BIOCHEMICAL LETTERS

The PL/IM 430 and the N 89 antibodies recognize two distinct 97 kDa sarco/endoplasmic-reticulum Ca^{2+} -ATPase proteins

Variations in the cytosolic Ca^{2+} concentration play an important role in regulating cell function [1]. Ca^{2+} signalling involves intracellular Ca^{2+} pools, the Ca^{2+} content of which is controlled by the SERCA-type Ca^{2+} pumps (sarco/endoplasmic-reticulum Ca^{2+} -ATPases) [2]. For a long time the SERCA system in non-muscle cells remained poorly characterized as compared with muscle cells. Recently, studies of phosphorylated-intermediate formation and Western-blot analysis of the SERCA proteins and controlled-proteolysis data resulted in the demonstration of the existence of two 97 kDa SERCA isoforms in human platelets and other non-muscle cells, in addition to the ubiquitous SERCA 2b isoform [3,4]. This was based on: (i) the 97 kDa SERCA 3 [5–8] giving rise to 80 and 25 kDa tryptic fragments, the latter being recognized by the N 89 antibody [5], and (ii) a new PL/IM 430-recognizable 97 kDa SERCA isoform giving rise to the 73, 68 and 40 kDa tryptic fragments [9]. However, a subsequent study, using platelets, contradicted these findings and suggested that the SERCA protein recognized by the PL/IM 430 antibody was the SERCA 3 protein [10]. This conclusion was based on similar recognition of non-specific degradation products of about 74, 70 and 40 kDa by the PL/IM 430 and N 89 antibodies. Results showing the SERCA 3-related 25 kDa fragment recognized by N 89 were, however, not presented.

In the present work, human cell lines were screened for the presence of the two 97 kDa SERCA proteins (Figure 1). We isolated mixed membrane fractions and studied SERCA expression by Western blotting using isoform-specific antibodies: the monoclonal IID8 (which recognizes SERCA 2b) [11] (Figure 1A), the monoclonal PL/IM 430 [12] (Figure 1B) and the polyclonal N 89 [5] (Figure 1C) antibodies. The T lymphoblastoid JUR E 6-1 (lane 1), the epithelial HeLa (lane 2) and the gastric-cancer KATO III (lane 3) cell lines were studied. As shown previously [13], in KATO III cells, only the SERCA 2b isoform was expressed, so we used this cell line as a negative control for the expression of the two 97 kDa SERCA isoforms. The SERCA 2b isoform appeared equally expressed in the three cell lines, as demonstrated by the similar recognition of the 100 kDa protein band with the IID8 antibody. In contrast, a clear difference was found between the expression levels of the SERCA 3 and the PL/IM 430-recognizable 97 kDa SERCA isoforms in JUR E 6-1 and HeLa cells. Indeed, JUR E 6-1 cells expressed exclusively the PL/IM 430-recognizable 97 kDa SERCA isoform, whereas HeLa cells expressed exclusively the SERCA 3 protein. It can therefore be concluded that the two 97 kDa SERCA isoforms are different and are differentially expressed in these cell lines.

To confirm these results further, we performed trypsinolysis of the JUR E 6-1 and HeLa cell membrane preparations and studied the fragmentation profiles of the SERCA isoforms by Western blotting (results not shown). The results revealed the presence of

the PL/IM 430-recognizable 73, 68 and 40 kDa fragments, but the absence of the 25 kDa fragment related to the SERCA 3 protein in JUR E 6-1 cell membranes. By contrast, for the HeLa cells, the trypsinolysed membranes were not recognized by the PL/IM 430 antibody, whereas the N 89 antibody revealed the presence of the SERCA 3-related 25 kDa fragment.

These results incited us to try to differentiate between the two 97 kDa SERCA isoforms in the haematopoietic system in which they were initially found to be concomitantly expressed. For this, we performed immunoprecipitation experiments (Figure 2) using the PL/IM 430 monoclonal antibody in human platelets (Figure 2A) and the megacaryocytic MEG 01 cell line (Figure 2B) [14] used as a new control not expressing the SERCA 3 protein. Indeed, similarly to JUR E 6-1 cells, the MEG 01 cell line was not recognized by the N 89 antibody (results not shown). The immunoprecipitates and their respective supernatants were tested by Western blotting using the PL/IM 430 and N 89 antibodies. Analysis of lanes 1 clearly showed the presence of the PL/IM

