to prepare S-100b protein from beef brain was essentially that used for calmodulin by Watterson et al. (1976). The 40–65% ammonium sulfate fraction, after the heat treatment, was applied to a DEAE-cellulose[1] column. The protein was eluted with a

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[1] Abbreviations: $NaDodSO_4$, sodium dodecyl sulfate; Gdn·HCl, guanidine hydrochloride; DTT, dithiothreitol; CD, circular dichroism; DEAE, diethylaminoethyl; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid.

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0–0.45 M NaCl gradient. The protein peak corresponding to S-100b was then applied to a Sephacryl S-200 gel filtration column. After the protein was run through this column, the main contaminants were calmodulin and the S-100a component. Final purification was achieved on a hydroxylapatite column (Bio-Rad), and the protein was eluted with a 0–0.65 M ammonium sulfate gradient.

Standard polyacrylamide gel electrophoresis was performed at pH 8.5 with the Tris–glycine buffer described by Schaub & Perry (1969). Sodium dodecyl sulfate ($NaDodSO_4$)–polyacrylamide gel electrophoresis and molecular weight determination were carried out according to Weber & Osborn (1969). Amino acid analyses were performed on a Beckman 121 amino acid analyzer as described earlier (Mani et al., 1974).

Protein concentrations were determined in the ultracentrifuge by employing the Rayleigh interference optical system, assuming 41 fringes equivalent to 10 mg/mL (Babul & Stellwagen, 1969). From a plot of the number of fringes vs. optical density, a value of 2.4 was established as the extinction coefficient, $E^{1\%}_{1cm,278nm}$, for this protein.

The absorption spectra of S-100b samples used were measured on a Cary 118C recording spectrophotometer over the wavelength range 350–250 nm. Difference spectra were obtained on the same instrument in 1-cm path-length cells. Aliquots of concentrated perturbant, either $CaCl_2$ or KCl, were added to the sample cell, while an equivalent volume of water was added to the reference cell. Protein concentrations employed were in the range 0.5–1.5 mg/mL. In most cases, the instrument was operated in the "Autoslit" mode and full-scale absorbance of 0.05. Scan speeds from 0.02 to 0.1 nm/s were employed, and the spectra were measured at 25 °C with a Lauda thermoregulator.

Ultracentrifuge studies were routinely done at 5 °C in a Beckman Spinco Model E ultracentrifuge equipped with a photoelectric scanner, multiplex accessory, and high-intensity light source. The Rayleigh interference optical system was also employed. Low-speed sedimentation equilibrium runs were performed according to Chervenka (1969). Sedimentation velocity experiments were carried out by employing the schlieren optical system with a rotor velocity of 60 000 rpm and a temperature of 20 °C. A partial specific volume of 0.73 mL/g was assumed in all the hydrodynamic calculations.

Circular dichroism measurements were made on a Cary Model 6001 circular dichroism attachment to a Cary 60 recording spectropolarimeter, in accordance with previously described methodology (Mani et al., 1974). A Perkin-Elmer Model MPF-44B recording spectrofluorometer was used for fluorescence measurements. The sample compartment was water jacketed and connected with a Lauda thermoregulator, and measurements were conducted at 25 °C. The instrument was operated in the ratio mode with 5-nm bandwidths for excitation and emission slits. The OD_{280nm} of the sample was 0.10 or less. All the solvents used in optical studies were routinely treated with Chelex 100. The protein was initially dialyzed vs. appropriate buffer in the presence of EDTA and was then subjected to exhaustive dialysis with at least four changes in the absence of EDTA.

Results and Discussion

Protein Purification and Characterization. After the heat treatment step, the supernatant was applied to a DEAE-cellulose column. Calmodulin and S-100 proteins were eluted together at 0.25 M NaCl, and the elution profile observed with this column (Figure 1) was very reproducible, resulting in the easy identification by conductivity measurements of the peak that included both calmodulin and S-100 proteins. In addition, the protein peak corresponding to fractions 120–140 (Figure 1) had the characteristic UV absorption spectrum of these proteins due to their high content of phenylalanine residues compared to tyrosine. Fractions 120–140 were then applied to a Sephacryl S-200 gel filtration column, and the observed elution profile is shown in Figure 1 (insert). Peak 1 material was a high molecular weight species, and the peak 2 material was essentially calmodulin and S-100 proteins. Protein eluted in peak 2 was now applied to a hydroxylapatite column, and the separated proteins were eluted with an ammonium sulfate gradient (Figure 2). Under these conditions calmodulin was eluted first in peak 1. Peak 2 contained mostly S-100a and S-100b components. Peak 3 material was S-100b and the protein was homogeneous according to polyacrylamide gel electrophoresis in the presence as well as the absence of $NaDodSO_4$ (Figure 3). A molecular weight of 10 500 was deduced from its mobility in 15% $NaDodSO_4$ gels with reference to the mobilities of several standard proteins. The molecular weights of the standard proteins ranged from 11 000 to 40 000, and these were parvalbumin (11 200), calmodulin (16 500), cardiac Tn-C (18 500), cardiac troponin-I (27 000), and cardiac troponin-T (36 000).

