

# Calcium- and pH-linked oligomerization of sorcin causing translocation from cytosol to membranes

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**Abstract** Sorcin, a cytosolic calcium-binding protein containing a pair of EF-hand motifs, undergoes a  $\text{Ca}^{2+}$ -dependent translocation to the cell membrane. The underlying conformational change is similar at pH 6.0 and 7.5 and consists in an increase in overall hydrophobicity that involves the aromatic residues and in particular the two tryptophan residues which become less exposed to solvent. The concomitant association from dimers to tetramers indicates that the tryptophan residues, which are located between the EF-hand sites, become buried at the dimer–dimer interface.  $\text{Ca}^{2+}$ -bound sorcin displays a striking difference in solubility as a function of pH that has been ascribed to the formation of calcium-stabilized aggregates.

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**Key words:** Sorcin; Calcium- and pH-dependent conformational change; Oligomerization

## 1. Introduction

Sorcin (SOLuble Resistance-related Calcium-bInding protein) is an EF-hand calcium-binding protein initially isolated from multidrug-resistant cells but expressed also in a number of normal tissues, such as cardiac and skeletal muscle, kidney and brain [1,2]. The 22-kDa subunit is organized in two different domains; the N-terminal one is 30% homologous to the corresponding domain of the calpain light chain where it is involved in heterodimer formation [3]. The C-terminal domain contains a pair of high affinity, EF-hand calcium-binding motifs [4] and a cAMP-dependent protein kinase recognition site which is phosphorylated in cultured cells by the protein kinase A catalytic subunit [5].

The biological role of sorcin in normal and transformed cells is as yet unknown. We established recently that, at physiological pH values, sorcin undergoes a calcium-dependent translocation from the cytosol to membranes at micromolar calcium concentrations and proposed that sorcin translocation is correlated to the functional activity of the protein [4]. The translocation process takes place in markedly different cells, like those of *E. coli* and heart, and can be reversed by ethylene glycol bis( $\beta$ -aminoethyl ether) (EGTA). The nature of the sorcin-binding site on membranes was not investigated although a hydrophobic type of interaction was suggested since calcium binding was shown to render sorcin soluble in the non-ionic detergent Triton X-114. Most recently, an inter-

action with specific protein targets was proposed by Meyers et al. [6], who observed co-precipitation of sorcin with the ryanodine receptor in an immunofluorescence study of cardiac myocytes, and by Brownawell and Creutz [7], who observed a calcium-dependent interaction between sorcin and immobilized annexin VII from bovine adrenal medullary tissue.

To characterize the calcium-dependent conformational change which provides the structural basis for the interaction between  $\text{Ca}^{2+}$ -bound sorcin and such diverse membrane proteins, a number of physicochemical properties of sorcin were studied in the absence and presence of calcium. Special attention was given to the overall hydrophobicity since the Triton X-114 experiments recalled above [4] indicate that sorcin, like many other EF-hand calcium-binding proteins [8], exposes hydrophobic patches upon calcium binding. The results obtained bring out the interplay between the binding of calcium and of protons and the existence of a ligand-linked oligomerization, a property that to our knowledge has not been observed in proteins with canonical 12-amino-acid EF hands.

## 2. Materials and methods

### 2.1. Expression and purification of sorcin

Expression of recombinant sorcin in *E. coli* BL21(DE3) and its purification were carried out as described in [4]. The concentration of the purified protein was determined spectrophotometrically at 280 nm using the molar extinction coefficient 29 400.

