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Time-resolved fluorescence of S-100a protein in the absence and presence of calcium and phospholipids

Giovanna Zolese [a], Ileana Giambanco [c], Giovanna Curatola [a], Gelsomina De Stasio [b] and Rosario Donato [c]

[a] *Istituto di Biochimica, Facoltà di Medicina, Università di Ancona, Ancona (Italy),* [b] *Istituto di Struttura della Materia, CNR, Frascati, Rome (Italy) and* [c] *Department of Experimental Medicine and Biochemical Sciences, Section of Anatomy, University of Perugia, Perugia (Italy)*

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We have used phase-modulation fluorescence lifetime measurements to study the single Trp residue of the Ca^{2+}-binding protein S-100a. Trp fluorescence decay was not exponential for the protein irrespective of the absence or presence of Ca^{2+}. Fluorescence decay was best described by Lorentzian lifetime distributions centered around two components (approx. 3 and 0.7 ns) for protein in absence of Ca^{2+} and one component (approx. 2.9 ns) for the protein in presence of 2 mM Ca^{2+}. Similar studies were performed with S-100a interacting with cardiolipin, phosphatidylserine or egg phosphatidylcholine, both in absence and in presence of 2 mM Ca^{2+}. Our data suggest that the conformation of the protein and its Ca^{2+}-binding properties vary depending on the characteristics of charge and structure of phospholipids.

Introduction

S-100 is a group of closely-related, small (21 kDa), acidic Ca^{2+}-binding proteins, S-100a_0, S-100a and S-100b, which are $\alpha\alpha$, $\alpha\beta$ and $\beta\beta$ in subunit composition, respectively (reviewed in Refs. 1,2). These proteins are structurally related to the Ca^{2+}-binding proteins of the EF-hand type, such as calmodulin, troponin C and parvalbumins. Both S-100 subunits exhibit unconventional binding sites in the N-terminal part of the polypeptide chain, corresponding to similar domains in the intestinal Ca^{2+}-binding protein (ICaBP). The site in the N-terminal part is one 30-residue putative 'EF-hand' calcium-binding domain (site I). A conventional Ca^{2+}-binding site, 28 residues long, is present in the C-terminal part of individual polypeptide chains (site II). The tertiary structure of canonical Ca^{2+}-binding sites has been predicted in a study of carp parvalbumin [3]. It has an helix-loop-helix conformation, the EF-hand, that has been shown to be characteristic of Ca^{2+}-regulated proteins of this type. It should be noted that the Ca^{2+}-binding loops of the C-terminal domains of both S-100 subunits share the same amino acids in the position corresponding to the Ca^{2+} ligands, and could be responsible for identical properties. These properties are similar to the EF hand prototype found in parvalbumin [4]. The major structural modification upon Ca^{2+} binding to the S-100a subunit (site IIa) is the exposure of a hydrophobic sequence [5]. This sequence, containing Trp-90, Phe- 88 and -89, Cys-84 and Tyr-73, might constitute the α-helix (helix D) essential for the Ca^{2+}-binding domain of the site II [5].

S-100 proteins are found in a soluble and a membrane-bound form and have the ability to interact with artificial and natural membranes [1,2]. The properties and the functions of the membrane-bound form of S-100 remain to be elucidated.

We have previously demonstrated that the $\beta\beta$-homodimer, S-100b, can interact with cardiolipin (CL) liposomes and assumes a new conformation in which its Ca^{2+}-binding properties are greatly enhanced [6]. S-100a protein is the $\alpha\beta$ heterodimer. The α-subunit is characterized by the presence of a single Trp residue, whose fluorescence characteristics in solution have been studied [5,7]. In the present work, the fluorescence of S-100a was studied by time-resolved techniques. The Trp fluorescence decay data were acquired by a frequency-domain fluorometer and were analyzed either in terms of a single- or double-exponential decay or as continuous distributions of lifetime values [8]. It

Correspondence to: G. Zolese, Istituto di Biochimica, Facoltà di Medicina Università di Ancona, Via Ranieri, 60100 Ancona, Italy.

is assumed that the proteins can exhibit a large number of conformations and that the rate of interconversion between them can be of the same order of magnitude of the excited-state decay rate. According to the approach proposed by Alcala et al. [8], this could result in a large distribution of Trp lifetimes, due to the different microenvironments sampled during the excited state. The aim of the present work is to establish how the interaction of S-100a with Ca^{2+} and phospholipids with different head groups affects the decay pattern of the Trp emission, in order to understand some molecular details concerning the S-100a-membrane interactions.

