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S-100a_0 protein stimulates the basal (Mg^{2+}-activated) adenylate cyclase activity associated with skeletal muscle membranes

G. Fanò, P. Angelella, D. Mariggiò, M.C. Aisa*, I. Giambanco* and R. Donato*

Institute of Cell Biology, Faculty of Sciences and **Section of Anatomy, Department of Experimental Medicine and Biochemical Sciences, University of Perugia, 06100 Perugia, Italy*

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S-100a_0 protein, the $\alpha\alpha$ isoform of the S-100 family, stimulates basal (Mg^{2+}-activated) adenylate cyclase (AC) activity associated with the sarcolemma, longitudinal tubules and terminal cisternae of rat skeletal muscle cells. The stimulatory effect of S-100a_0 on AC activity is maximal around 5μM S-100a_0 and half-maximal around 0.2 μM S-100a_0. Also, the stimulatory effect is greatest on the AC activity associated with the terminal cisternae than on the other membrane fractions studied. These data are discussed in relation to the subcellular localization of S-100a_0 in muscle cells.

Protein, S-100a_0; Adenylate cyclase; Enzyme regulation; (Muscle cell)

1. INTRODUCTION

The S-100 family comprises three closely related, 21 kDa, Ca^{2+}-binding proteins, S-100a_0, S-100a and S-100b, of subunit composition $\alpha\alpha$, $\alpha\beta$ and $\beta\beta$, respectively (reviews [1,2]). S-100 proteins belong to the superfamily of Ca^{2+}-regulatory proteins of the EF-hand type, on the basis of sequence homology with calmodulin, parvalbumin and troponin C [1,2]. S-100 proteins have been shown to regulate assembly-disassembly of microtubules [3-5], phosphorylation of a number of proteins [6-8], and a brain aldolase activity [9] Ca^{2+}-dependently. However, Ca^{2+}-independent effects of S-100 proteins on kinase and phosphoprotein phosphatase activities [10,11], microtubule assembly-disassembly [12] and protein phosphorylation [13] have been reported.

S-100 proteins are widely distributed in animal tissues but are not ubiquitous [1,2]. Also, individual S-100 isoforms are variously distributed in tissues [1,2]: S-100a_0 is abundant in skeletal and cardiac muscle cells and in the kidney, S-100a is found predominantly in the central nervous system and melanocytes, and S-100b is abundant in glial cells of the central and peripheral nervous system, melanocytes and adipocytes. In skeletal and cardiac muscle cells, S-100a_0 hs been localized to the sarcolemma, outer mitochondrial membranes, membranes of the sarcoplasmic reticulum, and the sarcoplasm facing all these membranes, by immunocytochemical and immunochemical techniques [14-16]. These sites of localization for S-100a_0 suggest that the protein might be involved in the regulation of one or more membrane activities.

Adenylate cyclase [ATP pyrophosphate-lyase (cyclizing), EC 4.6.1.1] (AC) is a membrane-bound enzyme complex which catalyzes the formation of the second messenger cyclic AMP (cAMP) from ATP. The activity of AC is regulated by a number of hormones acting through specific receptors located at the external surfaces of cells, as well as by proteins having GTPase activity (G-proteins) that are associated with cell membranes [17,18]. In addition, calmodulin regulates AC activity in a Ca^{2+}-dependent manner, at least in the nervous system [19]. In striated muscle cells, AC activity

Correspondence address: R. Donato, Section of Anatomy, Department of Experimental Medicine and Biochemical Sciences, Cas. Post. 81, 06100 Perugia Succ. 3, Italy

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has been localized to the sarcolemma and the sarcotubular system, as judged from ultrocytochemical techniques (review [20]). There is some uncertainty as to whether calmodulin affects the activity of AC in striated muscle cells [21].

We show here that S-100a_0 protein stimulates the basal (Mg^{2+}-activated) AC activity associated with the sarcolemma, longitudinal tubules, and terminal cisternae in skeletal muscles.

2. MATERIALS AND METHODS

S-100a_0 was purified from porcine heart [16]. TRK 432 cAMP kit was obtained from the Radiochemical Center (Amersham, England). ATP, GTP, DTT and BSA were from Sigma (St. Louis, MO).