It should be noted that in earlier studies, Isobe & Okuyama (1978) isolated S-100 protein using a DEAE-Sephadex A-50

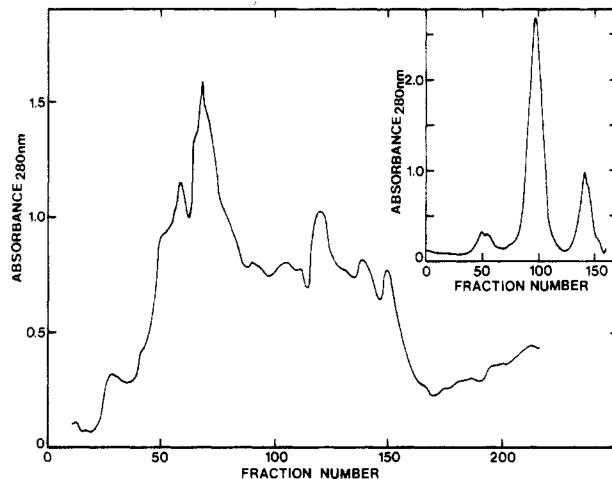

FIGURE 1: Elution diagrams of S-100 protein from DEAE-cellulose chromatography and from a Sephacryl S-200 gel filtration column (inset). Solvent systems used were 50 mM Tris-HCl buffer, pH 7.5, and 2 mM EDTA containing 2000 mL of a linear NaCl gradient from 0 to 0.45 M in the cellulose chromatography and 50 mM NH_4HCO_3 and 2 mM EDTA for the gel filtration column.

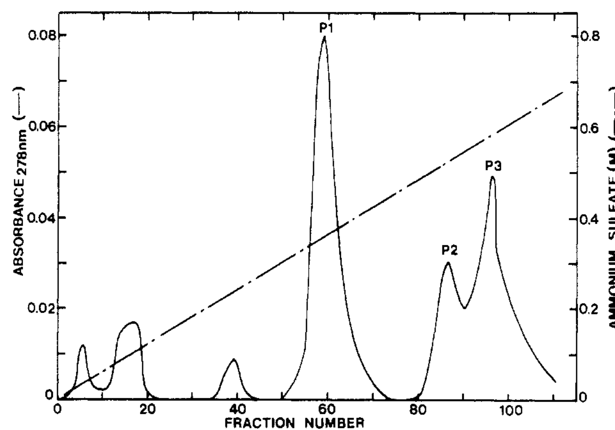

FIGURE 2: Elution profile of S-100b protein from a hydroxylapatite column (20 cm × 2 cm). Solvent system used was 50 mM Tris-HCl buffer, pH 8.0, 1 mM imidazole, and 0.1 M NaCl containing 1000 mL of a $(NH_4)_2SO_4$ gradient from 0 to 0.7 M.

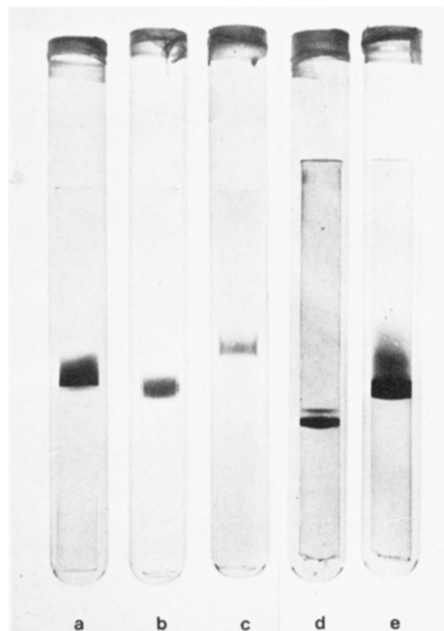

FIGURE 3: Electrophoresis of bovine brain S-100b protein, calmodulin, and parvalbumin. (a–c) 15% $NaDodSO_4$ gels: (a) parvalbumin; (b) S-100b; (c) calmodulin. (d and e) S-100b in Tris–glycine buffer, pH 8.6 (10% gels), in 0.1 mM EDTA and 0.1 mM Ca^{2+}, respectively.