Materials and Methods

Protein purification

S-100 protein, a mixture of S-100a plus S-100b, was purified from bovine brain and elecrophoretically characterized as described [9,10]. To separate S-100a from S-100b, bovine brain S-100 protein was loaded onto a DEAE-Sephacel A-50 column (1 × 10 cm) equilibrated with 20 mM Tris-HCl (pH 7.5), 1 mM EDTA, 5 mM 2-mercaptoethanol (buffer A). The column was washed with 50 ml of Buffer A, followed by 50 ml of Buffer A containing 0.2 M NaCl. Then the column was developed with a linear NaCl gradient (0.2–0.35 M) in a total of 100 ml of Buffer A. By a combination of spectroscopy, i.e., registration of absorption spectra of individual chromatographic fractions, and electrophoresis in 20% (w/v) acrylamide gels under nondenaturing conditions [11], we monitored the separation of S-100a (left tail of the chromatogram) from S-100b (right tail of the chromatogram). The absorption spectrum of S-100a is quite different from that of S-100b [4]. Also, the two proteins are characterized by different electrophoretic mobilities under native conditions [11]. The purified S-100a protein was exhaustively dialyzed against deionized, double-distilled water, and liophylized.

Sample preparation

Egg phosphatidylcholine (EPC), bovine hearth cardiolipin (CL) and phosphatidylserine (PS) were obtained from Avanti Polar (Birmingham, AL, USA).

The desired lipid, suspended in chloroform, was dried under nitrogen flux in a round bottom flask. Multilamellar liposomes (MLV) were formed by resuspending lipids, at 35°C, in 20 mM Tris-HCl, 0.1 mM EDTA (pH 7.34), at a concentration of 1 mg/ml. Before each measurement the lipid was incubated at 20°C for 1 h with the protein. The final concentrations of the lipid and S-100a in the sample were 0.2 mM and 14 μM, respectively.

Time-resolved fluorescence measurements

The decay of S100a protein was studied by a multifrequency phase fluorometer, using synchrotron radiation as light source. The Adone Storage Ring of the Progetto Utilizzazione Luce di Sincrotrone laboratory (Frascati, Italy) was used in connection with a commercially available electronic set up (ISS, La Spezia, Italy) in the configuration described by Gratton et al. [12]. Excitation was at 295 nm and the emission was through a LG-350 Corion filter, to avoid light-scattering contribution to lifetime measurements. Color errors due to photomultiplier response were minimized by the use of a solution of *p*-terphenyl in cyclohexane (lifetime 1 ns). The modulation frequencies were variable from 17 to 197 MHz. Data were acquired at 10 different frequen-

TABLE I

Lifetime analysis for S-100a

Fluorescence decay was measured in 20 mM Tris-HCl, 0.1 mM EDTA (pH 7.34), 20°C, with an excitation wavelength of 295 nm. Ca^{2+} was added from a concentrated solution up to 2 mM final concentration. For experimental details, see Materials and Methods. τ, exponential lifetimes; c, center and w, width of Lorentzian distribution analysis; f, fractional contribution to the total fluorescence, $f_1 + f_2 = 1$; α, pre-exponential terms associated with lifetimes, $\alpha_1 + \alpha_2 = 1$; χ^2, reduced χ^2.