A postmitochondrial membrane fraction derived from rat hindlimb muscles was further fractionated to obtain sarcolemmal membranes (R_1), longitudinal tubules (R_2) and terminal cisternae (R_4) [22]. All buffers contained 2 mM EGTA. The above membrane subfractions were characterized for Ca^{2+}-ATPase and Na^+,K^+-ATPase activities [22,23], Ca^{2+} fluxes [23] and polypeptide profiles [23]. AC activity was assayed as in [24]. Conditions were as described [25] except that 2 mM EGTA (unless stated otherwise) was included in the assay medium. Protein was measured as in [26] against a standard solution of BSA.

3. RESULTS AND DISCUSSION

Negligible cross-contamination of R_1, R_2 and R_4 subfractions was observed, on the basis of enzymic and functional criteria (table 1). As membrane subfractions were prepared in the presence of EGTA, no S-100a_0 was found associated with them (not shown), in accordance with previous data [16].

The basal (Mg^{2+}-activated) AC activity associated with R_1, R_2 and R_4 was 15.6, 7.4 and 3.6 pmol cAMP/min per mg protein, respectively (table 2). Thus, R_4 displayed the lowest AC activity. S-100a_0 (1 μM) gave rise to 52, 67 and 139% stimulation of AC activity associated with R_1, R_2 and R_4, respectively (table 2). Thus, most of the stimulatory effect of S-100a_0 was on R_4-associated AC activity. Under the same conditions, the stimulatory effect of S-100a_0 on R_4-associated AC activity was dose-dependent and half-maximal around 0.2 μM S-100a_0 (fig.2A). A similar dose dependency was observed with R_1 and R_2 subfractions (not shown). We have previously reported that S-100b protein results in dose-dependent inhibition of basal (Mg^{2+}-activated) AC activity in skeletal muscles [25]. Thus, in contrast to S-100b, S-100a_0 stimulates skeletal muscle AC activity in the presence of Mg^{2+}, irrespective of the membrane subfraction studied. When experiments were performed using R_4 and increasing $CaCl_2$ concentrations in the presence of Mg^{2+}, not only was AC activity progressively inhibited by Ca^{2+} (except for slight stimulation at 10 μM total $CaCl_2$), but no effect of S-100a_0 was observed (fig.2B). However, in the absence of other metals and presence of 0.2 mM EGTA, S-100a_0 produced dose-dependent stimulation of R_4-associated AC activity at 0.1 mM $CaCl_2$ (fig.2). A similar stimulatory effect of S-100b on skeletal muscle AC activity in the presence of Ca^{2+} alone has been previously observed [25].

S-100a_0 protein is abundant in striated muscle cells [16,27,28], where its overall concentration is 1–2 μM [16]. However, the S-100a_0 concentration in the proximity of skeletal muscle membranes is expected to be much higher, since practically all the

Table 1

Enzymic and functional characterization of R_1, R_2 and R_4 skeletal muscle membrane subfractions

Activity	Membrane subfractions		
	R_1	R_2	R_4
Ca^{2+}-ATPase	1.09 ± 0.02	13.68 ± 0.25	5.66 ± 0.12
Na^+,K^+-ATPase	1.08 ± 0.01	0.66 ± 0.30	0.27 ± 0.09
Ca^{2+} uptake	–	52 ± 13	78 ± 11
Ca^{2+} release	–	12 ± 4	16 ± 7

Ca^{2+}- and Na^+,K^+-ATPase activities are expressed as μmol P_i/min per mg protein; mean of six determinations ± SD. Ca^{2+} uptake and Ca^{2+} release are expressed as % available external $^{45}Ca^{2+}$; mean of six determinations ± SD. No determinations of Ca^{2+} uptake and release were made in the case of R_1

Table 2

Effect of S-100a_0 protein (1 μM) on adenylate cyclase activity in skeletal muscle membrane subfractions

Membrane subfractions	Adenylate cyclase activity	
	– S-100a_0	+ S-100a_0
R_1	15.6 ± 0.8	23.9 ± 1.1
R_2	7.4 ± 0.45	12.4 ± 0.65
R_4	3.6 ± 0.1	8.6 ± 0.4

The assay medium contained 5 mM $MgCl_2$ and 2 mM EGTA. The adenylate cyclase activity is expressed as pmol cAMP/min per mg protein. Mean of six determinations ± SD