column in the presence of 5% (v/v) ethanol. Since we wanted to avoid exposure of the protein to ethanol for prolonged periods of time, we developed the above method whereby we have successfully eliminated the use of ethanol during the course of purification. This is particularly important in our studies since we were interested in studying the effect of metal ions on protein conformation.

The mobility of S-100b in polyacrylamide gels in Tris–glycine buffer is shown in Figure 3d,e. The protein moves faster in the presence of EDTA. A decrease in mobility in the presence of Ca^{2+} could be due to a decrease in negative charge on the protein resulting from binding the cation. In this respect, S-100b behaves similarly to calmodulin but different from Tn-C. For example, Tn-C moves faster under similar conditions, and according to Head & Perry (1974), the decrease in hydrated volume associated with the conformational change that occurs when calcium ions are bound more than compensates for the charge effect. This behavior with skeletal Tn-C is also reflected in sedimentation velocity studies (Murray & Kay, 1972).

A comparison of the amino acid analysis of our preparation with that of Isobe & Okuyama (1978), as summarized in Table I, reveals excellent agreement between the two preparations. The ultraviolet absorption spectra of S-100b is characterized by the presence of several absorption maxima between 250 and 290 nm (Figure 4). The fine structure seen in the spectra stems from the fact that this protein has a high phenylalanine:tyrosine ratio (Table I) and the contribution of the phenylalanine is predominant, and is also reflected in the aromatic circular dichroism analysis of this protein (see below).

Ultracentrifugation. In the analytical ultracentrifuge, the protein sedimented as a single symmetrical boundary, and a $s_{20,w}$ value of 2.05 S was obtained for a 2 mg/mL protein concentration. Low-speed sedimentation equilibrium experiments were effected on the S-100b protein in a medium of 0.1 M Tris and 1 mM EDTA, at pH 7.5. The optical density vs. r^2 plot was linear and provided an estimate of the weight-average molecular weight over the whole cell, which was found to be 21 000 ± 500. In the presence of 2 mM Ca^{2+} the weight-average molecular weight distribution ranged from

Table I: Amino Acid Composition of Bovine Brain S-100b Protein Based on the Subunit Molecular Weight of 10 500[a]

amino acid	S-100b	PAP I-b[b]
Lys	8.3	8
His	4.7	5
Arg	1.0	1
Asp	10.6	9
Thr	3.5	3
Ser	5.0	5
Glu	19.2	19
Pro	0	0
Gly	4.8	4
Ala	5.7	5
Cys		2
Val	7.3	7
Met	2.7	3
Ile	4.0	4
Leu	8.5	8
Tyr	1.1	1
Phe	7.1	7
Trp	0	0

[a] The values are based on molar ratio. [b] From the amino acid sequence of Isobe & Okuyama (1978).

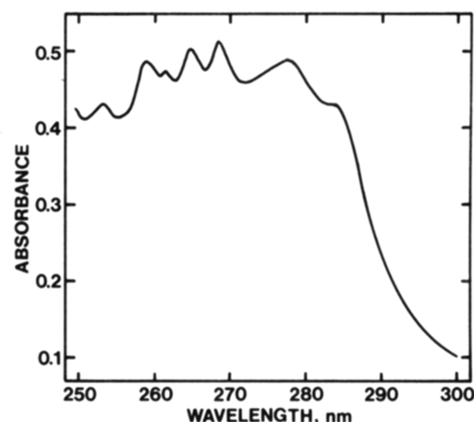

FIGURE 4: Ultraviolet absorption spectrum of S-100b in 0.1 M Tris-HCl buffer, pH 7.5, at room temperature.

