Hydrodynamic properties of bovine brain S-100 proteins

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The size and shape of S-100a and S-100b proteins in solution have been examined by gel filtration and ultracentrifugation in the presence and absence of Ca^{2+}. S-100a and S-100b proteins, in the absence of Ca^{2+}, have an intrinsic sedimentation coefficient, $s^0_{20,w}$ of 2.20 and 2.15 S, respectively and in 1 mM Ca^{2+} their $s^0_{20,w}$ values were decreased to 2.05 and 1.95 S, respectively, indicating an unfolding of the protein molecules. The Stokes radii of S-100a and S-100b ($-Ca^{2+}$) were 23.4 Å and 24.0 Å and they decreased to 22.2 Å and 22.3 Å in the presence of Ca^{2+}. The Ca^{2+} effect on S-100b > S-100a was in agreement with our earlier CD observations. Among the monovalent cations tested (K^+, Na^+ and Li^+) K^+ had the maximum effect on the Stokes radii and $s^0_{20,w}$ values of S-100 proteins. Since certain functions of the nervous system are accompanied by local changes in ionic concentrations of Ca^{2+}, Na^+ and K^+, it is conceivable that these respective conformational changes induced in S-100 proteins by these metals may be related to their function in the brain.

S-100 protein *Ca^{2+} effect* *K^+ effect* *Na^+ effect* *Li^+ effect* *CD* *Hydrodynamics*

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]. The exact biological function of this protein is unknown. The S-100 protein is a mixture of two components, S-100a and S-100b, with a subunit composition of $\alpha\beta$ and β_2, respectively [3]. The amino acid sequences of S-100 proteins [4,5] are similar to calcium binding proteins such as calmodulin, troponin-C and parvalbumin [6–9]. The α-subunit in S-100a possesses an extensive sequence homology (58%) with that of the β subunit.

S-100 proteins undergo a conformational change upon binding calcium and this has been demonstrated by UV difference spectroscopy, CD studies in the aromatic and far-UV spectral regions and fluorescence measurements [10–12]. The effect of K^+ on S-100 proteins is antagonistic to that of calcium [10,11]. The affinity of S-100a and S-100b proteins towards Ca^{2+} is similar and yet subtle differences exist in the microenvironment of specific chromophores. So far, no X-ray structural determination has been reported for S-100 proteins. Here, the structural properties of S-100a and S-100b proteins in solution are investigated by hydrodynamic methods. The results indicate that S-100 proteins undergo a conformational change in the presence of Ca^{2+}.

Abbreviations: CD, circular dichroism; UV, ultraviolet

2. MATERIALS AND METHODS

S-100a and S-100b proteins were prepared from bovine brain as in [10,11]. Protein concentrations were determined using an $E^{1\%}_{1cm,278nm}$ of 5.4 and 2.4 for S-100a and S-100b proteins, respectively.

2.1. *Sedimentation studies*

M_r values were measured by the low-speed sedimentation equilibrium method as in [10]. Sedimentation coefficients were established at 60000 rpm with Kel-F-type single sector cells and Schlieren optics. For a given protein concentra-

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tion, data were collected for both the plus (+) and the minus (−) Ca^{2+} states in a single run with two cells and a 1° positive-wedge window. Solutions for these experiments were prepared by adding EDTA or $CaCl_2$ (5 μl) to 0.5 ml protein solution prior to the run. The Stokes radius ($R_{s,sed}$) was calculated from the extrapolated value of $s^o_{20,w}$ at infinite dilution ($s^o_{20,w}$) by the relationship:

$$R_{s,sed} = M_r(1 - \bar{v}\varrho)/(6\pi N\eta_o s^o_{20,w}) \quad (1)$$

where: $\bar{v}$ is the partial specific volume, ϱ is the solvent density, N is Avogadro's number and η_o is the solvent viscosity in P.

2.2. *Densitometry*

Density measurements of the S-100 protein and buffer solutions were carried out at 20°C with a digital density meter (DMA 60 and DMA 601; Anton Paar) calibrated with dry air and water. Temperature control was maintained to within 0.01°C with a Lauda/Brinkmann K-2/R circulating water bath. Density data were used to calculate partial specific volume ($\bar{v}$) values as in [13].

