

S-100a₀ protein stimulates Ca²⁺-induced Ca²⁺ release from isolated sarcoplasmic reticulum vesicles

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S-100a₀ protein, the αα-isoform of the S-100 family, stimulates Ca²⁺-induced Ca²⁺ release from terminal cisternae isolated from rat skeletal muscle cells. The stimulatory effect of S-100a₀ is maximal at ~5 μM S-100a₀ and half maximal at ~0.1 μM S-100a₀, at 1.8 μM free Ca²⁺ in the presence of 5 mM Mg²⁺ plus 0.1 M KCl. The effect of the protein on Ca²⁺-induced Ca²⁺ release is completely inhibited by the calcium release blocker, ruthenium red.

Protein S-100a₀; Ca²⁺ release; Regulation; (Muscle cell)

1. INTRODUCTION

Ca²⁺-induced Ca²⁺ release has been hypothesized to be one possible mechanism by which small increases in the concentration of sarcoplasmic Ca²⁺ in the proximity of the sarcoplasmic reticulum (SR) in muscle cells will trigger the release from SR of the additional Ca²⁺ required for muscle contraction [1–6]. The terminal cisternae of SR represent the subcellular compartment involved in Ca²⁺-induced Ca²⁺ release [4,7,8]. This activity is brought about by a distinct molecular entity, the Ca²⁺ release channel protein, which has been purified to homogeneity [9]. Ca²⁺-induced Ca²⁺ release is stimulated by adenine nucleotides [10] and inhibited by Mg²⁺, ruthenium red, and the ubiquitous intracellular Ca²⁺ receptor, calmodulin [11–13].

S-100a₀ protein is the αα-isoform of S-100 proteins, a group of 3 closely related Ca²⁺-binding proteins of the EF-hand type [14,15]. High levels of the S-100α, but not the S-100β, subunit are expressed in striated muscle cells [16–18]. Im-

munocytochemical and immunochemical analyses revealed that S-100a₀ protein is found associated with the sarcolemma and with SR membranes, and in the sarcoplasm facing these membranes [18,19], suggesting the possibility that this protein might be involved in the regulation of one or more membrane activities. We report here that S-100a₀ protein stimulates Ca²⁺-induced Ca²⁺ release from SR in the presence of Mg²⁺.

2. MATERIALS AND METHODS

S-100a₀ protein was purified from porcine heart [18].

Terminal cisternae (R4) were obtained as in [20] from rat hindlimb muscles and washed twice with 0.2 mM EGTA. R4 was shown to be highly enriched in terminal cisternae by a number of criteria [20–23]. R4 vesicles (0.25 mg of protein/ml) were actively loaded with CaCl₂ in 20 mM imidazole, 0.2 mM EGTA, 20 mM potassium oxalate, 5 mM ATP, 5 mM MgCl₂, 0.18 mM CaCl₂, 0.1 M KCl, pH 7.4, in the presence of 5 μM ⁴⁵CaCl₂ (specific activity 1 mCi/nmol) at 25°C. Ca²⁺-induced Ca²⁺ release was studied by Millipore filtration technique. After the plateau of Ca²⁺ uptake had been attained (6 min), the medium was replaced with the release solution. This consisted of the above medium minus ATP, containing 1.8 μM free Ca²⁺ unless stated otherwise plus or minus other additions as indicated. The released Ca²⁺ was calculated on filtered solutions by taking the differences between the value measured at the end of Ca²⁺ uptake and those recorded at the time points indicated

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after the addition of the release solution. Protein was measured as in [24] against a standard solution of bovine serum albumin.

3. RESULTS AND DISCUSSION

In the presence of $1.8 \mu\text{M}$ free Ca^{2+} and 5 mM MgCl_2 , S-100a₀ protein produced a time- and dose-dependent stimulation of Ca^{2+} release from R4 vesicles, with half-maximal effect around $0.1 \mu\text{M}$ S-100a₀ (figs 1 and 2). The extent of Ca^{2+} release was $\sim 70\%$ stimulated by $1 \mu\text{M}$ S-100a₀, whereas the initial rate of Ca^{2+} release was $\sim 65\%$ stimulated, under the above conditions. In the presence of $180 \mu\text{M}$ free Ca^{2+} (table 1), the percent Ca^{2+} -induced Ca^{2+} release in the absence of S-100a₀ protein was $\sim 70\%$ smaller than at $1.8 \mu\text{M}$ free Ca^{2+} , as expected [10]. Under the same conditions, in the presence of $1 \mu\text{M}$ S-100a₀ protein a strong stimulation of Ca^{2+} release was observed at all time points considered (table 1).