### 2.2. Determination of $\text{Ca}^{2+}$ affinity

$\text{Ca}^{2+}$ -binding constants were obtained from titrations of sorcin in the presence of the fluorescent  $\text{Ca}^{2+}$  chelator, 2-[[2-bis-(carboxymethyl)amino]-5-methylphenoxy]methyl]-6-methoxy-8-bis-(carboxymethyl)amino]quinoline (Quin 2) according to [9]. The Quin 2 concentration was determined from the absorbance of the  $\text{Ca}^{2+}$  complex at 240 nm ( $\epsilon_M = 42\,000 \text{ mol}^{-1} \text{ cm}^{-1}$ ). All solutions were prepared in 100 mM Tris-HCl at pH 7.5 using doubly distilled water and were stored in plastic containers with a dialysis bag containing Chelex 100. In the titration experiments, Quin 2 and sorcin were at a concentration of 25  $\mu\text{M}$  (total volume 2 ml); the  $\text{Ca}^{2+}$  solution (10 mM) was added in aliquots of a few  $\mu\text{l}$ . After each  $\text{Ca}^{2+}$  addition, the solution was allowed to equilibrate for 2 min at 25°C. The experiments were carried out in a Fluoromax spectrofluorimeter; excitation was at 339 nm (slit 0.5 nm). The increment of the emission signal due to  $\text{Ca}^{2+}$  binding to Quin 2 was followed at 492 nm (slit 0.5 nm). The overall binding constant,  $K_{ov}$ , was obtained by fitting the experimental data with a program written with the MATLAB program (The Math Works, Natick, MA).

### 2.3. Determination of the calcium dissociation rate constant

The rate of calcium dissociation from sorcin was determined at 20°C in a stopped flow apparatus (Applied Photophysics, Leatherhead, UK) in 100 mM Tris-HCl buffer at pH 7.5 pre-treated with Chelex 100.  $\text{Ca}^{2+}$ -bound sorcin (80  $\mu\text{M}$ ) was mixed with Quin 2 (440  $\mu\text{M}$ ) and the reaction was followed at 360 nm where the absorbance of Quin 2 decreases upon calcium binding. As reported in [10], binding of any free  $\text{Ca}^{2+}$  to Quin 2 takes place in the dead time of the instrument (3 ms).

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**Abbreviations:** Quin 2, 2-[[2-bis-(carboxymethyl)amino]-5-methylphenoxy]methyl]-6-methoxy-8-bis(carboxymethyl)amino]quinoline; EGTA, ethylene glycol bis( $\beta$ -aminoethyl ether)

#### 2.4. Circular dichroism spectra

CD spectra were recorded on a Jasco J-710 spectropolarimeter in the far-UV (195–240 nm) and near-UV (250–350 nm) regions at 25°C, unless otherwise stated, in 0.1 M sodium acetate at pH 6.0 and in 0.1 M Tris-HCl at pH 7.5. The buffers contained 2 mM EGTA or sufficient calcium to saturate sorcin. The  $\alpha$ -helical content was calculated from the ellipticity value at 222 nm [11].

#### 2.5. Hydrophobic interaction chromatography experiments

Hydrophobic interaction chromatography (HIC) experiments were performed at room temperature using either a Phenyl- or an Octyl-Sepharose column (0.7×2.5 cm) connected to an FPLC apparatus (Pharmacia, Uppsala, Sweden). The experiments were carried out in 50 mM Tris-HCl at pH 7.5 and in 50 mM sodium acetate or BisTris-HCl at pH 6.0 in the absence or presence of 1 mM EGTA. The calcium concentration of both buffers in the absence of EGTA was 2  $\mu$ M as determined by atomic absorption spectroscopy. Sorcin (1 mg/ml) was applied to the HIC column after dialysis against the equilibration buffer; elution was carried out with water.

#### 2.6. Interaction of sorcin with liposomes

Small unilamellar vesicles (SUV) were prepared as described in [12]. Phosphatidylcholine, phosphatidylserine, phosphatidylglycerol and cardiolipin were dissolved in an appropriate volume of buffer (50 mM Tris-HCl at pH 7.0 containing 0.1 M NaCl or 50 mM sodium acetate at pH 6.0 containing 0.1 M NaCl) to give a final concentration of 10 mg/ml. After stirring for 30 min at 25°C, the lipidic dispersion was sonicated (1 min/ml) in a MSE Soniprep 150 sonicator while maintaining the temperature at 4°C. The interaction of sorcin with SUV was studied at pH 7.0 and 6.0. Liposomes and sorcin were mixed to yield a final concentration of 4 and 1 mg/ml, respectively, and were incubated for 5 min at room temperature. Thereafter, 1 ml of the mixture was loaded onto a Sephadex G-75 (Pharmacia, Uppsala, Sweden) column (1×60 cm) in order to separate the unbound protein from liposomes and any liposome-bound sorcin. The presence of liposome-bound sorcin was monitored spectrophotometrically and by SDS-PAGE [13].

