Volume 257, number 2, 380–382 FEB 07800 November 1989

Interaction of smooth muscle caldesmon with S-100 protein

Elena V. Skripnikova and Nikolai B. Gusev

Department of Biochemistry, School of Biology, Moscow State University, Moscow 119899, USSR

Received 15 September 1989

The interaction of caldesmon with certain Ca-binding proteins was investigated by means of electrophoresis under non-denaturating conditions. In the presence of Ca^{2+} calmodulin, troponin C and S-100 protein form a complex with caldesmon. No complex formation takes place in the absence of Ca^{2+}. Lactalbumin and pike parvalbumin (p*I* 4.2) do not interact with caldesmon independently of Ca-concentration. Both S-100 protein and calmodulin effectively inhibit phosphorylation of caldesmon by Ca-phospholipid-dependent protein kinase. At low ionic strength S-100 protein reverses the inhibitory action of caldesmon on the skeletal muscle acto-heavy meromyosin ATPase more effectively than calmodulin. It is supposed that in certain tissues and cell compartments the proteins belonging to the S-100 family are able to substitute for calmodulin in the caldesmon-dependent regulation of actin and myosin interaction.

Caldesmon; Calmodulin; Protein, S-100; Protein kinase C phosphorylation

1. INTRODUCTION

Caldesmon is a calmodulin- and actin-binding protein [1] involved in the regulation of the contractile activity of non-muscle and smooth-muscle cells [2,3]. It is supposed [2,3] that at low Ca^{2+} concentrations caldesmon inhibits the sliding of actin and myosin filaments. Ca-saturated calmodulin interacts with caldesmon and by this means reverses the inhibitory effect of caldesmon. At the same time complete reversion of the inhibitory effect of caldesmon is observed only at a very large excess of calmodulin [2,4] and only under very specific incubation conditions [5]. Moreover Ca-sensitive thin filaments of smooth muscle contain very small quantities of calmodulin [6]. All these facts lead to the hypothesis that not calmodulin but another Ca-binding protein is involved in the regulation of smooth muscle contraction [5]. This paper is devoted to the investigation of caldesmon interaction with some Ca-binding proteins and especially with S-100 protein.

2. MATERIALS AND METHODS

Ca-binding proteins were isolated by earlier described methods: calmodulin [7] and S-100 protein [8] from bovine brain, troponin C [9] from rabbit skeletal muscle. Pike skeletal muscle parvalbumin (p*I* 4.2) was kindly donated by Dr E.A. Permyakov, Institute of Biophysics of USSR Academy of Sciences. Lactalbumin was purchased from Sigma. Caldesmon and tropomyosin were isolated from duck gizzard according to Lynch and Bretscher [10]. A method described earlier [11] was used for the isolation of Ca-phospholipid-dependent protein kinase (protein kinase C) from rat brain. Actin and HMM were isolated from rabbit skeletal muscle [12,13]. The homogeneity of proteins was checked by SDS-gel electrophoresis [14].

Interaction of caldesmon with Ca-binding proteins was investigated by means of polyacrylamide gel electrophoresis under non-denaturing conditions [15]. Earlier, this method was successfully used for the detection of interaction of caldesmon and calmodulin or its peptides [1,16].

The ATPase activity of acto-HMM was measured in the incubation mixture of the following composition: 20 mM imidazole-HCl (pH 7.0), 5 mM $MgCl_2$, 0.1 mM $CaCl_2$ and 2 mM ATP. As a rule the incubation mixture (400–500 μl) contained 3.3 μM skeletal muscle F-actin, 0.6 μM smooth muscle tropomyosin, 0.3 μM of skeletal muscle HMM and varying quantities of caldesmon and Ca-binding proteins. After incubation for 5–15 min at 25°C, the reaction was stopped with trichloroacetic acid and inorganic phosphate was determined by the Hers and Van Hoof method [17]. Protein concentration was determined either by molar extinction coefficients reported in the literature or by the method of Spector [18] using bovine serum albumin as a standard. The molecular masses of the following proteins were taken to be 350 kDa for HMM, 140 kDa for caldesmon, 70 kDa for tropomyosin, 43 kDa for actin, 21 kDa for S-100 protein, 18 kDa for calmodulin and troponin C, 14.4 kDa for lactalbumin and 11.5 kDa for pike parvalbumin.