A: discrete component analysis of data							
Sample	τ_1	f_1	α_1	τ_2	f_2	α_2	χ^2
S-100 in buffer	1.92	1	1	–	–	–	757
	3.96	0.63	0.28	0.92	0.37	0.72	9.9
S-100 + 2 mM Ca^{2+}	2.52	1	1	–	–	–	482
	4.29	0.71	0.40	1.16	0.29	0.60	10
B: Lorentzian distributions of decay times							
Sample	c_1	w_1	f_1	c_2	w_2	χ^2	
S-100 in buffer	2.01	2.60	1	–	–	4.14	
	3.04	1.86	0.73	0.94	0.69	2.02	
S-100 + 2 mM Ca^{2+}	2.82	2.30	1	–	–	1.15	
	2.89	2.10	0.99	< 0.01	0.08	0.85	

cies with the uncertainties of 0.2° and 0.004 for phase angles and modulation ratios, respectively. Data were fitted with a sum of exponential decay components using non-linear least-square analysis or as continuous distributions of lifetime values. In both cases, the analysis software was provided by ISS according to models and equations described by Alcala et al. [12]. The reduced χ^2 value was used to judge the goodness of fit [13]. All measurements were performed at 20°C.

Steady-state fluorescence measurements

Polarization measurements were performed in a Perkin-Elmer MPF66 spectrofluorometer. For polarization measurements, Trp was excited at 295 nm and fluorescence was collected at 360 nm. A series of parallel experiments were performed in unlabeled PL suspensions to measure the contribution of light-scattering to fluorescence measurements. Results of fluorescence are presented as means ± S.D. All measurements were made at 20°C.

For emission spectra, Trp was excited at 295 nm and emission spectra were corrected for the background and Raman scattering.

Results

Measurements in absence of phospholipids

The fluorescence decay of S-100a was measured at 20°C in 20 mM Tris-HCl (pH 7.34), both in the presence or in the absence of 2 mM Ca^{2+} (Table I). This Ca^{2+} concentration was chosen because under these conditions Ca^{2+}-binding sites on S-100 are completely saturated [4]. In the single exponential analysis, the interaction with Ca^{2+} causes a 31% increase in the lifetime value, compared to the value obtained in absence of Ca^{2+}. However, when the fluorescence decay is described by two exponentials, there is an 8 and 26% increase in longer and shorter lifetime, respectively, while the fractional intensity associated with the first one is only slightly increased. The best fit to the fluorescence decay of NATA is by a single exponential, however, in the case of S-100a the large χ^2 values obtained indicated that the Trp decay is unsatisfactorily described by a single-exponential and double-exponential analysis, also if an increase of fit is obtained with the double-exponential model. The best fit was obtained using a bimodal Lorentzian distribution of lifetimes: about 80–90%, compared to the biexponential analysis. Gaussian and Uniform distribution analysis did not yield good fits for the same data (not shown). These results are in agreement with recent literature data reporting comparison among uniform, Gaussian and Lorentzian distributions, for the analysis of Trp fluorescence decay in several proteins. These reports showed that Lorentzian distributions better describe the observed emission decay [8,13]. The Lorentzian distribution is characterized by a center of lifetimes distribution and a full-width at half maximum [14]. The distributions were normalized and defined only in the positive lifetime domain. At 20°C, the fluorescence decay of S-100a in absence of Ca^{2+} was characterized by two broad distribution of lifetimes,

TABLE II

Fluorescence decay fitting parameters for S100a

The protein (14 μM) was incubated for 1 h at 20°C, with MLV of the desired lipid (0.2 mM), T = 20°C, pH 7.34, no Ca^{2+}. For symbols and other conditions, see Table I.