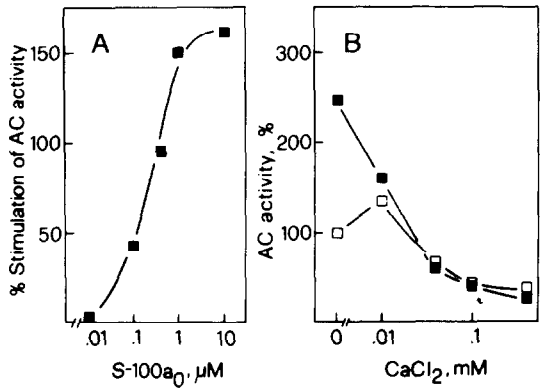

Fig.1. Characterization of the effect of S-100a_0 protein on adenylate cyclase activity in R_4 subfraction. (A) Dose dependency of the stimulatory effect of S-100a_0 in the presence of 5 mM $MgCl_2$ and absence of Ca^{2+} (2 mM EGTA). Values are expressed as percent stimulation of basal AC activity, which was 3.3 pmol cAMP/min per mg protein. Maximal SD was 5% (n = 5). (B) AC activity in R_4 subfraction in the presence of 5 mM $MgCl_2$, 2 mM EGTA and increasing concentrations of $CaCl_2$ as indicated plus (■) or minus (□) 1 μM S-100a_0. Values are expressed as the percent of AC activity in the absence of $CaCl_2$ and of S-100a_0 (3.9 pmol cAMP/min per mg protein). Maximal SD was 8% (n = 3).

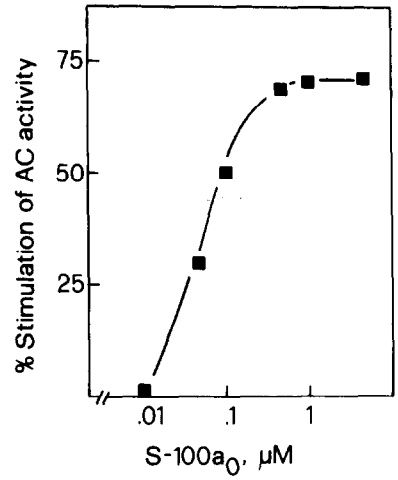

Fig.2. Effect of increasing concentrations of S-100a_0 protein on AC activity in R_4 subfraction in the absence of $MgCl_2$ and presence of 0.2 mM EGTA plus 0.1 mM $CaCl_2$. Values are expressed as percent stimulation of the AC activity measured in the absence of S-100a_0 (2.3 pmol cAMP/min per mg protein). Maximal SD was 6% (n = 3).

S-100a_0 is detected in the sarcoplasm and in association with muscle membranes and since no S-100a_0 is found associated with contractile elements [16]. Interestingly, S-100a_0 only affects the basal (Mg^{2+}-activated) AC activity, and has no effects when Ca^{2+} is added at increasing concentrations in the presence of Mg^{2+}. Thus, if the S-100a_0 effect we have registered were to be physiologically relevant, then it would seem that S-100a_0 stimulates AC activity in striated muscle cells in the absence of Ca^{2+} transients. Precedent exists for Ca^{2+}-independent effects of S-100 proteins on protein phosphorylation and dephosphorylation and on microtubule assembly-disassembly [10–13], in spite of their being Ca^{2+}-binding rpoteins of the EF-hand type. Yet, in the presence of Ca^{2+} alone, S-100a_0 stimulates AC activity. This univocal effect of S-100a_0 on the AC activity in the presence of individual metals contrasts with what has been observed with S-100b on the same system, i.e. inhibition of Mg^{2+}-activated AC activity and stimulation of AC activity in the presence of Ca^{2+} [25]. We tentatively conclude that the similar effects of S-100a_0 and S-100b proteins on AC activity in the presence of Ca^{2+} alone presumably depend on the Ca^{2+}-binding properties of individual S-100 isoforms, whereas the opposite effects in the presence of Mg^{2+} and absence of Ca^{2+} presumably reflect different sites of action of individual isoforms.

The present results and those in a previous report [25] represent the first functional correlation of binding of S-100 proteins to natural membranes [1,2]. Future studies should elucidate the mechanism of action of S-100 proteins on the AC system as well as the interactions between S-100 proteins and individual components of the AC system, including the G-proteins.

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