3. RESULTS

The interaction of caldesmon with Ca-binding proteins was investigated by using electrophoresis under non-denaturing conditions. At alkaline pH values Ca-binding proteins having low molecular weights and isoelectric points possess high electrophoretic mobilities (fig.1, lanes 2,4,6,8,10). Moreover, the electrophoretic mobility of some Ca-binding proteins depends on their saturation with Ca^{2+} (cf. lanes 2,4,6,8,10 on gels A and B on fig.1). Although the isoelectric point of caldesmon is close to 5.6–5.7 [19], this protein has rather a high

Correspondence address: N.B. Gusev, Department of Biochemistry, School of Biology, Moscow State University, Moscow 119899, USSR

Abbreviation: HMM, heavy meromyosin

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/89/$3.50

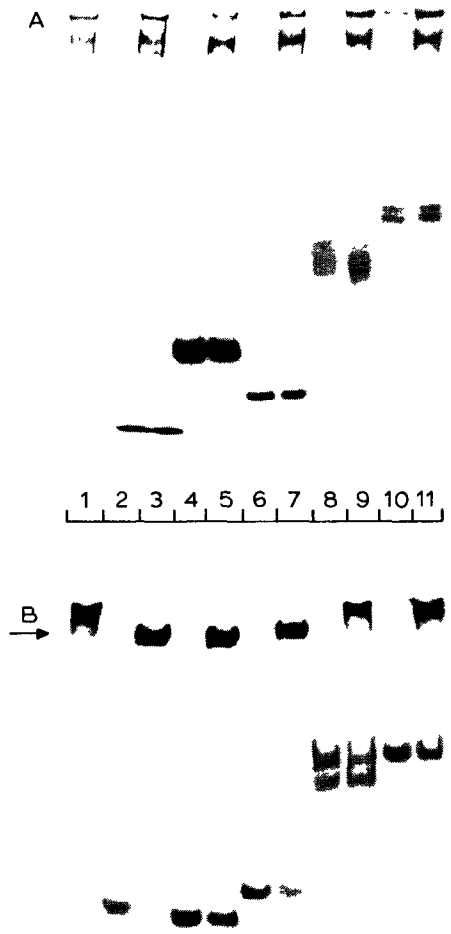

Fig.1. Interaction of caldesmon with different Ca-binding proteins as revealed by polyacrylamide gel electrophoresis performed in the presence of 2 mM EDTA (A), or 0.1 mM $CaCl_2$ (B). Lanes: 1, isolated caldesmon; 2, isolated S-100; 3, mixture of S-100 and caldesmon; 4, isolated troponin C; 5, mixture of troponin C and caldesmon; 6, isolated calmodulin; 7, mixture of calmodulin and caldesmon; 8, isolated lactalbumin; 9, mixture of lactalbumin and caldesmon; 10, isolated parvalbumin; 11, mixture of parvalbumin and caldesmon. Caldesmon (7 μM) was loaded on each track, the Ca-binding protein:caldesmon molar ratio was equal to 3.

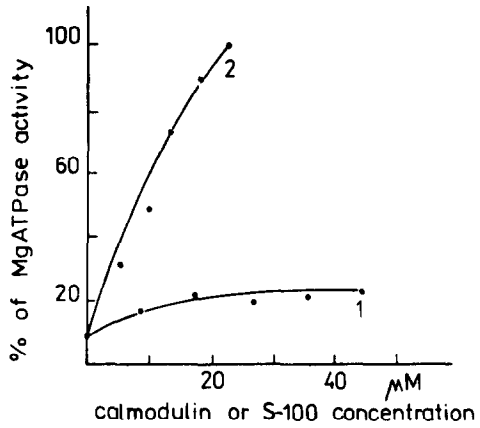

Fig.2. Calmodulin (1) and S-100 (2) induced reversion of the inhibitory action of caldesmon on acto-HMM ATPase. The ATPase activity was measured under conditions, described in section 2; protein concentrations were actin 3.7 μM, tropomyosin 0.7 μM, HMM 0.3 μM and caldesmon 1 μM. The ATPase activity of acto-HMM in the absence of caldesmon was equal to 3 s^{-1} and was taken for 100%.