Cytochrome *c* oxidase vesicles (COV) were prepared according to the cholate dialysis procedure [14]. Typically, 50 mg/ml of purified asolectin in HEPES-NaOH 0.1 M at pH 7.3 and containing 25 mM cholic acid were gently stirred at room temperature for 30 min under nitrogen. The suspension was clarified by sonication under nitrogen at 4°C; cytochrome *c* oxidase (10  $\mu$ M in heme) was added and the suspension dialysed extensively against the same HEPES-NaOH buffer containing 2 mM EGTA or 2 mM CaCl<sub>2</sub>. Sequential mixing stopped flow experiments were carried out in an Applied Photophysics apparatus. The COV solution (10  $\mu$ M in heme) was mixed with sorcin (9  $\mu$ M) in the same calcium-containing buffer (first mixing) and the resulting solution was mixed with reduced cytochrome *c* (60  $\mu$ M, second mixing). The oxidation of cytochrome *c* was followed at 550 nm. As a control, the effect of the ionophore valinomycin was recorded.

#### 2.7. Sedimentation velocity experiments

Sedimentation velocity experiments were performed in a Beckman Optima XL-A ultracentrifuge on sorcin dialysed against 100 mM Tris-HCl at pH 7.5 containing 2 mM EGTA or against 100 mM sodium acetate at pH 6.0 containing 2 mM EGTA or 2 mM EGTA+2.1–2.5 mM calcium. The experiments were carried out at 10–40°C and at 40 000 rev/min. The movement of the protein toward the bottom of the cell was determined by absorption scans along the centrifugation radius at a wavelength of 280 nm. Sedimentation coefficients were corrected to  $s_{20,w}$  using standard procedures. The  $s_{20,w}$  values were correlated to the state of association of the protein assuming that the *n*-mers are spherical and that sedimentation coefficient is proportional to  $(M_r)^{2/3}$  [15].

### 3. Results

#### 3.1. Calcium affinity

Calcium titrations were carried out at pH 7.5 and 25°C in 0.1 M Tris-HCl buffer in the presence of Quin 2. As soon as saturation of the protein is exceeded, a precipitate forms such that the estimate of the higher asymptote is difficult. In Fig. 1,

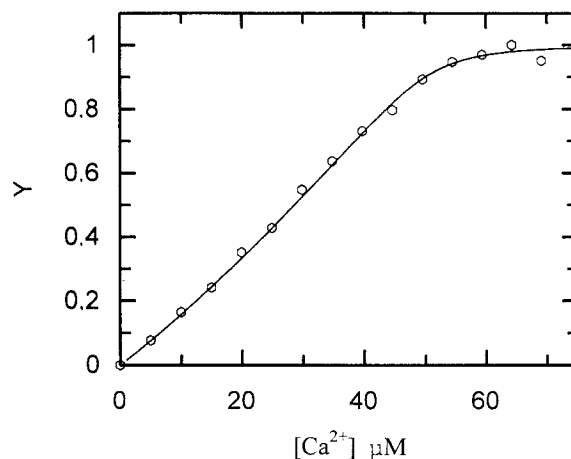


Fig. 1. Indirect titration of sorcin with calcium in the presence of Quin 2. The degree of saturation of Quin 2 is plotted as a function of total calcium concentration. Conditions: 25  $\mu$ M sorcin and Quin 2 in 0.1 M Tris-HCl at pH 7.5 and 25°C.

the data have been plotted in terms of the degree of saturation of Quin 2 as a function of the total calcium concentration and have been fitted with an overall affinity constant,  $K_{ov}$ ,  $2 \times 10^{12} \text{ M}^{-2}$ .

Preliminary experiments indicated that, at pH 6.0, Ca<sup>2+</sup>-bound sorcin is soluble when the temperature is increased to 40°C. Direct fluorescence titrations were carried out at this temperature. The decrease of intrinsic protein fluorescence, plotted in terms of the degree of calcium saturation as a function of the total calcium concentration is shown in Fig. 2. The experimental data yield  $2 \times 10^8 \text{ M}^{-2}$  as the overall affinity constant,  $K_{ov}$ . This value is significantly lower than that measured at pH 7.5 in line with the structure of the EF-hand calcium-binding sites.