2.3. *Gel chromatography*

Analytical gel filtration experiments were performed at room temperature in a 95 × 1.1 cm column packed with Sephacryl S-200 (Pharmacia). Elution volumes were determined gravimetrically, followed by collection of 0.4-ml fractions in the region of interest. The following gel standards and Stokes radii (R values) were used: catalase, 52 Å [14]; lactate dehydrogenase, 41 Å [15]; bovine serum albumin, 35 Å [14]; ovalbumin, 28.4 Å [16]; α-chymotrypsinogen, 21.9 Å [17]; TN-C, 26.3 Å [18]; myoglobin, 19.8 Å [16]; cytochrome c, 17.2 Å [14]. The void volume (V_o) and the total included volume (V_T) were measured with Blue dextran and potassium chromate, respectively. S-100 proteins and gel markers were run individually. The partition coefficient (σ) was calculated from the elution volume by the relationship:

$$\sigma = (V_T - V_o)/(V_T - V_o) \quad (2)$$

The Stokes radius of S-100 protein ($R_{s,gel}$) was then calculated from a standard curve of log R_s *vs* σ, as in [14]. Experiments in the presence of Ca^{2+} were done by equilibrating the same column using the same buffer, i.e., 0.1 M Tris–HCl buffer (pH 7.5) and 0.2 M salt (KCl or NaCl or LiCl) and 1 mM Ca^{2+}, and recalibrating with the protein standards. Translational frictional ratios (f/f_{min}) were calculated from the experimental Stokes radii obtained by gel filtration ($R_{s,gel}$) or sedimentation velocity ($R_{s,gel}$) by using the relationship:

$$f/f_{min} = R_s/R_o = R_s/[3M_r\bar{v}/(4\pi N)]^{1/3} \quad (3)$$

where R_o is the Stokes radius of the equivalent unhydrated sphere of M_r and partial specific volume $\bar{v}$. The frictional ratio due to asymmetry (f/f_o) was calculated by separating the contribution of particle hydration according to:

$$f/f_{min} = (f/f_o)[1 + w/(\bar{v}\varrho)]^{1/3} \quad (4)$$

where w is the degree of hydration expressed as g water bound/g protein. Axial ratios for both prolate and oblate ellipsoids were generated from frictional ratio estimates by using tabulated data [19].

3. RESULTS

The Stokes radii of S-100a and S-100b proteins in 0.1 M Tris–HCl buffer (pH 7.5) and 0.2 M salt (KCl or NaCl or LiCl) were determined by using a calibrated Sephacryl S-200 column. S-100 proteins were eluted in a single symmetrical peak and in the protein concentration range used (1–4 mg/ml), there was no significant effect on the elution volume. In the absence of Ca^{2+}, the Stokes radii of S-100a and S-100b proteins are 23.4 and 24.0 Å, respectively, in 0.1 M KCl Tris buffer and this value decreased to 22.2 and 22.3 Å in the presence of Ca^{2+} (table 1) for the two respective proteins. These results suggest that S-100 proteins undergo a conformational change in the presence of Ca^{2+}. The effect of different monovalent cations on the Stokes radius of S-100 proteins is tabulated in table 1. In the absence of Ca^{2+} the Stokes radii of S-100a are 23.4, 24.4 and 25.5 Å in KCl, NaCl and LiCl medium, respectively, and for S-100b the values are 24.0, 24.7 and 25.8 Å in the corresponding media. Thus, S-100 proteins assume a more compact structure in the presence of KCl and the effect of monovalent cations is of the order $K^+ > Na^+ > Li^+$. In [20] a similar effect was observed based on fluorescence studies. It also becomes apparent from our sedimentation velocity studies that S-100b assumes a more compact structure in the

Table 1

Physical parameters of S-100 proteins

Protein	KCl		NaCl		LiCl	
	$-Ca^{2+}$	$+Ca^{2+}$	$-Ca^{2+}$	$+Ca^{2+}$	$-Ca^{2+}$	$+Ca^{2+}$
S-100b						
$R_{s,gel}$ (Å)	24.0	22.3	24.7	23.6	25.8	24.4
$s^{o}_{20,w}$ (S)	2.15	1.95	1.93	1.78	1.8	1.7
f/f_{min}	1.31	1.20	1.35	1.27	1.41	1.31
S-100a						
$R_{s,gel}$ (Å)	23.4	22.2	24.4	23.4	25.5	24.6
$s^{o}_{20,w}$ (S)	2.20	2.05				
f/f_{min}	1.26	1.19	1.32	1.26	1.39	1.32