A: discrete component analysis of data

Sample	τ_1	f_1	α_1	τ_2	f_2	α_2	χ^2
S100+EPC	1.99	1	1	–	–	–	821
	4.41	0.60	0.26	1.00	0.40	0.74	18
S100+CL	1.99	1	1	–	–	–	1524
	5.53	0.60	0.21	0.94	0.40	0.79	62
S100+PS	1.83	1	1	–	–	–	1177
	4.00	0.66	0.26	0.74	0.34	0.74	27

B: Lorentzian distributions of decay times

Sample	c_1	w_1	f_1	c_2	w_2	χ^2
S100+EPC	2.08	2.85	1	–	–	1.77
	2.14	2.76	0.99	< 0.01	0.07	1.98
S100+CL	1.72	4.68	1	–	–	4.56
	1.80	4.14	0.97	< 0.01	0.05	3.44
S100+PS	1.72	3.45	1	–	–	9.9
	2.27	2.65	0.95	< 0.01	0.05	2.0

centered at 3.04 ns and 0.94 ns, respectively, while only the longer lifetime (2.89 ns) was evident in presence of 2 mM Ca^{2+} (Table I). In the first case, 73% of the fluorescence was associated with this longer lifetime, while in presence of Ca^{2+} this percentage increased to 99%. Moreover, the distribution width of 3 ns component was only slightly increased in presence of Ca^{2+}. The meaning of the very fast component centered at < 0.01 ns, which was detected in the presence of 2 mM Ca^{2+}, remains unexplained. A very fast component < 0.01 ns will be present also in other measurements (see Tables I–IV). Castelli et al. [15] suggested that scattered excitation light could result in the appearance of fast (0.03 ns) decay components, and this could be the origin of the short lifetime also found in some of our measurements. However, no increase of fit was obtained, in these samples, by a three-component distribution analysis including a very short lifetime (0.001 ns), to eliminate light scattering contribution. Since the origin of this fast component is unknown and, when present, it accounts for only the 1–14% of the fluorescence decay, it will be no more discussed here.

Measurements in the presence of phospholipids (PLs)

The fluorescence decay of S-100a was measured in the presence of zwitterionic or anionic lipids, in the presence or absence of 2 mM Ca^{2+}. Table II shows the results obtained in absence of Ca^{2+}. In the presence of PLs, the χ^2 values obtained by the double-exponential analysis were particularly high (Table IIA). A large increase of fit (about 90%, in each case) was obtained using the Lorentzian distribution of lifetimes. No increase of fit was obtained using Gaussian or Uniform distribution analysis (not shown). In each case, the 95–100% of fluorescence intensity was associated with a lifetime component that was 2.08 ns, 1.80 ns and 2.27 ns in presence of EPC, CL and PS, respectively. When EPC or PS were added to the sample, the center and the width of the distribution were similar to the unimodal distribution obtained for S-100a in the absence of PLs, suggesting that the impossibility to obtain two distinct lifetimes could be dependent by the mobility of the protein molecule, decreased by the interaction with the PLs. In the presence of CL, the S-100a fluorescence decay was clearly best fitted by an unimodal distribution characterized by a center value (1.80 ns) similar to that obtained with the other PLs (14–26% difference). On the contrary, the width was largely increased, suggesting an increase of the number of different microenvironments sampled by Trp during its excited lifetime. Also when Ca^{2+} is present in the sample, in each case the best fit was obtained when the Lorentzian distribution of lifetimes was used to describe the Trp fluorescence decay (Table III). Acceptable χ^2 values were obtained with an unimodal distribution of lifetimes: the central value was similar to the longer lifetime obtained for S-100a in the presence of 2 mM Ca^{2+} and absence of PLs, whereas the width of distribution was dependent from the nature of PL considered.

Steady-state fluorescence measurements

The fluorescence polarization of S-100a on excitation at 295 nm is reported in Table IV. Under our

TABLE III

Fluorescence decay fitting parameters for S100a

pH 7.34, T = 20°C in presence of liposomes and 2 mM Ca^{2+}. For symbols, see Table I. All other conditions are described in the legend of Table I and in Materials and Methods.