#### 3.2. Rate of calcium dissociation

Fig. 3 reports the time course of calcium dissociation from sorcin, measured in a stopped flow apparatus upon mixing the protein in 100 mM Tris-HCl at pH 7.5 with Quin 2 in molar excess. The reaction, followed at 360 nm where Ca<sup>2+</sup>-free and Ca<sup>2+</sup>-bound Quin 2 absorb differently, is monophasic and the absorbance change corresponds to the release of two calcium ions per sorcin molecule. Therefore, the first-order rate constant,  $k_{off}$ , applies to calcium dissociation from both binding sites. The  $k_{off}$  value,  $2.7 \text{ s}^{-1}$ , and the overall affinity constant yield an average calcium on rate,  $k_{on}$ , of  $\approx 3 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$ .

#### 3.3. Hydrophobic interaction experiments

Previous experiments [4] indicated that, at neutral pH values, sorcin partitions mainly in the hydrophobic phase of the non-ionic detergent Triton X-114 when calcium-saturated but remains in the aqueous phase in the presence of EGTA.

The hydrophobicity of sorcin and its calcium dependence have been investigated in detail by means of hydrophobic matrices of different properties, viz. Octyl-Sepharose, of pure hydrophobic character, and Phenyl-Sepharose, in which also the aromatic character as well as lack of charge play a role. Adsorption of a hydrophobic protein to these matrices is achieved in a high ionic strength buffer and elution in a low ionic strength one or simply in water. Strongly hydrophobic

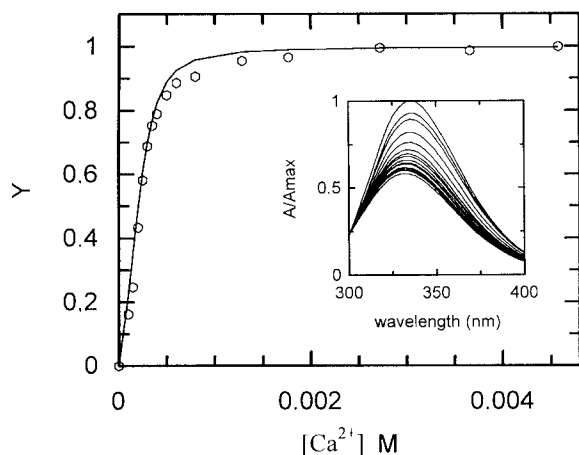


Fig. 2. Direct titration of sorcin with calcium at pH 6.0. The degree of saturation of sorcin is plotted as a function of total calcium concentration. The inset shows the quenching of the intrinsic protein fluorescence as a function of added calcium. Conditions: 15  $\mu$ M sorcin in 100 mM Na-acetate buffer at 40°C.

proteins will interact with these matrices even in buffers of low ionic strength [16].

The results obtained at pH 7.5 and 6.0 are presented in Table 1. At pH 7.5, sorcin interacts differently with Octyl- and Phenyl-Sepharose. Thus, essentially all the protein binds to Octyl-Sepharose in the 50 mM equilibrating buffer both in the presence and in the absence of calcium, while between 30% (in the presence of calcium) and 50% (in the presence of EGTA) elutes from Phenyl-Sepharose. In line with these findings, upon elution with water, more sorcin is released from the Octyl- than from the Phenyl-Sepharose column. Full recovery, however, is never obtained. At pH 6.0, the interaction with both matrices is much stronger than at pH 7.5 although usually a decrease in pH weakens hydrophobic interactions. Table 1 shows that practically no protein is eluted with the equilibrating buffer nor with water irrespective of the presence of calcium. Elution of sorcin with water can be achieved only after equilibration of the columns at pH 7.5. Again, full recovery of sorcin is not obtained.