Solvent system used: 0.1 M Tris–HCl buffer (pH 7.5) and 0.2 M salt (KCl or NaCl or LiCl)

presence of KCl since the observed $s^{o}_{20,w}$ value in KCl is 2.15 as opposed to 1.8 S in LiCl (table 1). In order to test if the observed effect of monovalent cations on the S-100 protein is specific, calmodulin was used as a control. The Stokes radius of calmodulin in a NaCl medium was 24.9 Å ($-Ca^{2+}$) and 22.8 Å in the presence of Ca^{2+}. However, there was no significant effect of monovalent cations on the Stokes radius of calmodulin. Hence, the observed effect of K^+, Na^+ and Li^+ on S-100 proteins is due to specific interaction of these monovalent cations on S-100 proteins.

In the presence of Ca^{2+}, S-100 proteins tend to sediment slowly in the ultracentrifuge (table 1), suggesting that they unfold in the presence of Ca^{2+} and this observation is consistent with our earlier CD studies in which we noted a decrease in the ellipticity value at 222 nm as a result of Ca^{2+} addition. In this respect S-100 proteins are different from other calcium-binding proteins such as TN-C. With TN-C, addition of Ca^{2+} results in an increase in the $s^{o}_{20,w}$ accompanied by an increase in negative ellipticity at 222 nm suggesting that the TN-C molecule is more compact in the presence of Ca^{2+}. Calcium has a more pronounced effect on S-100b compared with S-100a. With S-100b, the drop in Stokes radius as a consequence of Ca^{2+} addition in a KCl medium is 1.7 Å, whereas with S-100a the drop is only 1.2 Å. Similarly, in LiCl medium the changes are 1.4 and 0.9 Å for S-100b and S-100a, respectively (table 1). This finding is also in agreement with our earlier CD studies [12]. With S-100b the drop in negative ellipticity at 222 nm, as a result of Ca^{2+} addition, was nearly 1800 $deg \cdot cm^2 \cdot dmol^{-1}$ while with S-100a, the decrease was only around 1100 $deg \cdot cm^2 \cdot dmol^{-1}$.

The effect of Ca^{2+} on the M_r behaviour of S-100 protein was studied in the ultracentrifuge. The apparent weight average M_r of S-100b in the absence of Ca^{2+} was 21000 [10]. In the presence of 2 mM Ca^{2+} the weight average M_r distribution ranged from 20000 to 27000. It is concluded from these experiments that S-100b does not undergo extensive aggregation in the presence of Ca^{2+}. Moreover, $s^{o}_{20,w}$ values for both S-100a and S-100b proteins decrease upon the addition of Ca^{2+} and hence it is unlikely that they undergo any aggregation.

In view of the importance of the partial specific volume term in M_r and hydrodynamic calculations, the value of this parameter was measured by densitometry for the S-100 protein in the presence and absence of Ca^{2+}. This determination was carried out for only one protein concentration and hence the values reported are only preliminary; nonetheless they indicate trends. Since S-100a and S-100b proteins possess 58% sequence homology, we decided to use S-100 protein, which is a mixture of a and b, for the $\bar{v}$ determinations. The partial specific volume for S-100 in 0.1 M Tris (pH 7.5) and 0.2 M KCl, 1 mM EDTA was 0.707 ml/g and in the presence of Ca^{2+} the $\bar{v}$ value was 0.74 ml/g. Thus it appears that the partial specific volume of

S-100 protein is affected by Ca^{2+}. The value of 0.707 observed in the $-Ca^{2+}$ state may seem low but similar observations have been made with other calcium-binding proteins [18,21]. The low partial specific volume may be due to electrostriction of water around the numerous charged amino acid side-chains of S-100 protein [22]. When Ca^{2+} is added to S-100, the observed $\bar{v}$ increase may be the result of charge neutralization and/or due to the induced conformational change in the protein molecule because of which some side-chain groups may now be occupying different positions resulting in an alteration of the net charge on the protein. The Stokes radii ($R_{s,sed}$) calculated from $s^{o}_{20,w}$ are 23.2 Å ($-Ca^{2+}$) and 22.5 Å ($+Ca^{2+}$) for S-100a and 23.7 Å ($-Ca^{2+}$) and 22.8 Å ($+Ca^{2+}$) for S-100b using the above mentioned $\bar{v}$ value of S-100. These values are in excellent agreement with those obtained from gel filtration (table 1). This consistency suggests that the Ca^{2+}-induced behaviour of the S-100 protein is due to a conformational change and is not an artifact arising out of a particular method of analysis.