A: discrete component analysis of data

Sample	τ_1	f_1	α_1	τ_2	f_2	α_2	χ^2
S100 + EPC	2.66	1	1	–	–	–	418
	4.57	0.69	0.39	1.32	0.31	0.61	13
S100 + CL	2.83	1	1	–	–	–	582
	6.46	0.56	0.25	1.66	0.44	0.75	33
S100 + PS	2.55	1	1	–	–	–	565
	4.61	0.69	0.37	1.21	0.31	0.63	22

B: Lorentzian distributions of decay times

Sample	c_1	w_1	f_1	c_2	w_2	χ^2
S100 + EPC	3.00	2.19	1	–	–	1.97
	3.02	2.19	0.996	1.32	0.05	2.36
S100 + CL	3.27	2.95	1	–	–	11
	3.00	1.78	0.86	< 0.01	0.26	2.10
S100 + PS	2.88	2.61	1	–	–	4.51
	2.98	2.23	0.99	0.01	0.05	5.65

TABLE IV

Rotational relaxation times of S-100a protein from polarization of the intrinsic fluorescence, pH 7.34

Conditions	Polarization P (±0.005)	$\langle\tau\rangle$ (ns) [a] (±0.16)	Φ [b] (ns)
S-100 in buffer	0.137	1.77	4.61
+2 mM Ca^{2+}	0.138	2.40	6.34
S-100+EPC	0.151	1.87	5.95
+EPC+2 mM Ca^{2+}	0.133	2.58	6.35
S-100+CL	0.194	1.87	11.50
+CL+2 mM Ca^{2+}	0.146	2.85	8.45
S-100+PS	0.186	1.60	8.61
PS+2 mM Ca^{2+}	0.154	2.45	8.14

[a] Average lifetime in ns calculated as $\langle\tau\rangle = \alpha_1\tau_1 + \alpha_2\tau_2$.

[b] Φ, rotational relaxation time, calculated from the Perrin equation. For the calculation of the relaxation time, $P_0 = 0.28$ is the limiting polarization for S-100 [6].

experimental conditions, the presence of 2 mM Ca^{2+} did not change the S-100 polarization, while the average lifetime was significantly increased. Relaxation times (Φ) were calculated using the polarization values (P) and the average lifetime $\langle\tau\rangle$ by the Perrin equation [5,7]. For the protein in buffer (no additions), the mean rotational relaxation time was comparable to the value calculated for a protein of similar molecular weight [17]. This indicates that Trp residue in S-100a has little internal rotational freedom, that is slightly decreased in presence of calcium, in accordance with previous data [5]. In fact, in the presence of Ca^{2+}, Baudier and Gerard [5] found a small decrease in polarization, an increase in lifetime and an increase in rotational relaxation time. These data were interpreted as indicating the occurrence of stabilization of the protein structure. In the presence of EPC liposomes without Ca^{2+}, the polarization value differed only slightly from the corresponding value obtained without liposomes and returned to the control value in the presence of Ca^{2+}. The calculated rotational relaxation times were similar to those obtained for the protein in the presence of Ca^{2+}; i.e., 5.95 ns in the absence and 6.35 ns in the presence of Ca^{2+}. The rotational relaxation time obtained in the presence of EPC liposomes without Ca^{2+} indicated that the overall protein rotations were affected by the interaction of S-100a with the PL bilayer, whereas the effect of Ca^{2+} was not changed by the presence of EPC. The overall protein rotations are more affected by negative lipids, both in absence and in presence of Ca^{2+}, as indicated by the rotational relaxation times obtained (Table IV), suggesting that the interaction depends on the structural and chemico-physical characteristics of PLs.

The average amplitude of rotation (Θ) for the restricted motion of the Trp can be calculated by polarization, using the following equation [6]:

$$\cos^2\Theta = 1/3(1+2/(1+3\tau/\Phi))$$

Using the data in Table IV, this equation gave a value of 36° for the protein in buffer, irrespective of the presence or absence of Ca^{2+}, and was almost unchanged in the presence of EPC, again irrespective of the absence (35°) or presence (37°) of Ca^{2+}. Also, in presence of CL and PS the average amplitude of rotation is only slightly affected (28°/29° and 34°/35° in CL and PS, in absence and in presence of Ca^{2+}, respectively). These data could indicate that the amplitude of the overall motion of the protein, as well as the average of local motions, was unaffected either by Ca^{2+} or by PLs.

