LOCATION OF THE PERIPHERAL PROTEINS IN SARCOPLASMIC RETICULUM VESICLES

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1. Introduction

The composition and the molecular organization of sarcoplasmic reticulum (SR) membrane from skeletal muscle has been investigated extensively for the last few years [1]. It is now well established that the protein pattern of this membrane is relatively simple. In addition to the Ca^{2+}, Mg^{2+}-dependent ATPase (105 000 daltons), a typical integral membrane protein, SR from fast skeletal muscle contains also two peripheral proteins, called high affinity calcium binding protein (HACBP) (55 000 daltons) and calsequestrin (45 000 daltons) [1]. The function of two latter proteins is still not clear and their location in the membrane is a matter of controversy [2–7].

The extraction of the SR vesicles with the solution of EDTA leads to the release of both peripheral proteins [5,8]. EDTA, however, makes the vesicles leaky and permeable to macromolecules [8]; therefore, this approach cannot bring any information about the location of these proteins in the membrane. For that reason in the present work a non-penetrating chelating resin Chelex 100 was used instead of EDTA in order to localize the peripheral proteins in the SR vesicles.

2. Materials and methods

The light and heavy subfraction of SR vesicles were obtained from fast rabbit skeletal muscle as in [9].

Chelex 100 (1310-Rad Laboratories, Richmond, CA) was prepared according to Seidel and Gergely [10]. The vesicles of light or heavy subfraction suspended in 20 mM imidazole, pH 7.2 and 100 mM KCl (called throughout this paper imidazole–KCl buffer) at protein conc. 5 mg/ml were shaken for 3 min with Chelex 100 suspended in the same buffer (10 mg Chelex 100/30 ml). Chelex 100 was removed by centrifugation at 1500 × *g* for 10 min and then the suspension was centrifuged at 100 000 × *g* for 1 h.

In some experiments the vesicles of light or heavy subfraction suspended in the imidazole–KCl buffer were extracted with 1 mM EDTA at pH 8.0 for 30 min at 0°C with constant stirring and then centrifuged for 1 h at 100 000 × *g*. The pellets and the supernatants after Chelex 100 or EDTA treatment were used for the further studies.

Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–gel electrophoresis) in 7.5% slab-gel was carried out according to [11] at a current of 100 mA/plate. Protein content was determined according to [12].

3. Results and discussion

SR vesicles can be separated [9] by an additional sucrose density gradient centrifugation into 2 subfractions, the heavy one, consisting of normally oriented, right-side-out vesicles, and the light one containing most probably, inside-out vesicles. The protein pattern of both subfractions is virtually the same although they differ from each other in the relative proportions of calsequestrin and HACBP (fig.1).

In the present work these 2 subfractions have been used for the studies on the location of the peripheral proteins.