Since the SR vesicles used in these experiments were actively loaded with Ca^{2+} in the presence of ATP, experiments were conducted to examine the possibility that the S-100a₀ effect described above were due to an effect of the protein on the Ca^{2+} -ATPase activity associated with R4. In the presence of $1.8 \mu\text{M}$ free Ca^{2+} , the specific activity of the enzyme measured in the absence of S-100a₀ protein was $0.94 \mu\text{mol P}_i/\text{min}/\text{mg}$ of protein, and that of the enzyme measured in the presence of $1 \mu\text{M}$ S-100a₀ protein was $0.91 \mu\text{mol P}_i/\text{min}/\text{mg}$ of protein. Thus S-100a₀ protein did not affect the ATPase activity in R4 vesicles.

The stimulatory effect of S-100a₀ protein on Ca^{2+} -induced Ca^{2+} release was prevented by the Ca^{2+} release channel blocker, ruthenium red (table 2). Also, ruthenium red, when added to R4 vesicles induced to release Ca^{2+} in the presence of $1.8 \mu\text{M}$ free Ca^{2+} plus $1 \mu\text{M}$ S-100a₀ protein, blocked further S-100a₀-dependent Ca^{2+} -induced Ca^{2+} release (fig.3). The reverse experiment was also performed in which S-100a₀ protein was added to R4 vesicles actively loaded with Ca^{2+} and then induced to release Ca^{2+} in the presence of ruthenium red. No significant effect of S-100a₀ protein was registered under these conditions (fig.3).

S-100 proteins (S-100a₀, S-100a, and S-100b) are Ca^{2+} -binding proteins of the EF-hand type, and are $\alpha\alpha$, $\alpha\beta$, and $\beta\beta$ in subunit composition, respectively [14,15]. S-100 proteins are widely, but dif-

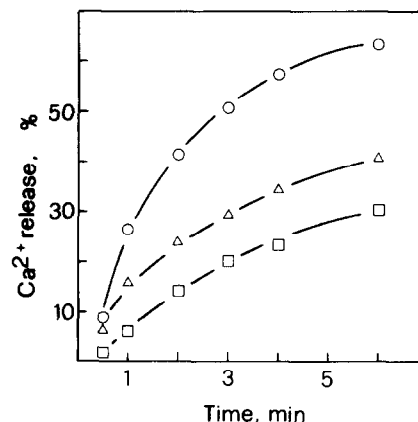


Fig.1. Time course of Ca^{2+} -induced Ca^{2+} release from R4 vesicles in the absence of additions (Δ) and in the presence of either $1 \mu\text{M}$ S-100a₀ protein (\circ) or $60 \mu\text{M}$ ruthenium red (\square). Results are expressed as the percent Ca^{2+} release vs time. At the end of Ca^{2+} uptake, vesicles contained $49 \pm 6 \text{ nmol Ca}^{2+}/0.1 \text{ mg protein}$. Maximal SD was $\pm 5\%$ ($n = 3$).

ferently distributed in animal cells. They have been shown to regulate a number of activities, including the assembly-disassembly of microtubules, several kinase and phosphoprotein phosphatase activities, the phosphorylation of a number of proteins by interacting with the substrates rather than with the kinases, a brain aldolase activity, and the adenylate cyclase activity (reviewed in [14,15]).

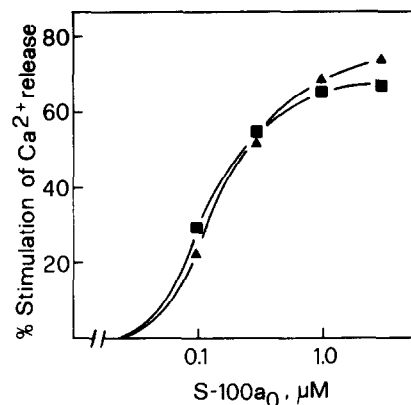


Fig.2. Effect of increasing concentrations of S-100a₀ protein on Ca^{2+} -induced Ca^{2+} release from R4 vesicles. The release solution contained increasing concentrations of S-100a₀ protein. Results are expressed as the percent stimulation of Ca^{2+} release 2 min (\blacktriangle) and 4 min (\blacksquare) after the starting of Ca^{2+} release. At the end of Ca^{2+} uptake, R4 vesicles contained $45 \pm 5 \text{ nmol Ca}^{2+}/0.1 \text{ mg protein}$. Maximal SD was $\pm 6\%$ ($n = 3$).