20 000 to 27 000, suggesting that the protein might be undergoing slight aggregation, at this concentration of cation. Dannies & Levine (1969) reported the minimum molecular weight as 7000 on the basis of $NaDodSO_4$–polyacrylamide gels, and since they observed a molecular weight of 21 000 in native solvents in the ultracentrifuge, they suggested that the protein was made up of three polypeptide chains. However, Isobe & Okuyama (1978) came up with a value of 10 500 as the subunit molecular weight on the basis of their amino acid sequence data. We carried out sedimentation equilibrium studies in the ultracentrifuge under denaturing conditions using 6 M Gdn·HCl, and the observed molecular weight in this medium represents the minimum or subunit molecular weight. A plot of ln y vs. r^2 under denaturing conditions was linear, and the slope term yielded a molecular weight of 10 400 ± 300, indicating that the protein exists as a dimer of 21 000 molecular weight in native solvents. The subunit molecular weight of 10 400 obtained in the ultracentrifuge is in excellent agreement with our 15% $NaDodSO_4$ gel results. Sedimentation equilibrium experiments in 6 M Gdn·HCl were also carried out in the presence and absence of the reducing agent DTT, and the results obtained were virtually the same, suggesting that the two polypeptide chains are not held together by covalent forces.

Circular Dichroism Studies. Far-ultraviolet CD studies on S-100b protein revealed that the conformation of the protein depends on the metal ions present, particularly Ca^{2+} and K^+.

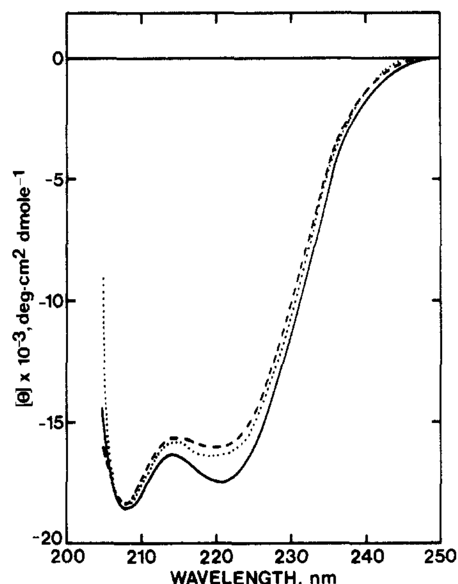

FIGURE 5: Far-ultraviolet CD spectra of S-100b in (—) 0.1 M Tris, pH 7.5, in (--) 0.1 M Tris, pH 7.5, and 1.6 mM Ca^{2+}, and in (···) 0.1 M Tris, pH 7.5, 1.6 mM Ca^{2+}, and 90 mM KCl.

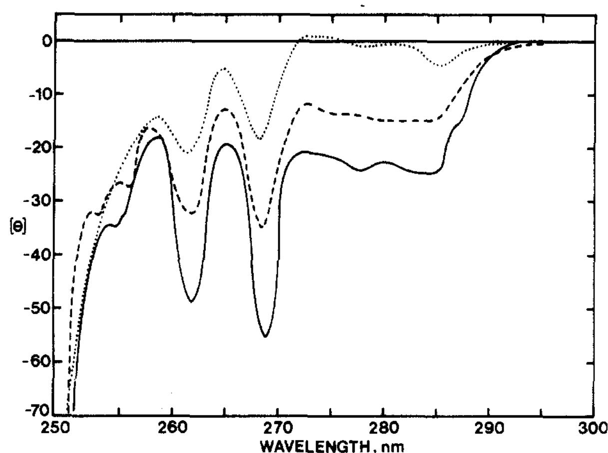

FIGURE 6: Aromatic CD spectra of S-100b in (—) 0.1 M Tris, pH 7.5, in (···) 0.1 M Tris, pH 7.5, and 1.6 mM Ca^{2+}, and in (--) 0.1 M Tris, pH 7.5, 1.6 mM Ca^{2+}, and 90 mM KCl.

Figure 5 represents typical far-ultraviolet circular dichroism spectra of S-100b in the absence and presence of Ca^{2+}. Also included in the figure are spectra of the protein in the presence of both Ca^{2+} and K^+. In the absence of Ca^{2+} and K^+, the $[\theta]_{222nm}$ is nearly −16 000 ± 300 deg cm² dmol⁻¹, while the addition of 1.6 mM $CaCl_2$ causes a 10% decrease to −14 300 ± 300 deg cm² dmol⁻¹. Analysis of the CD data according to the Chou–Fasman (Chou & Fasman, 1977) method indicates a decrease in apparent α-helical content from 58 to 52%. The effect of Ca^{2+} on the conformation of this protein is very different from its effect on other calcium binding proteins such as Tn-C, calmodulin, and parvalbumin (Murray & Kay, 1972; Walsh et al., 1979). With the latter proteins an increase in apparent α-helix content was observed with calcium addition, whereas with S-100b we have noted a decrease in α helix. Addition of K^+ to the protein in the presence of Ca^{2+} seems to indicate a slight recovery of the secondary structure, but the effect of K^+ is more obvious in the aromatic CD region.