#### 3.4. Interaction of sorcin with liposomes

The possibility that sorcin might interact directly with the lipidic component of the membranes was explored using liposomes as model systems. SUV, prepared with phosphatidylcholine, phosphatidylserine, phosphatidylglycerol and cardio-

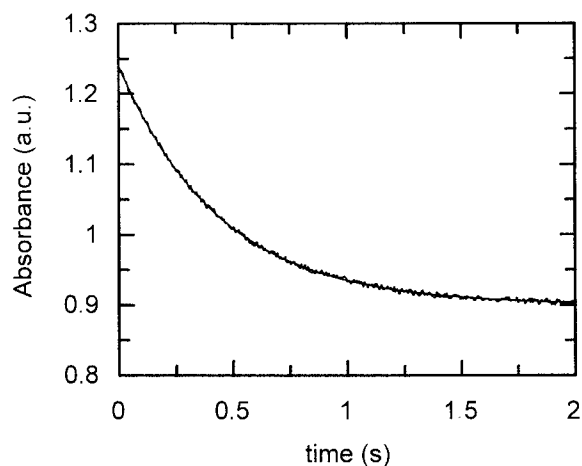


Fig. 3. Time course of calcium dissociation from sorcin. Sorcin (80  $\mu$ M) was mixed in a stopped flow apparatus with Quin 2 (440  $\mu$ M). The absorbance change was followed at 360 nm. Conditions: 0.1 M Tris-HCl at pH 7.5 at 25°C.

lipin, were subjected to chromatography on a Sephadex G-75 column after incubation with sorcin in 50 mM Tris-HCl at pH 7.5 or 50 mM sodium acetate at pH 6.0, in the presence of stoichiometric amounts of calcium. No evidence of interaction was obtained upon analysis of the eluate by absorption spectroscopy and SDS-PAGE (data not shown).

The absence of interaction was confirmed in a different kind of experiment. COVs were employed as they allow one to monitor changes in liposome permeability by following the rate of oxidation of reduced cytochrome *c* by cytochrome *c* oxidase in air [17,18]. Fig. 4 shows that, at variance with the ionophore valinomycin, sorcin in calcium-containing buffer does not affect the rate of cytochrome *c* oxidation with respect to the control.

#### 3.5. Circular dichroism experiments

The hydrophobic chromatography experiments reported above indicate that: (i) at physiological pH values the sorcin molecule has hydrophobic properties both when  $\text{Ca}^{2+}$ -free and when  $\text{Ca}^{2+}$ -bound; (ii) at pH 6.0 the aromatic residues are more accessible, especially in the  $\text{Ca}^{2+}$ -bound protein. In order to establish whether this difference in behaviour could be ascribed solely to the change in the ionization of the protein and/or to a conformational change, cd spectra were measured in the far- and near-UV region.

Table 1

Fractional amount of  $\text{Ca}^{2+}$ -free and  $\text{Ca}^{2+}$ -bound sorcin eluted from hydrophobic chromatography columns at pH 7.5 and 6.0

Eluting solution	$\text{Ca}^{2+}$ -free sorcin		$\text{Ca}^{2+}$ -bound sorcin	
	Octyl-Sepharose	Phenyl-Sepharose	Octyl-Sepharose	Phenyl-Sepharose
pH 7.5				
Buffer A	0.02	0.49	0.04	0.33
H <sub>2</sub> O	0.50	0.15	0.56	0.16
pH 6.0				
Buffer B	0	0	0	0
H <sub>2</sub> O	0.08	0.08	0	0
Buffer A	0	0.30	0.02	0.28
H <sub>2</sub> O	0.50	0.20	0.41	0.16

Sorcin (1 mg/ml) was applied to the columns after dialysis against the equilibrating buffer (buffer A or B) and was eluted with water at room temperature. For  $\text{Ca}^{2+}$ -free sorcin: buffer A, 50 mM Tris-HCl at pH 7.5 containing 1 mM EGTA; buffer B, 50 mM sodium acetate buffer or BisTris-HCl at pH 6.0 containing 1 mM EGTA. For  $\text{Ca}^{2+}$ -bound sorcin: buffer A, 50 mM Tris-HCl at pH 7.5; buffer B, 50 mM sodium acetate buffer or BisTris-HCl at pH 6.0; the buffers contain 2  $\mu$ M calcium as determined by atomic absorption spectroscopy.

The far-UV cd spectra show that, at pH 6.0 and 7.5, in the absence of calcium, the secondary structure of the protein is unchanged and that the  $\alpha$ -helix content corresponds to  $\approx 50\%$ . The near-UV cd spectra were measured at pH 7.5 and 6.0 on the  $\text{Ca}^{2+}$ -free protein and as a function of added calcium. The latter experiments were carried out at 35–40°C in order to increase solubility of the  $\text{Ca}^{2+}$ -bound protein. In the presence of EGTA, there are no significant differences at the two pH values (Fig. 5A). Two sharp negative bands are visible at 262 and 268 nm, attributable to phenylalanines; the peaks characteristic of the tyrosyl fine structure at 283 and 276 nm, corresponding to the 0–0 and to the 0–1 (0–0+800  $\text{cm}^{-1}$ ) transition, respectively, are likewise negative and rather weak. At 292 nm, a sharp, well-resolved, fairly intense peak attributable to tryptophans is present.