Steady-state fluorescence spectra (emission maximum 342 nm, Fig. 1) of S-100a in buffer are in agreement with previous data [19], showing an increase of intensity and a red shift (approx. 2 nm), when 2 mM Ca^{2+} is present in the sample. In absence of Ca^{2+}, the presence of the lipids causes an increase of fluorescence intensity, but the emission wavelength is red-shifted only in the presence of CL, suggesting an higher polarity for Trp environment in the presence of this PL. When the Ca^{2+} is present in the medium, each spectrum is increased in intensity and red-shifted, although the modification of the spectrum is different

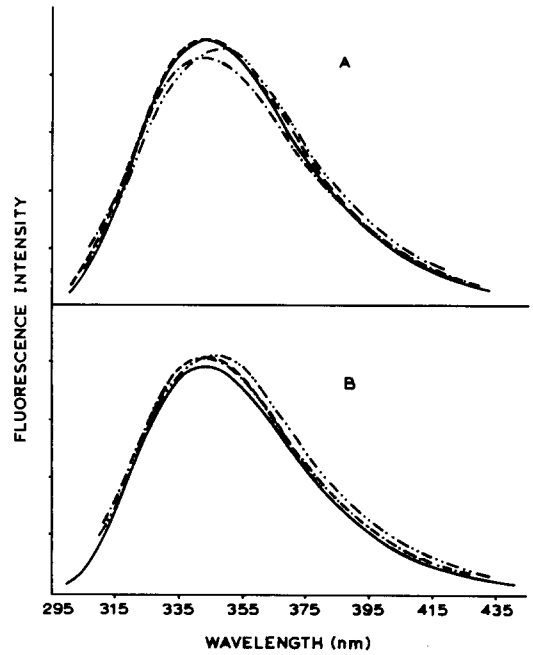

Fig. 1. Steady-state fluorescence emission spectra of S-100a in absence of Ca^{2+} (A) and in presence of 2 mM Ca^{2+} (B). Excitation wavelength was 295 nm. All measurements were made at 20°C in 20 mM Tris-HCl, 0.1 mM EDTA (pH 7.34). S-100a in buffer (———), in the presence of EPC (— — —), PS (-·-·-) or CL (-···-···).

with the type of PL. These findings imply that the environment of Trp residue is modified by the presence of PLs, depending from the type of lipid considered.

Discussion

Several factors, including exposure to water molecules, many other moieties intrinsic to the protein structure and/or excited state processes, can affect the fluorescence decay of tryptophan residues in proteins. These factors largely influence the measured fluorescence lifetime, which varies by more than a factor of 100 in different proteins [18]. The Trp fluorescence decay is usually described in terms of exponential components. Each component is usually associated with a single Trp residue. However, even proteins with a single Trp can have a complex decay [18], due to conformational heterogeneity. In the case of S-100a protein, under our experimental conditions, an improvement of data fitting has been obtained using the distributional approach introduced by Alcala et al. [8]. For this reason, we used the distribution fit as a more convenient way to directly compare the fits to data obtained for S-100a. The distributional approach assumes that proteins can experience a large number of conformations and that the rate of interconversion between them can be of the same order of magnitude as the excited-state decay rate. The number of average protein conformations, as well as the rate of interconversion between them, determines the characteristics of the lifetime distribution [8]. The possibility for Trp to probe a multiplicity of environments can give origin to an heterogeneity in the fluorescence decay and is interpreted in terms of width of lifetime distributions. According to this approach, the two relatively large distributions (centered around 3.04 and 0.94 ns), obtained for S100a, indicate that the protein can experience a large number of different microenvironments during its excited-state lifetime, suggesting an high degree of the protein flexibility related to Trp microenvironment. Our data, showing two decay times for S100a, are in agreement with previous results [19]. The presence of the second lifetime in S100a has been interpreted as due to the existence of complexed Trp, interacting with neighboring quenching groups [19]. Previous data demonstrated that an excess of Ca^{2+}, e.g., 4 mM, causes a partial exposure of the Trp residue to the solvent, that is associated with an increase of the quantum yield [3]. Moreover, in similar conditions, it has been shown an increase of decay times and a large decrease of percentage contribution of the short component [19]. These results were related to a decrease of the dynamic quenching, due to the presence of other amino-acid residues in the Trp environment [3]. Our results, for S-100a interacting with Ca^{2+}, are consistent with this hypothesis, since the longer value of lifetime obtained and the larger distributional width of lifetimes indicate a remarkable change in the properties of the microenvironment surrounding the fluorophore. In fact, a broader distribution of decay lifetimes would be expected for Trp residues free to move through large angles, and experiencing a larger number of microenvironments during its excited state lifetime. Such a situation is more likely for residues on the surface of proteins or in water filled pockets [7].