Table 1

Ca²⁺-induced Ca²⁺ release from R₄ vesicles in the presence of 180 μ M free Ca²⁺ with or without S-100a₀ protein

Additions	Time (min)		
	0.5	2	4
None	0.7 \pm 0.2	2.9 \pm 0.3	5.0 \pm 0.4
S-100a ₀ (1 μ M)	1.7 \pm 0.5	6.2 \pm 0.6	10.6 \pm 1.2

Conditions were as described in the legend to fig.1, except that the free Ca²⁺ concentration in the release medium was 180 μ M. Figures represent the percent Ca²⁺ release at the time points indicated ($n = 3 \pm$ SD). At the end of Ca²⁺ uptake (6 min) R₄ vesicles contained 62 \pm 4 nmol Ca²⁺/0.1 mg⁻¹ protein

Table 2

Ca²⁺-induced Ca²⁺ release from R₄ vesicles in the presence of various combinations of S-100a₀ protein and ruthenium red

Substances	Percent Ca ²⁺ release in 4 min
None	30.7 \pm 2.3
S-100a ₀ (1 μ M)	58.9 \pm 3.5
Ruthenium red (60 μ M)	20.3 \pm 1.6
Ruthenium red (60 μ M) + S-100a ₀ (1 μ M)	19.8 \pm 1.8

Conditions were as described in the legend to fig.1. Substances were added to the release solution to the final concentrations indicated. At the end of Ca²⁺ uptake (6 min) R₄ vesicles contained 47 \pm 6 nmol Ca²⁺/0.1 mg⁻¹ protein ($n = 3 \pm$ SD)

Thus S-100 proteins are candidate to constitute a multifunctional protein fraction. As anticipated, striated muscle cells express almost exclusively the $\alpha\alpha$ -isoform (S-100a₀). In these cells, the S-100a₀ concentration is estimated to be 1–2 μ M [18], but it is expected to be considerably higher in the proximity of muscle membranes, since no S-100a₀ protein is found associated with the contractile elements [18]. S-100a₀ protein has been recently reported to stimulate the basal (Mg²⁺-activated) adenylate cyclase activity in muscle membranes [23]. The data presented in this report suggest that this protein might also play a role in the regulation of Ca²⁺-induced Ca²⁺ release from SR. S-100a₀ protein stimulates Ca²⁺-induced Ca²⁺ release from R₄ vesicles in the presence of 1.8 μ M free Ca²⁺ plus 0.1 M KCl, i.e. under conditions where this protein in solution would not bind Ca²⁺ [14,15]. Thus our findings suggest that, if S-100a₀ protein exerts its effects on this system because of its

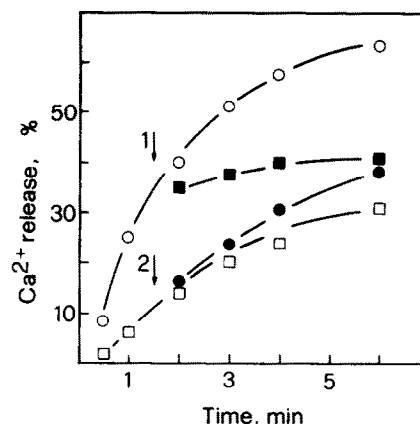


Fig.3. Effects of additions of ruthenium red or S-100a₀ protein on Ca²⁺-induced Ca²⁺ release from R₄ vesicles in the presence of S-100a₀ protein or ruthenium red, respectively. (○) 1.5 min after starting Ca²⁺ release in the presence of 1 μ M S-100a₀ protein (arrow 1), ruthenium red was added to 60 μ M (■). (□) 1.5 min after starting Ca²⁺ release in the presence of 60 μ M ruthenium red (arrow 2), S-100a₀ protein was added to 1 μ M (●). Ruthenium red blocked the stimulatory effect of S-100a₀ protein on Ca²⁺-induced Ca²⁺ release, whereas S-100a₀ protein did not significantly change the inhibitory effect of ruthenium red on Ca²⁺-induced Ca²⁺ release. Results are expressed as the percent Ca²⁺ release vs time. At the end of Ca²⁺ uptake, R₄ vesicles contained 42 \pm 6 nmol Ca²⁺/0.1 mg protein. Maximal SD was \pm 7% ($n = 3$).

Ca²⁺-binding properties, as is reasonable to assume, then the conclusion can be drawn that the Ca²⁺-binding affinity of S-100a₀ protein increases by several orders of magnitude once it has come into contact with its targets. This suggestion is supported by recent observations on interactions between S-100 proteins and tubulin [25], microtubule-associated τ -proteins and mellitin [26], artificial membranes [27], and hydrophobic matrices [18], all of which strongly indicate that the Ca²⁺-binding affinities of individual S-100 isoforms depend on their conformation and increase upon their binding to targets. At relatively high free Ca²⁺ concentrations, Ca²⁺ release from SR occurs at a reduced rate and to a much smaller extent than at low free Ca²⁺ concentrations [10]. The observation that S-100a₀ protein stimulates Ca²⁺-induced Ca²⁺ release by 100% at 180 μ M free Ca²⁺ clearly indicates that the protein does not act on this system by chelating free Ca²⁺.

Interestingly, S-100a₀ protein and calmodulin appear to exert opposite effects on Ca²⁺-induced Ca²⁺ release, in spite of their belonging to the same

superfamily of Ca^{2+} -binding proteins of the EF-hand type. There is evidence that calmodulin binds to the Ca^{2+} release channel protein [12,21,28]. We are working at identifying the molecular target(s) of S-100a₀ protein in terminal cisternae.

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