Upon addition of calcium, the behaviour at pH 7.5 and 6.0 differs. At pH 6.0, the cd spectrum becomes progressively less negative such that  $\text{Ca}^{2+}$ -bound sorcin is characterized by a positive cd spectrum (Fig. 5B). The tryptophan peak is blue-shifted relative to that of the  $\text{Ca}^{2+}$ -free protein and overlaps with the tyrosine band(s) whose relative intensity is significantly increased. At pH 7.5, the cd spectrum is essentially unchanged until sorcin becomes saturated with calcium and precipitates when calcium is in excess. The cd spectrum of the protein that remains in solution after centrifugation resembles that measured on  $\text{Ca}^{2+}$ -bound sorcin at pH 6.0.

In order to test the contribution of electrostatic and hydrophobic interactions to the precipitation of  $\text{Ca}^{2+}$ -bound sorcin, the effect of EGTA and 0.2%  $\beta$ -octylglucoside was studied in 0.05 M Tris-HCl buffer at pH 7.5. Whereas EGTA reverses precipitation,  $\beta$ -octylglucoside has no effect. It produces a general decrease of the ellipticity in the near-UV region due to the general decrease in solvent polarity, with no significant shifts in the peaks of the aromatic residues.

### 3.6. Sedimentation velocity experiments

Sorcin sediments as a single band with  $s_{20,w} = 3.5\text{--}3.7\text{ S}$  in Tris-HCl buffer at pH 7.5 containing 2 mM EGTA and 10–

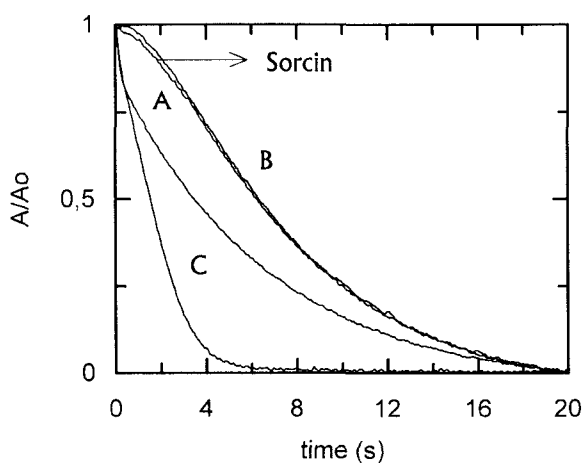


Fig. 4. Effect of sorcin on the time course of COV-catalyzed cytochrome *c* oxidation. The COV solution (10  $\mu\text{M}$ ) was mixed with sorcin (9  $\mu\text{M}$ ) in 0.1 M HEPES-NaOH buffer at pH 7.5 containing 2 mM  $\text{CaCl}_2$  and the resulting solution was mixed with reduced cytochrome *c* (60  $\mu\text{M}$ ) in a sequential mixing stopped flow experiment. The reaction was followed at 550 nm. Controls: (A) without sorcin in 2 mM EGTA; (B) without sorcin in 2 mM  $\text{CaCl}_2$ ; (C) effect of the ionophore valinomycin at 10  $\mu\text{M}$ .