The behavior of Trp fluorescence was also studied in presence of different types PLs. S-100 proteins have been reported to interact with both artificial and natural membranes [6,7,20–23], although structural and functional characteristics of these membrane-bound forms need further investigations.

Under our experimental conditions, the interaction of S-100a with EPC results in a decrease in the rotational mobility of the overall protein (Table IV), evident in absence of Ca^{2+}. In the presence of EPC, the fluorescence decay of S-100a is best fitted by one-component Lorentzian distributional analysis, where the center of the distribution is similar to that shown by S-100a in buffer with no additions, with a small increase in the width of distribution. This behavior could be due to a decrease in the mobility of the protein in its membrane-bound state, which also modifies the mobility of Trp residue in its unmodified microenvironment. This hypothesis is also confirmed by the calculated average amplitude of rotation (see Results), which suggests that the average motions of Trp are unchanged in the presence of EPC liposomes. Similar results were obtained in the presence of PS liposomes. On the contrary, CL liposomes induce a larger increase of lifetime distributional width, indicating a larger number of microenvironments for the Trp residue. A dependence from the type of PL used is also shown in the presence of 2 mM Ca^{2+}, suggesting that the conformation of the protein and its Ca^{2+}-binding properties could be strictly related to the interaction with PLs, with different characteristics of charge and structure. These results are in line with fluorescence emission spectra (Fig. 1) and with our previous data on interaction of S-100b protein with CL liposomes by different experimental approaches [6]. We have proposed that S-100b can interact through its hydrophobic cluster (residues 7–19) together with its cationic site (residues 20–33) with the negatively-charged CL liposomes in the absence of Ca^{2+} [21]. The interaction of S-100b with CL liposomes in the absence of Ca^{2+} produces large changes in the secondary structure of the protein [6], which are similar to those produced by Ca^{2+} on both S-100b and S-100a, i.e., a decrease in the α-helical content of the proteins [6,24], as well as an increase in the Ca^{2+}-binding affinity. On the other hand, Ca^{2+}

increases the Trp exposure to water in S-100a and its average lifetime value [5].

Our fluorescence data on S-100a suggest that the interaction with liposomes in absence of Ca^{2+} significantly modifies the Trp environment only in the case of CL liposomes, whereas in presence of EPC or PS liposomes the average conformation around the Trp residue is almost unchanged, although the rate of fluctuation of the protein is likely modified. The interaction with liposomes does not modify the Ca^{2+} effect when the PL is EPC, while Trp fluorescence decay presents different characteristics, as compared to S-100a in buffer, when the PL is CL or PS. This suggests that the protein conformation could be strictly dependent on the type of PL used. Although other experimental evidence is necessary to prove this possibility, our data suggest that the protein could assume different conformations upon interaction with membranes of different PL composition. Previous work on binding of S-100 protein to brain membranes indicates that both membrane proteins and phospholipids play a role in the binding process [25,26]. The functional meaning of S-100 binding to membranes is largely unknown, although data on effects of S-100 proteins on adenylate cyclase activity, on Ca^{2+}-induced Ca^{2+} release and on regulation of liposome aggregation and fusion have been presented [22,27–29], suggesting an involvement of S-100 protein in the regulation of membrane trafficking and/or the structural organization of membranes.

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