Figure 6 reveals the effects of adding Ca^{2+} and K^+ on the aromatic CD spectrum of S-100b protein. The ellipticity of the protein is negative between 250 and 300 nm. The two bands at 284 and 276 nm can be assigned to the single tyrosine residue. The two well-resolved CD bands at 268.5 and 261.5 nm can be assigned unambiguously to the phenylalanine residues. Binding of Ca^{2+} to S-100b results in a dramatic change in the CD spectrum above 250 nm. It is obvious that the single tyrosine is affected by the addition of Ca^{2+} in the 280-nm region. The ellipticity values at 268.5 and 261.5 nm are also affected. For example, $[\theta]_{268.5nm}$ decreases from −55 to −18 deg cm² dmol⁻¹ upon the addition of Ca^{2+}. If K^+ is then added to the protein containing Ca^{2+}, the observed aromatic CD spectrum is again altered and the $[\theta]_{268.5nm}$ value is −40 deg cm² dmol⁻¹, suggesting that the effect of K^+ is antagonistic to the Ca^{2+} effect. These findings are in agreement with the earlier fluorescence studies of Calissano et al. (1969) on the mixture of S-100 proteins, where they noted that monovalent cations antagonized the effect of Ca^{2+}, the effects being in the order $K^+ > Na^+ > Li^+$. It should be noted in our studies that the apoprotein contained 2–3 μM K^+, as determined by atomic absorption measurements, and the concentration of K^+ used was generally in the range of 50–100 mM, implying that the observed effect of K^+ on the protein operates under physiological concentrations of available K^+ in the brain. In this respect, the behavior of this protein is unique in the sense that one does not observe a K^+ effect on the conformation of other calcium binding proteins unless one uses abnormally high concentrations of K^+ of the order of 0.5 M (McCubbin & Kay, 1973). Whether the inhibitory (agonist) effect of K^+ is the result of a specific competition between the monovalent cation and Ca^{2+} or from nonspecific modulation of Ca^{2+} binding by electrostatic shielding of negatively charged binding sites is not clear. Certainly the high concentration of K^+ involved makes the latter a plausible possibility. However, Mg^{2+} (1.6 mM) had no significant effect on the protein conformation, and the fact that Ca^{2+} was able to induce a conformational change in the protein in the presence of Mg^{2+} suggests some sort of specificity with regard to divalent cations.

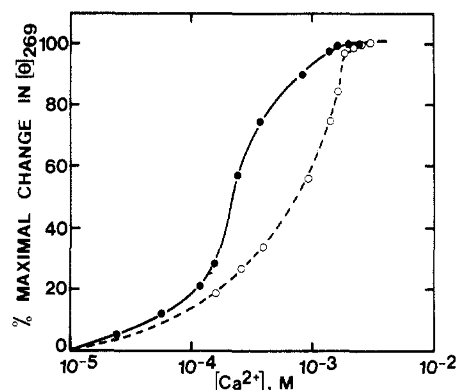

FIGURE 7: Percent change in ellipticity at 269 nm as a function of Ca^{2+} concentration for S-100b in 0.1 M Tris-HCl buffer, pH 7.5 (●), and in 0.1 M Tris-HCl buffer, pH 7.5, and 90 mM KCl (○).

Ca^{2+} titration in the aromatic CD region (Figure 7) suggests that the affinity of this protein for Ca^{2+} (K_D) is 2×10^{-4} M and is lowered significantly ($K_D = 8 \times 10^{-4}$ M) by the presence of K^+. A K_D value of 2×10^{-4} M is in good agreement with the findings of Calissano et al. (1969) on the mixture of S-100 proteins. The fact that the conformation assumed by the protein is dependent upon the presence of these cations (Ca^{2+} and K^+) indicates that changing conformation may be the way S-100 responds to local changes in ionic parameters.