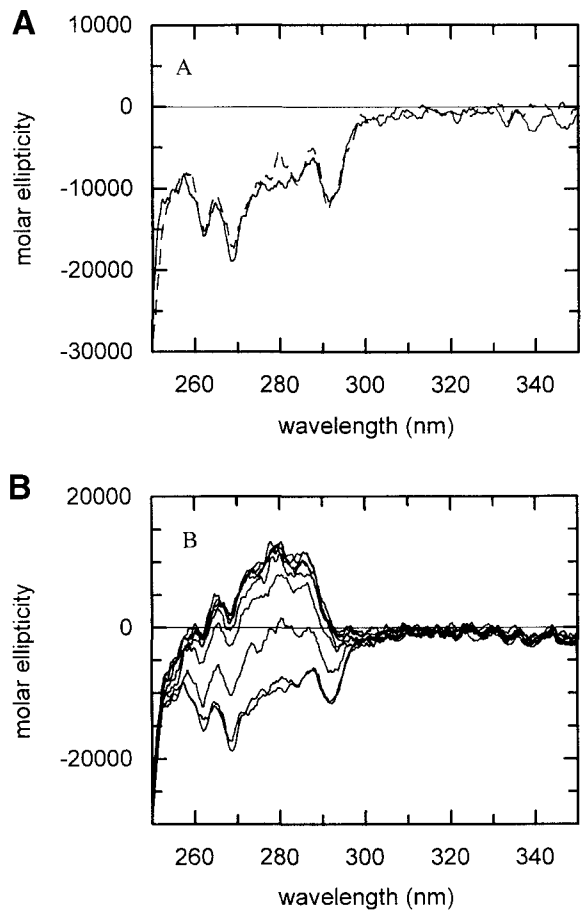


Fig. 5. Near-UV cd spectra of sorcin. (A) At pH 6.0 (---) and 7.5 (—) for the  $\text{Ca}^{2+}$ -free form; (B) at pH 6.0 as a function of total calcium concentration from 0 to 3.6 mM. Conditions: 0.1 M Na-acetate at pH 6.0 containing 2 mM EGTA, 40°C or 0.1 M Tris-HCl at pH 7.5 containing 2 mM EGTA, 40°C.

20°C. The  $s_{20,w}$  value indicates that sorcin is essentially all dimeric as suggested by previous gel-filtration data [19]. At pH 6.0, sedimentation velocity runs were performed over the temperature range 25–40°C. In the presence of 2 mM EGTA, sorcin is dimeric as at pH 7.5. In the presence of  $\text{Ca}^{2+}$ , measurements were carried out only at 35–40°C, where the protein is soluble; the  $s_{20,w}$  value increases to 6.0–6.6 S, a value indicative of tetramer formation assuming a spherical shape of the molecule [15].

## 4. Discussion

The potential function of sorcin in normal and multidrug-resistant cells is most likely related to the calcium-dependent translocation from cytosol to membranes recognized recently [4] and that has been proposed to involve specific target proteins, viz. the ryanodine receptor in cardiac myocytes [6] and annexin VII in adrenal medullary tissue [7]. The translocation process must have its structural basis in a major calcium-dependent conformational change that results in an increase in surface hydrophobicity. This is indicated by the observation that sorcin, when  $\text{Ca}^{2+}$ -bound, becomes soluble in the detergent phase of Triton X-114 [4]. The present work brings out that the exposure of hydrophobic surfaces is determined by a

complex interplay between the binding of calcium and of protons and changes in the state of association.

Sorcín in the  $\text{Ca}^{2+}$ -free form has physico-chemical properties that remain constant between pH 7.5 and 6.0: it is predominantly dimeric, has an  $\alpha$ -helix content of  $\approx 50\%$  and a negative cd spectrum in the near-UV region. The  $\text{Ca}^{2+}$ -bound form displays a striking difference over the same pH range, viz. a difference in solubility. Thus,  $\text{Ca}^{2+}$ -bound sorcín is soluble at pH 6.0 when temperature is increased to 35–40°C. At pH 7.5, it forms high molecular weight aggregates that precipitate in the presence of excess (free) calcium even at 35–40°C. Since precipitation can be reversed by addition of EGTA but is not influenced by  $\beta$ -octylglucoside, it may be envisaged that sorcín aggregates are stabilized by calcium ions which chelate-specific negatively charged groups located on the surface of the molecule. These groups may be identified tentatively with carboxylates in a cluster of negative charges since they are protonated at pH 6.0 and deprotonated at pH 7.5.