SDS–gel electrophoresis shows that the extraction

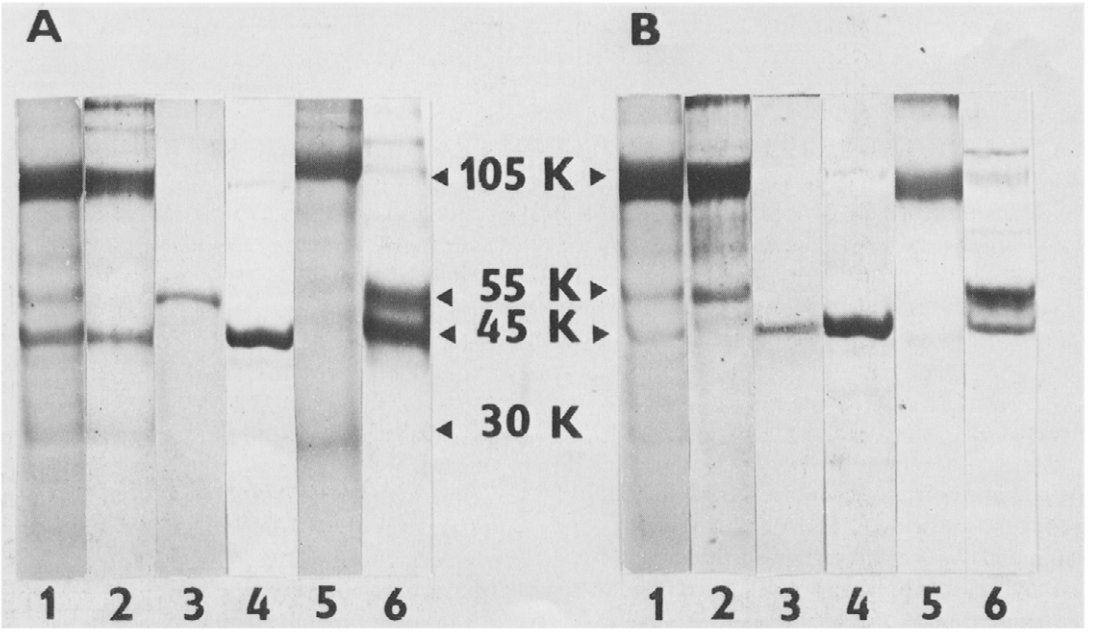

Fig.1. Treatment of the vesicles of heavy (A) or light (B) SR subfraction with Chelex 100 or EDTA. Vesicles were treated with Chelex 100 or extracted with EDTA as in section 2 and then centrifuged for 1 h at 100 000 × *g*. The protein pattern of the supernatants and the pellets was analyzed in SDS–slab gel electrophoresis carried out according to [11]. (1) Intact SR vesicles; (2) proteins remaining in SR membrane after Chelex 100 treatment; (3) proteins released from the membrane by Chelex 100 treatment; (4) calsequestrin purified according to [18]; (5) pellet after EDTA extraction; (6) supernatant after EDTA extraction; 105 K, Ca^{2+}, Mg^{2+}-ATPase; 55 K higher affinity calcium binding protein; 45 K, calsequestrin; 30 K, glycoprotein.

with EDTA causes the release of both peripheral proteins from either subfraction (fig.1). In contrast, the treatment with Chelex 100 leads to the release of HACBP only from the heavy subfraction and of calsequestrin only from the light one (fig.1).

The presence of calsequestrin in the supernatant obtained after Chelex 100 treatment of the vesicles of light subfraction and its absence in the supernatant obtained from the vesicles of heavy subfraction has been proved by the Ouchterlony double diffusion test [13] with the antiserum against calsequestrin (fig.2).

It is known that the extraction of the SR vesicles

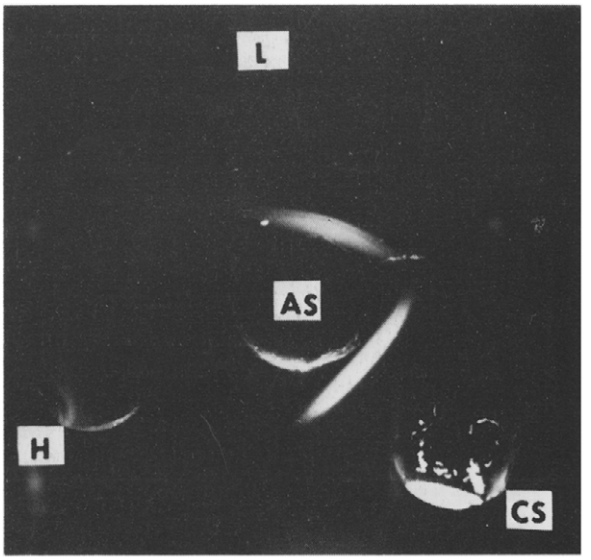

Fig.2. Ouchterlony double diffusion test in an agarose plate. The well labeled AS contained antiserum from a sheep immunized against rabbit calsequestrin; the well labeled CS contained rabbit calsequestrin; the well labeled H and L contained the supernatant after Chelex 100 treatment from the vesicles of heavy and light SR subfractions, respectively.

with EDTA causes an increase of the activity of Ca^{2+}, Mg^{2+}-dependent ATPase [8]. Contrary to that Chelex 100 treatment does not lead to any changes in the ATPase activity. This observation indicates the lack of damage of the vesicles during Chelex 100 treatment.

The results show that in normally oriented vesicles (present in the heavy subfraction) HACBP is located on the external surface of the membrane, whereas calsequestrin is bound to the inner surface. Moreover, the data furnish another evidence to the previous assumption [9] that the light SR subfraction contains the vesicles with the opposite orientation of the membrane than the vesicles present in the heavy subfraction.

The results of this work indicating the internal location of calsequestrin in normally oriented vesicles remain in agreement with the findings of many authors studying the location of the peripheral proteins in SR [3–6,14,15]. Some authors [3–6] have published data suggesting that HACBP is also present inside the vesicles. The results of this work show, however, that in right-side-out vesicles HACBP is bound to the outer surface of the membrane.

The proteins present on the external surface of the vesicles of each subfraction, i.e., HACBP in the heavy subfraction and calsequestrin in the light one, seem to be rather loosely bound. One can assume that the difference in the proportions of these 2 peripheral proteins between the subfractions (see fig.1) is due to the partial removal, during the purification procedure, of this protein, which is externally located in the given subfraction. This assumption may explain why the carefully washed preparations of SR obtained [15–17] contained virtually no HACBP, although this protein was present in the crude SR fraction. The most plausible explanation is that HACBP, which according to the results of this work is located on the external surface of the vesicles, was removed during the washing procedure. Scrupulous analysis of the densitometric tracings of the gels of the SR vesicles labeled with the nonpenetrating reagent diazonium salt of [^{35}S]sulfanilic acid, presented [15], is also in favour of the external location of HACBP. Even in the case where there was virtually no visible protein band in the gel corresponding to HACBP, the incorporation of the radioactivity in this region was quite high.

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