molecular weight. Therefore, isolated caldesmon has a relatively low electrophoretic mobility. In the absence of Ca^{2+} the electrophoretic mobility of caldesmon is unaffected by the presence of Ca-binding proteins, i.e. all proteins retain their original mobilities (fig.1A). At the same time, in the presence of Ca^{2+} certain Ca-binding proteins (calmodulin, troponin C and S-100) form a complex with caldesmon. The complex formed has an electrophoretic mobility intermediate between that of isolated caldesmon and the corresponding Ca-binding protein. The formation of this band (fig.1, arrow) is accompanied by a decrease in the intensity of the band corresponding to isolated caldesmon and Ca-binding proteins. The data presented indicate that in the presence of Ca^{2+} calmodulin, troponin C and S-100 protein are able to form a tight complex with caldesmon. It is worthwhile mentioning that far from all Ca-binding proteins are able to interact with caldesmon. For example, regardless of Ca^{2+} concentration neither lactalbumin nor parvalbumin are able to interact with caldesmon (fig.1, lanes 8–11). Thus, among the five Ca-binding proteins tested only three (calmodulin, troponin C and S-100) interact with caldesmon. Taking into account that calmodulin and S-100 protein are present in the smooth muscle, we investigated the interaction of caldesmon with S-100 protein in more detail.

It is well known that Ca-phospholipid-dependent protein kinase (protein kinase C) phosphorylates caldesmon [11,20]. The sites phosphorylated by this enzyme are located in the C-terminal part of caldesmon involved in the interaction with calmodulin [11]. This seems to be the reason for the fact that calmodulin induces a 40–60% inhibition of caldesmon phosphorylation catalyzed by protein kinase C. An addition of a 4–6-fold molar excess of S-100 protein over caldesmon leads to a 1.5–2-fold decrease of its phosphorylation by protein kinase C. Thus, the inhibitory effect of S-100 on caldesmon phosphorylation is comparable with that of calmodulin. This finding indirectly indicates that calmodulin and S-100 protein interact with the same site in the caldesmon structure.

In search for a more direct evidence of the interaction of S-100 with caldesmon, we studied the regulatory effect of caldesmon on acto-HMM ATPase. Under the conditions used, the addition of caldesmon up to an actin:caldesmon molar ratio of 3.5:1 causes a 70–90% inhibition of acto-HMM ATPase (fig.2). Such an inhibitory effect of caldesmon is comparable to that

described in the literature [2,4]. Calmodulin used even at rather high concentrations only slightly reverses caldesmon inhibition. At the same time by using lower concentrations of S-100 we were able to completely reverse the inhibitory effect of caldesmon. The data obtained indicate that, at low ionic strength and at a high caldesmon:actin molar ratio, S-100 protein is able to eliminate the inhibitory action of caldesmon on the ATPase activity of acto-HMM.

4. DISCUSSION

Summing up one can conclude that S-100 protein is able to interact with isolated caldesmon, inhibits phosphorylation of caldesmon by protein kinase C and reverses the inhibitory action of caldesmon on the ATPase activity of acto-HMM. Thus, under certain conditions, S-100 protein may be involved in caldesmon-dependent regulation of actin and myosin interaction. If this assumption is correct, a question arises as to whether the concentration of caldesmon and S-100 protein are comparable. Caldesmon concentration in smooth muscle is close to 12 μM [2,4]. The corresponding value for S-100 protein seems to be close to 0.5–1 μM [21]. At the same time smooth muscle contains rather a high concentration of calmodulin (about 30 μM) [4]. If these values are correct, then S-100 protein will not effectively compete with calmodulin for the interaction with caldesmon. However there are some doubts about the true concentration of S-100 in smooth muscle. These doubts are due to the fact that a number of closely related proteins are now known under the common name of 'S-100 protein' [22]. In our investigation we used an unfractionated mixture of proteins mainly composed of a so-called S-100b protein. There is a vast variety of S-100 isoforms in different tissues [21]. The estimation of concentration of S-100 is mainly based on immunological approaches. Therefore using antibodies to a certain isoform of S-100 protein it is difficult to determine the exact content of S-100. Moreover, recently published data indicate that caldesmon is involved in the regulation of actin and myosin interaction not only in smooth muscle but in certain non-muscle cells [3] and may take part in the capping of receptors [19] as well as in hormone secretion [23]. The latter two processes are closely related to the membrane which contains rather large quantities of bound S-100. Thus, we may suppose that, at least in the compartment close to the surface membrane, S-100 protein may be involved in the caldesmon-dependent regulation of actin and myosin interaction. Moreover in a recently published paper by Marston et al. [5] it was shown that the fraction of Ca-binding protein isolated from smooth muscle and composed of S-100, calcimedins and calmodulin reverses the inhibitory action of caldesmon on the acto-myosin ATPase more effectively than isolated calmodulin. Thus, further investigation of the S-100 and caldesmon interaction seems to be meaningful.