Ultraviolet Difference Spectroscopy. The difference in the absorption properties of S-100b protein between 250 and 300 nm, induced in the presence of metal ions (Ca^{2+} and K^+), is shown in Figure 8. The dominant difference peaks at 287 and 280 nm in the presence of Ca^{2+} arise from the perturbation

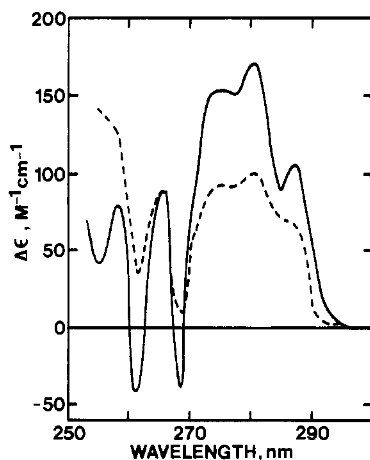

FIGURE 8: UV difference spectra of S-100b produced by increasing the concentration of Ca^{2+}. Chelex-treated S-100b in 0.1 M Tris, pH 8.0, was used for these studies. The data were corrected for dilution and are expressed as the difference in molar absorption, $\Delta\epsilon$. The temperature was 25 °C. (—) 1.6 mM $CaCl_2$; (---) 1.6 mM $CaCl_2$ and 90 mM KCl.

of the single tyrosyl chromophore. It is evident from the figure that one or more of the phenylalanine chromophores are also perturbed when calcium is bound. This lends support to the similar inference drawn from the CD data. The sign of the tyrosyl difference peak (i.e., a "red shift") suggests that the chromophore is in a less polar environment in the presence of Ca^{2+} (Donovan, 1969).

Alternatively the perturbations may be due to local charge effects upon binding calcium. The binding of calcium by carboxylate groups would reduce the free negative charge on the latter, which might result in changes in the geometry of the binding site and reorientation of the phenolic group. Similar observations have been noted for skeletal and cardiac Tn-C (Hincke et al., 1978) and porcine intestinal calcium binding protein (CaBP) by Dorrington et al. (1978).

Difference spectra generated upon adding K^+ to protein containing calcium clearly indicate that tyrosine and phenylalanine chromophores are perturbed. In the presence of K^+ the magnitude of the tyrosine difference peak is considerably reduced, from +160 to around +90 ($\Delta\epsilon$ values in M^{-1} cm^{-1}), suggesting that tyrosine moves to a relatively polar environment.

Fluorescence Spectroscopy. Since S-100b contains no tryptophan, the observed fluorescence spectrum will be that of tyrosine. Upon excitation at 280 nm, the emission maximum occurs between the 327- and 330-nm range, with a shoulder near 306 nm (Figure 9), indicating that the protein possesses an abnormal tyrosine. Since the protein exists as a dimer in benign medium, the fluorescence results suggest that out of the two tyrosine residues one might be behaving abnormally with emission maximum centered around 327–330 nm. This would imply that the two tyrosine residues in the protein molecule probably are not in the same environment. In order to obtain an estimate of the fluorescence yield due to the single tyrosine in the protein, we excited tyrosine of comparable concentration under the same instrumental settings. The fluorescence output from the single tyrosine in the protein was nearly 30% of standard tyrosine. Since most tyrosine residues in proteins have a fluorescence yield of only one-tenth that of free tyrosine (Chen, 1973), the protein is atypical not only in its emission peak but also in its fluorescence yield. Secondary and tertiary structures must play a crucial role in creating a microenvironment that gives rise to this anomaly. In this context, it should be mentioned that the value

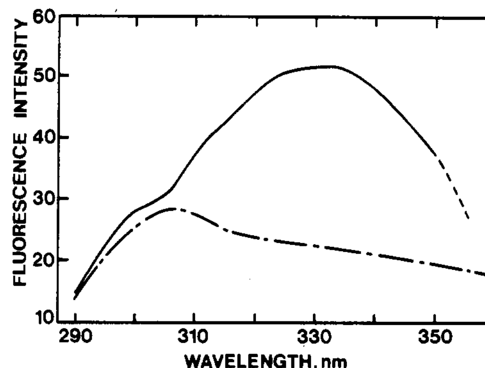

FIGURE 9: Fluoresence emission spectra of S-100b in 0.1 M Tris-HCl buffer, pH 7.5, at 25 °C (—) and in 0.1 M Tris-HCl buffer, pH 7.5, containing 6 M guanidine hydrochloride at 25 °C (–•–).

of 2.4 obtained for the extinction coefficient ($E^{1\%}_{1cm,278nm}$) in the analytical ultracentrifuge is considerably higher than that calculated for a protein containing a single tyrosine with a subunit molecular weight of 10 500.