The effect of pH on the solubility of  $\text{Ca}^{2+}$ -bound sorcín brings out clearly the linkage between the binding of calcium and of protons but does not provide insight into the conformational change from the  $\text{Ca}^{2+}$ -free to the  $\text{Ca}^{2+}$ -bound form. Information on this point is provided by the near-UV cd spectra and sedimentation velocity data. Upon saturation of sorcín with calcium, the near-UV cd spectrum becomes positive demonstrating that a generalized change in the environment of the aromatic residues takes place (Fig. 5B). Of special interest is the shift towards lower wavelengths of the cd peak assigned to tryptophan residues since the only two tryptophans of the sorcín sequence are located between the two EF-hand calcium-binding sites in the C-terminal domain. The observed shift indicates that in the  $\text{Ca}^{2+}$ -bound protein tryptophans become less exposed to solvent. The sedimentation velocity data bring out that, concomitant with the near-UV cd change, the association from a predominantly dimeric to a tetrameric state takes place both at pH 6.0 and at pH 7.5. This finding and the similarity of the near-UV cd spectrum of  $\text{Ca}^{2+}$ -bound sorcín at the two pH values indicate that the change in solubility of the protein over this pH range is due solely to the ionization of specific residues.

The  $\text{Ca}^{2+}$ -linked formation of tetramers, taken together with the blue shift in the cd peak assigned to tryptophans, suggests that these residues and hence the C-terminal domain become buried at the dimer–dimer interface of  $\text{Ca}^{2+}$ -bound sorcín. It follows that the new hydrophobic surface that is created upon calcium binding is embedded in the interior of the molecule, a conclusion in apparent contrast with the observation that calcium binding renders sorcín soluble in the detergent phase of Triton X-114 [4]. The hydrophobic chromatography results clarify this point and in addition provide information into the possible mode of interaction of  $\text{Ca}^{2+}$ -bound sorcín with targets on the cell membrane. The interaction with Octyl-Sepharose is so strong that differences related to calcium binding are vanishingly small although an enhancement of the interaction at pH 6.0 relative to pH 7.5 can be seen clearly. The experiments with Phenyl-Sepharose are more useful as the interaction of sorcín with this matrix is less strong and hence displays a calcium and pH dependence. Consistent with the Triton X-114 experiments just recalled, at pH 7.5,  $\text{Ca}^{2+}$ -bound sorcín interacts more strongly with Phenyl-Sepharose than the  $\text{Ca}^{2+}$ -free form; moreover, at pH

6.0, the interaction is significantly enhanced for both forms of the protein due to the decrease in net charge. The most likely explanation of these results is that the hydrophobic surface which contributes to the dimer–dimer interface in the  $\text{Ca}^{2+}$ -bound sorcín tetramer and contains the tryptophan residues is used by the protein also for the interaction with the HIC matrix, with Triton X-114 or with hydrophobic surfaces of target proteins on the cell membrane. It may be envisaged therefore that the interaction with the target competes with tetramer formation. In the cell, the carboxylate groups that are ionized at pH 7.5 and stabilize the calcium-chelated sorcín aggregates in the absence of a membrane, are likely to be involved in the interaction with the target thereby conferring specificity to the interaction itself and preventing precipitation of sorcín. These ideas on the mode of the sorcín–membrane interaction in turn suggest that the lipid moiety does not participate directly in the process. The experiments carried out with two membrane model systems, SUV and COV, indicate that this is indeed the case.

Last, a discussion of the equilibrium and dynamics of calcium binding to sorcín is in order, also in relation to the possible role of sorcín as a regulatory protein proposed in previous work on myocytes [4,6]. The binding affinity ( $10^6 \text{ M}^{-1}$ ) and the on rate of the binding reaction ( $3 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$  at 0.1 M ionic strength) are consistent with the contention that sorcín acts as an EF-hand ‘sensor’ protein [20]. Among the ‘sensor’ proteins, members of the S100 family, like calyculin, share distinctive features with sorcín. Thus, sorcín, like calyculin [21,22], undergoes a calcium-induced dimerization through hydrophobic contacts and in addition interacts with an annexin, annexin VII in bovine adrenal medulla [7]. Moreover,  $\text{Ca}^{2+}$ -free sorcín, though hydrophilic, exposes sufficient hydrophobic groups at physiological pH that may allow interaction with hydrophobic matrices in a calcium-independent manner as reported for calmodulin [23].

In conclusion, the characteristics of the calcium-induced conformational change and, in particular, the unexpected oligomerization of  $\text{Ca}^{2+}$ -bound sorcín, revealed by the present study, provide focus for future experiments aimed at the understanding of sorcín function.

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