Circular dichroism studies on the zinc-induced conformational changes in S-100a and S-100b proteins

Rajam S. Mani and Cyril M. Kay

Medical Research Council Group in Protein Structure and Function, Department of Biochemistry, University of Alberta, Edmonton, Alberta T6G 2H7, Canada

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The effect of Zn^{2+} binding on the circular dichroism (CD) spectra of brain-specific S-100a and S-100b calcium-binding proteins has been examined. In the presence of Zn^{2+}, S-100a undergoes a conformational change and the decrease in ellipticity at 222 nm, as a result of Zn^{2+} addition, was nearly 1400 deg·cm^{-2}·dmol^{-1}, whereas with S-100b there was no significant conformational change. Ca^{2+} was able to bind to S-100 proteins in the presence of Zn^{2+} and the two metal-ion binding sites on the proteins appear to be different. In the presence of Zn^{2+}, K^+ had no significant effect on the conformation of S-100 proteins. Ca^{2+} and Zn^{2+} binding induce different environments around the tyrosine residues in S-100a, whereas with S-100b, similar changes were observed for the single tyrosine residue, using either metal.

S-100 protein *Ca^{2+} effect* *Zn^{2+} effect* *CD*

1. INTRODUCTION

The highly acidic water-soluble S-100 protein [1] is considered mainly a nervous tissue-specific protein found primarily in the cytoplasm of glial cells [2]. Authors in [3] have demonstrated the presence of S-100 protein in continuous cell lines of human malignant melanoma. The biological function of this protein is unknown; however, existing literature suggests a role for it in the function or development of the nervous system [4–6]. S-100 protein is actually a mixture of two components, S-100a and S-100b, with a subunit composition of $\alpha\beta$ and β_2 respectively [7]. The amino acid sequences of S-100 proteins [8,9] are similar to calcium-binding proteins such as calmodulin, troponin-C and parvalbumin [10–13]. The α-subunit in S-100a possesses an extensive sequence homology (58%) with that of the β-subunit. The α-subunit is characterized by the presence of a single tryptophan (Trp-90) whereas the β-subunit has no tryptophan.

S-100 proteins undergo a conformational change upon binding Ca^{2+} [14–16], and K^+ acts as an antagonist to the Ca^{2+} effect. S-100 proteins have a lower affinity for calcium, when K^+ is present. Authors in [17] have developed a zinc-dependent affinity chromatographic procedure for separating the S-100 proteins. We here employed CD measurements to study the structural changes induced by zinc on S-100a and S-100b proteins and the observed effects clearly demonstrate that these two proteins interact differently with zinc.

Abbreviations: CD, circular dichroism; UV, ultraviolet; Tris, tris(hydroxymethyl)aminoethane; EDTA, ethylenediaminetetraacetic acid; DTT, dithiothreitol

2. EXPERIMENTAL

S-100a and S-100b proteins were prepared from bovine brain as in [15,16]. CD measurements were made on a Jasco J500C instrument fitted with a DP500N data process unit. The instrument was standardized with *d*-10-camphorsulfonic acid and pantoyl lactone. The solvents used in CD measurements were routinely passed through a Chelex-100 column. The proteins were initially

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dissolved in appropriate buffer in the presence of 1 mM EDTA and, thereafter, were subjected to exhaustive dialysis with at least 4 changes against the solvent that had been passed through the Chelex-100 column.