4. DISCUSSION

The binding of Ca^{2+} to S-100 proteins has been studied in the past using CD, difference spectroscopy, fluorescence spectroscopy and equilibrium dialysis with labelled calcium [11,12,20]. Here, we demonstrated that the binding of Ca^{2+} and monovalent cations affects the overall hydrodynamic shape of both S-100a and S-100b proteins. This effect of monovalent cations

Table 2

Structural parameters of equivalent ellipsoids of revolution based on hydrodynamic properties of S-100 proteins

	Hydration [w(g/g)]	Prolate axial ratio[a] (Calcd. from R_s)	Oblate axial ratio (Calcd. from R_s)
S-100b (NaCl)			
$-Ca^{2+}$	0	6.6 (128)[b]	7.5 (72)
	0.25	4.5 (99)	4.95 (62)
	0.50	3.3 (80)	3.45 (55)
	0.75	2.3 (64)	2.35 (48)
$+Ca^{2+}$	0	5.5 (115)	6.10 (68)
	0.25	3.7 (88)	3.93 (59)
	0.50	2.45 (68)	2.50 (50)
	0.75	1.24 (43)	1.26 (40)
S-100a (NaCl)			
$-Ca^{2+}$	0	6.1 (123)	6.9 (70)
	0.25	4.2 (96)	4.5 (61)
	0.50	2.9 (75)	3.05 (53)
	0.75	2.0 (59)	2.0 (47)
$+Ca^{2+}$	0	5.2 (111)	5.8 (67)
	0.25	3.5 (86)	3.7 (58)
	0.50	2.3 (65)	2.4 (50)
	0.75	1.0 (37)	1.0 (37)

[a] Axial ratio values were calculated from the Stokes radius (R_s) by using eq. 3 and 4

[b] The longest axis ($2a$) in Å was evaluated from $a = R_o\,(r_1^2\,r_2)^{1/3}$ where r_1 is the ratio of the longest axis to the intermediate axis and r_2 is the ratio of the intermediate axis to the shortest axis. For a prolate ellipsoid, r_1 is the axial ratio and r_2 is unity, while for an oblate ellipsoid, r_2 is the axial ratio and r_1 is unity

on S-100 protein is specific since no such effect has been observed with other calcium-binding proteins, namely calmodulin, TN-C or parvalbumin especially in the low concentration level of metal ion used (i.e., 10–20 mM KCl). This conclusion is also borne out in our present investigation by the fact that monovalent cations had no effect on the elution volume of calmodulin on our gel filtration column.

Axial ratios for both prolate and oblate ellipsoids were calculated from the Stokes radius using equations 3 and 4 and from frictional ratio estimates by using tabulated data [19]. Table 2 lists the various combinations of hydration and axial asymmetry for S-100a and S-100b proteins derived from the experimental values of R_s. By setting the degree of hydration (w) to zero, the maximum possible asymmetry of S-100 proteins can be obtained. A more reasonable estimate of w, however, is the calculated value of 0.47 g/g of the S-100b protein estimated from the amino acid composition as in [23]. With this estimate of hydration, an axial ratio of 3–4 would be expected for S-100b in the absence of Ca^{2+} (table 2). This corresponds to a width of about 55 Å for an oblate ellipsoid or about 80 Å for a prolate ellipsoid. In 1 mM free Ca^{2+}, the dimensions are decreased to 50 Å for an oblate ellipsoid model and 68 Å for a prolate ellipsoid. A similar trend was observed with the S-100a protein (table 2). However, the interpretation of hydrodynamic data in terms of ellipsoidal models should not be taken too literally. Moreover, it is not known whether S-100 proteins function free in solution, or attached to some receptor. In this connection, about 7% of the S-100 protein is found bound to the cellular membranes obtained from brain [24,25] and this would obviously involve some interaction with the membrane components which in turn could result in altering the shape and conformation of the S-100 proteins.

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