Acknowledgements: The authors are grateful to Drs A.V. Vorotnikov and G.M. Pron'ko for providing preparation of protein kinase C and HMM. We thank Professor S.E. Severin for valuable discussions and Mrs R.L. Birnova for linguistic advice.

REFERENCES

[1] Sobue, K., Muramoto, Y., Fujita, M. and Kakiuchi, S. (1981) Proc. Natl. Acad. Sci. USA 78, 5652–5655.
[2] Marston, S.B. and Smith, C.W.J. (1985) J. Muscle Res. Cell Motil. 6, 669–708.
[3] Sobue, K., Kanda, K., Tanaka, T. and Ueki, N. (1988) J. Cell. Biochem. 37, 317–325.
[4] Smith, C.W.J., Pritchard, K. and Marston, S.B. (1987) J. Biol. Chem. 262, 116–122.
[5] Marston, S.B., Pritchard, K., Redwood, C. and Taggart, M. (1988) Biochem. Soc. Trans. 16, 494–497.
[6] Ngai, P.K., Scott-Woo, G.C., Lim, M.S., Sutherland, C. and Walsh, M.P. (1987) J. Biol. Chem. 262, 5352–5359.
[7] Gopalakrishna, R. and Anderson, W.B. (1982) Biochem. Biophys. Res. Commun. 104, 830–836.
[8] Isobe, T., Nakajima, T. and Okuyama, T. (1977) Biochim. Biophys. Acta 491, 222–232.
[9] Gusev, N.B., Barskaya, N.V., Verin, A.D., Duzhenkova, I.V., Khuchua, Z.A. and Zheltova, A.O. (1983) Biochem. J. 213, 123–129.
[10] Lynch, W. and Bretscher, A. (1986) Methods Enzymol. 134, 37–42.
[11] Vorotnikov, A.V., Shirinsky, V.P. and Gusev, N.B. (1988) FEBS Lett. 236, 321–324.
[12] Spudich, J.A. and Watt, S. (1971) J. Biol. Chem. 246, 4866–4871.
[13] Okamoto, J. and Sekine, T. (1985) J. Biochem. 98, 1143–1145.
[14] Laemmli, U.K. (1970) Nature 227, 680–685.
[15] Schaub, M.C. and Perry, S.V. (1969) Biochem. J. 115, 993–1004.
[16] Yazawa, M., Yagi, K. and Sobue, K. (1987) J. Biochem. 102, 1065–1073.
[17] Hers, H. and Van Hoof, F. (1966) Methods Enzymol. 8, 525–532.
[18] Spector, T. (1978) Anal. Biochem. 86, 142–146.
[19] Walker, G., Kerrick, W.G.L. and Bourguignon, L.Y.W. (1989) J. Biol. Chem. 264, 496–500.
[20] Umekawa, H. and Hidaka, H. (1985) Biochem. Biophys. Res. Commun. 132, 56–62.
[21] Kato, K., Kimura, S., Haimoto, H. and Suzuki, F. (1986) J. Neurochem. 46, 1555–1560.
[22] Van Eldik, L.J. and Zimmer, D.B. (1988) in: Calcium and Calcium-Binding Proteins (Gerday, C. et al. eds) pp.114–127, Springer, Berlin.
[23] Burgoyne, R.D., Cheek, T.R. and Norman, K.M. (1986) Nature 319, 68–70.