3. RESULTS

Typical far-UV CD spectra of S-100a in 0.1 M Tris–HCl buffer (pH 7.5) in the absence and presence of Zn^{2+}, are shown in fig.1. In the absence of Zn^{2+} the $[\theta]_{222\ nm}$ is nearly -16800 ± 300 deg·cm^2·dmol^{-1}, while the addition of Zn^{2+} causes about an 8% decrease to -15400 ± 300 deg·cm^2·dmol^{-1}. The effect of Zn^{2+} on the conformation of S-100a is similar to that produced by Ca^{2+} except that with Ca^{2+}, the observed decrease in ellipticity at 222 nm was only 700–800 deg·cm^2·dmol^{-1}. In contrast to Ca^{2+}, the Zn^{2+} effect on S-100a was not pH-dependent when tested between pH 7.0 and 8.5. The observed Zn^{2+} effect could be reversed by adding excess EDTA. Zn^{2+} is capable of inducing a conformational change in the presence of 1 mM Ca^{2+} and this is shown as an insert in fig.1. In this experiment, Ca^{2+} was first added to the apoprotein, followed by Zn^{2+} addition. In another series of experiments, Zn^{2+} was added initially to the apoprotein and subsequently, Ca^{2+} was added. In both cases, the observed final CD spectrum was essentially the same and also the magnitude of the change induced by one metal ion was not influenced by the presence of the other, implying that Ca^{2+} and Zn^{2+} probably bind at different sites on the protein. Authors in [18] have come to a similar conclusion based on their fluorescence studies. It is obvious from the figure (insert 1) that K^+ had no significant effect on the protein in the presence of Zn^{2+}. Since K^+ does not act as an antagonist in the presence of Zn^{2+}, one may conclude that K^+ and Zn^{2+} bind at different sites. The alternative possibility is that both cations bind to the same site; however, since Zn^{2+} has a binding constant (K_d) of 10^{-8}–10^{-6} M for S-100 proteins [18], it is preferentially bound to the protein. Of course, one should not rule out the possibility that K^+ may alter the binding affinity of Zn^{2+} for S-100a.

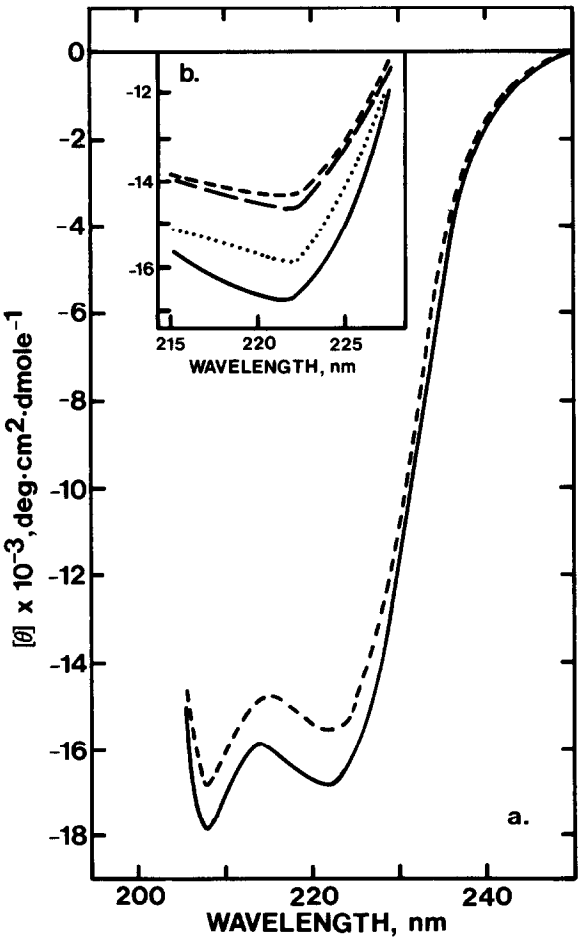

Fig.1. (a) Far-UV CD spectra of S-100a in 0.1 M Tris, pH 7.5 (——), and in 0.1 M Tris, pH 7.5 and 0.5 mM Zn^{2+} (– – –). (b) Far-UV CD spectra of S-100a in the region of 222 nm in 0.1 M Tris, pH 7.5 (——); in 0.1 M Tris and 1 mM Ca^{2+}, pH 7.5 (···); in 0.1 M Tris, 1 mM Ca^{2+} and 0.5 mM Zn^{2+} (— —) and in 0.1 M Tris, 1 mM Ca^{2+}, 0.5 mM Zn^{2+} and 0.1 M KCl (– – –).

Fig.2 represents typical far-UV CD spectra of S-100b in the absence and presence of Zn^{2+}. Addition of Zn^{2+} to S-100b produced an increase in ellipticity at $[\theta]_{222\ nm}$ of nearly 400 deg·cm^2·dmol^{-1} while the experimental error is $\pm 300°$. This small difference in ellipticity was reproducible; however, it is clear that Zn^{2+} did not induce a gross conformational change in S-100b. The presence of Zn^{2+} had no significant effect on the magnitude of the calcium-induced conformational change.