Figure 1 Cell-dependent expression of the PL/IM 430- and N 89-recognizable SERCA

Mixed 100 000 g membrane fractions were isolated from different cell lines. A 50 μg portion of each membrane preparation was analysed by SDS/8% PAGE and electroblotted on to nitrocellulose sheets. Immunodetection of SERCA 2b (A) was performed using the IID8 monoclonal antibody, detection of the PL/IM 430-recognizable 97 kDa SERCA using the PL/IM 430 monoclonal antibody (B) and SERCA 3 was revealed using the polyclonal anti-(SERCA 3) antibody termed N 89 (C). Lanes 1–3 represent membranes isolated from T-lymphoblastoid JUR E 6-1, epithelial HeLa and gastric-cancer KATO III cell lines respectively. The molecular masses of the proteins, estimated using standard 'rainbow coloured' markers, are indicated on the left. This Figure shows results representative of four experiments.

Figure 2 Dissociation between the PL/IM 430-recognizable SERCA and SERCA 3 by immunoprecipitation

Human platelets and MEG 01 cell membrane proteins (100 μ g) were treated for immunoprecipitation using the PL/IM 430 monoclonal antibody. The immunoprecipitates and the corresponding supernatants obtained after concentration were reduced, analysed by SDS/11% PAGE, electroblotted on to nitrocellulose sheets and treated for immunoblotting using either the monoclonal PL/IM 430 or the polyclonal anti-(SERCA 3) N 89 antibodies. (A) Human platelets: lane 1, Western blot of the PL/IM 430 immunoprecipitate using the PL/IM 430 antibody; lane 2, Western blot of the supernatant using the N 89 antibody. (B) MEG 01 cells: lane 1, Western blot of the PL/IM 430 immunoprecipitate using the PL/IM 430 antibody; lane 2, Western blot of the supernatant using the N 89 antibody; lane 3, Western blot of the PL/IM 430 immunoprecipitate using the N 89 antibody. The molecular masses of the proteins estimated using standard 'rainbow coloured' markers, are indicated on the right. The Figure shows results representative of four experiments.

430-recognizable 97 kDa SERCA isoform in the immunoprecipitates. The immunoprecipitations were complete, since no residual PL/IM 430-recognizable 97 kDa SERCA isoform was detected in the supernatants (results not shown). The SERCA 3 protein was detected in the supernatant of platelet immunoprecipitates, but was not found in MEG 01 cell supernatant (lanes 2), confirming the lack of SERCA 3 protein in these latter cells. Moreover, these immunoprecipitation experiments allowed us to demonstrate further the isoform specificity of the PL/IM 430 (lane 1) and N 89 (lane 3) antibodies, since no N 89 recognition could be detected on this PL/IM 430 immunoprecipitate, which is devoid of SERCA 3 protein.

In conclusion, these results provided a clear differentiation between the PL/IM 430-recognizable 97 kDa SERCA isoform and the 97 kDa SERCA 3 protein in different cell types, and

confirmed the SERCA isoform specificity of the PL/IM 430 and N 89 antibodies. Consequently, these results are not compatible with the existence of an unique 97 kDa SERCA isoform recognized by both the PL/IM 430 and N 89 antibodies [10]. On the contrary, these antibodies recognize distinct SERCA entities: N 89 recognizes the SERCA 3 isoform, whereas PL/IM 430 recognizes another 97 kDa SERCA protein not yet identified in the SERCA family. Consequently, previous findings [9] and the present work strongly support the notion of the original expression of a multi-SERCA system in platelets and other non-muscle cells, in agreement with the existence of multiple Ca^{2+} pools described for these cells.

Christine LACABARATZ*, Elisabeth CORVAZIER*, Tünde KOVÁCS†, Régis BOBE*, Frank WUYTACK‡, Béla PAPP* and Jocelyne ENOUF*

*Institut National de la Santé et de la Recherche Médicale U 348, IFR Circulation Lariboisière, Hôpital Lariboisière, 8 rue Guy Patin, 75475 Paris Cedex 10, France, †The National Institute of Haematology and Immunology, Daroczi ut 24, H-1113 Budapest, Hungary, and ‡Laboratorium voor Fysiologie, Katholieke Universiteit, Campus Gasthuisberg, 3000 Leuven, Belgium

§ To whom correspondence should be addressed.

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