To rule out the possibility that the observed emission maximum might be due to contamination with the S-100a subunit, which has a single tryptophan, we dialyzed the S-100b protein against 6 M Gdn·HCl. When the protein in 6 M Gdn·HCl was excited at 280 nm, the emission maximum occurred at 306 nm (Figure 9), indicating that our earlier observations in a native solvent are due to the presence of an abnormal tyrosine.

Our findings with the S-100b protein are not unique since other proteins that lack tryptophyl residues but have tyrosyl ones are known that fluoresce with a long-wavelength tyrosyl component. For example, cattle adrenodoxin has a single tyrosyl residue and fluoresces with a 330-nm peak from the native conformer but has a 304-nm tyrosyl fluorescence when denatured (Lim & Kimura, 1980). Similarly, two cytotoxins from the venom of the Indian cobra, each with four tyrosyl groups, have a 345-nm fluorescence component from a denatured conformer (Szabo et al., 1978). Although the origin of this anomaly is unclear in precise chemical terms, it has been suggested, with the above systems, that the anomalous fluorescence may result from the presence of a buried tyrosine residue(s) in these proteins and its subsequent excited singlet state protolysis may be due to an appropriate acceptor group such as an aspartic and/or glutamic acid residue. A similar explanation may be invoked for the S-100b protein since its isoelectric point is 4.1 and it possesses a net negative charge of 14/subunit at neutral pH. It is certainly conceivable that intramolecular interactions between the phenolic hydroxyl group of the tyrosine residue and a carboxyl group of either an aspartic acid or a glutamic acid residue might occur.

Acknowledgments

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Circular Dichroism and Nuclear Magnetic Resonance Studies on the Complexation of Valinomycin with Calcium†

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ABSTRACT: Complexation of valinomycin (VM) with the divalent cation Ca^{2+} in a lipophilic solvent, acetonitrile (CH_3CN), has been studied by using circular dichroism and proton and carbon-13 nuclear magnetic resonance (^1H NMR and ^{13}C NMR). From analyses of the spectral data, it is concluded that VM forms a 2:1 (peptide–ion–peptide) sandwich complex with Ca^{2+}, at low concentration of VM. At moderate concentrations of the salt, in addition to the sandwich complex, an equimolar (1:1) complex different from those observed for potassium and sodium is also observed. At very large concentrations of the calcium salt, the data suggested a complex with a conformation similar to that of the free VM in polar solvents. Possible conformations for the sandwich and the equimolar VM–calcium complexes are proposed.

Our understanding of the molecular basis of ion transport across biological membranes is far from complete. However, over the last 2 decades, considerable work has been carried out in different laboratories on the structure and the conformation of ion-binding macrocyclic antibiotics (ionophores) and their cation complexes (Ovchinnikov et al., 1974; Ovchinnikov, 1979; Ovchinnikov & Ivanov, 1974) with a hope to get more insight into their transmembrane ion-transporting properties. Among the ionophores acting as ion carriers, valinomycin (VM), a 36-membered cyclic depsipeptide, *cyclo*-(L-Val-D-Hyi-D-Val-L-Lac)$_3$,[1] plays an important role in selective enhancement of permeability of biological membranes for potassium ions (Pressman, 1968; Tosteson et al., 1967). The circular dichroism (CD) and nuclear magnetic resonance (NMR) studies on VM and its cation complexes have shown that the selectivity of a particular cation by this molecule depends on the nature of the ligands and the conformational states of the molecule (Haynes, 1969; Ivanov et al., 1969; Ohinishi & Urry, 1969; Grell et al., 1973; Patel & Tonelli, 1973; Davis & Tosteson, 1975). The conformation of the free VM has been shown to be highly solvent dependent (Patel & Tonelli, 1973; Ovchinnikov, 1974; Bystrov et al., 1977). A host of literature is available regarding the conformational studies of the complexation of VM with monovalent cations like K^+, Na^+, Rb^+, Cs^+, and Tl^+ (Bystrov et al., 1977; Ovchinnikov, 1974; Davis & Tosteson, 1975; Neupart-Laves & Dobler, 1975; Ohinishi & Urry, 1969). To get more insight into the molecular basis of the ion-transporting properties, it is important also to study the complexing ability of VM with

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[1] Abbreviations: Val, valine; Hyi, hydroxyisovaleric acid; Lac, lactic acid.

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