Fig.3 reveals the effect of Zn^{2+} on the aromatic CD spectrum of S-100a at pH 7.5. The negative band at 295 nm is due to the single tryptophan. The two positive bands at 284 and 277 nm can be

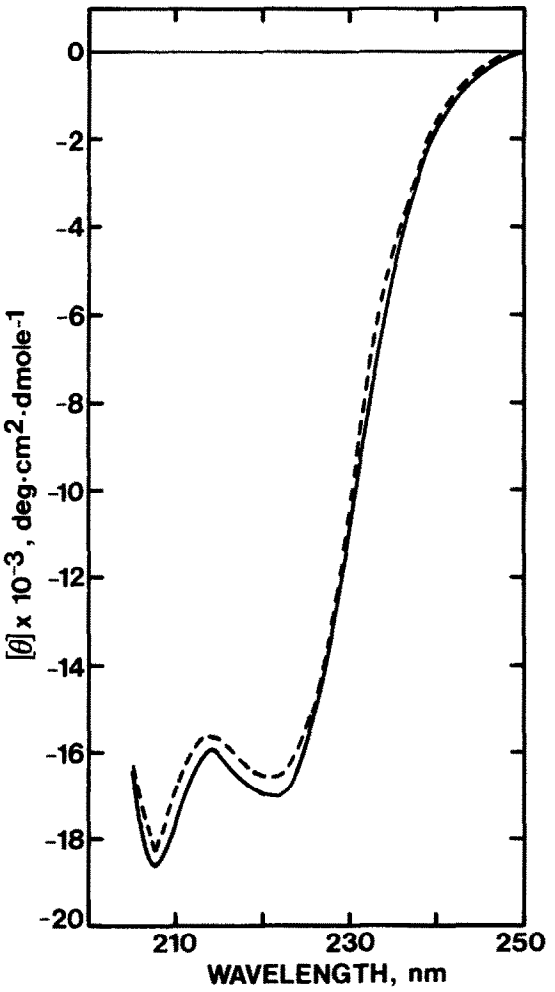

Fig.2. Far-UV CD spectra of S-100b in 0.1 M Tris, pH 7.5 (——) and in 0.1 M Tris, pH 7.5 and 0.5 mM Zn^{2+} (– – –).

assigned to the tyrosine residues while the two bands at 268.5 and 261.5 nm can be attributed to the phenylalanine residues. From the observed spectrum, one can conclude that the tryptophan band at 295 nm and the tyrosine residues in the 280 nm region are perturbed by the addition of Zn^{2+}. Ellipticity values at 268.5 and 261.5 nm are also affected. When Ca^{2+} was added to S-100a containing Zn^{2+}, the aromatic chromophores were further perturbed and, as an example, the ellipticity value in the 280 nm region was reduced from nearly $+50$ deg·cm^2·dmol^{-1} to about $+25$ deg·cm^2·dmol^{-1}, again demonstrating that these two metal ions can interact with the protein in the

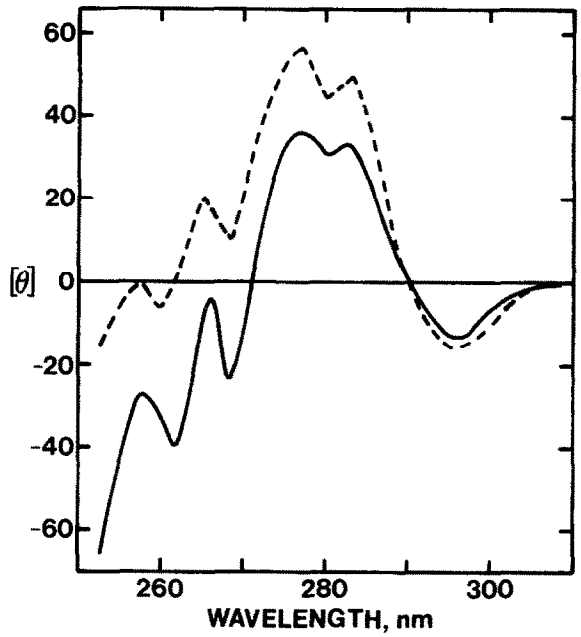

Fig.3. Aromatic CD spectra of S-100a in 0.1 M Tris, pH 7.5 (——) and in 0.1 M Tris, pH 7.5 and 0.5 mM Zn^{2+} (– – –).

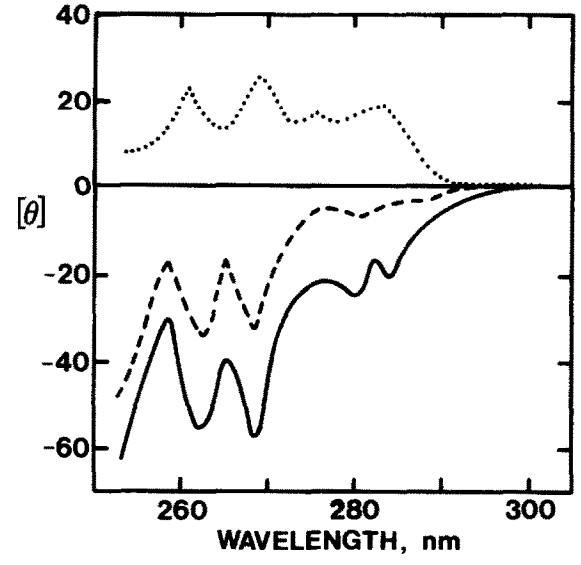

Fig.4. Aromatic CD spectra of S-100b in 0.1 M Tris, pH 7.5 (——) and in 0.1 M Tris, pH 7.5 and 0.5 mM Zn^{2+} (– – –). Difference CD spectra generated by subtracting the protein spectrum in the presence of Zn^{2+} from the apoprotein (···).

presence of each other. The aromatic CD spectrum of S-100b in the absence and presence of Zn^{2+} is shown in fig.4. The ellipticity of the protein is negative between 250 and 300 nm. The two bands at 284 and 278 nm are due to the tyrosine residue and the position of the other two bands are characteristic of phenylalanine residues. Addition of Zn^{2+} results in alteration of the microenvironment around the single tyrosine and one or more of the 7 phenylalanine residues, and this is apparent in the difference spectrum generated by subtracting the protein spectrum in the presence of Zn^{2+} from the apoprotein spectrum (fig.4).

4. DISCUSSION

The fact that the two S-100 proteins can be separated from each other using Zn^{2+} affinity column suggested to us that S-100 proteins bind Zn^{2+} differently. It has become apparent from our CD studies that S-100 proteins do indeed respond differently to Zn^{2+}. In the presence of Zn^{2+}, S-100a undergoes a gross conformational change, resulting in a net 8% loss in ellipticity at $[\theta]_{222\ nm}$, whereas the cation had no significant effect on S-100b, as revealed by far-UV CD measurements. Binding of Ca^{2+} to S-100 proteins is pH dependent around neutrality (pH 7.0–8.5) and with S-100a, the Ca^{2+}-induced change can be reversed by EDTA only when DTT is present in the solvent system, suggesting the involvement of sulfhydryl groups [16]. However, the zinc effect is not pH-dependent and with S-100a, the Zn^{2+}-induced conformational change can be reversed by adding excess EDTA even in the absence of DTT.

Far-UV CD studies of calcium binding to S-100 proteins in the presence of zinc have shown that Ca^{2+} and Zn^{2+} ions can bind simultaneously to the proteins. We have also shown here that the order of addition of metal ion has no significant effect on the observed final CD spectrum, implying that these two metal ions probably bind at different sites. In the presence of Zn^{2+}, K^+ had no significant effect on S-100 proteins whereas only when Ca^{2+} was present did it act as an antagonist [15,16]. Hence the K^+ binding site on S-100 proteins must be in close proximity to the calcium-binding one, whereas the Zn^{2+} binding site must be located away from the K^+ binding site. Alternatively, once Zn^{2+} is bound, in view of its higher affinity, that site is no longer available to K^+.

Near-UV CD measurements indicate that Ca^{2+} and Zn^{2+} induce different environments around the tyrosine residues in S-100a. For example, the ellipticity value in the 280 nm region becomes more positive (i.e., from +34 in the apoprotein to $+50\ deg \cdot cm^2 \cdot dmol^{-1}$ in the presence of Zn^{2+}) whereas with Ca^{2+}, the $[\theta]_{280\ nm}$-value decreases to about $+20\ deg \cdot cm^2 \cdot dmol^{-1}$. In contradistinction with S-100b, binding of Ca^{2+} and Zn^{2+} ions leads to similar changes in the environment of the tyrosine residue. From our CD studies, it is obvious that Zn^{2+} has a pronounced effect on the secondary structure and the aromatic environment of S-100a, whereas with S-100b it has only a subtle effect on the microenvironment of the aromatic groups. The observed differences in the behaviour of S-100 proteins towards Zn^{2+} may be related to their specific, hitherto unknown, functions.

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