Purification of an inositol 1,4,5-trisphosphate-binding calreticulin-containing intracellular compartment of HL-60 cells

Christian VAN DELDEN,*§ Cécile FAVRE,* András SPÄT,† Erich CERNY,† Karl-Heinz KRAUSE* and Daniel P. LEW*

*Division of Infectious Diseases and †Fondation pour Recherches Médicales, Geneva University Hospital, CH-1211 Geneva 4, Switzerland, and ‡Department of Physiology, Semmelweis University, Budapest, Hungary

To investigate the identity of Ins(1,4,5) P_3 -sensitive intracellular Ca^{2+} stores in myeloid cells, we have developed a method that yields subcellular fractions highly enriched in Ins(1,4,5) P_3 binding. HL-60 cells were disrupted by nitrogen cavitation, and subcellular fractions were obtained by differential centrifugation, followed by Percoll- and sucrose-density-gradient separations. A subcellular fraction enriched 26-fold in Ins(1,4,5) P_3 -binding sites was obtained. This fraction showed no enrichment in plasma-membrane markers and only a comparatively moderate enrichment (7-fold) in endoplasmic-reticulum markers. The ratio between specific enrichment of Ins(1,4,5) P_3 binding and endoplasmic-reticulum markers in the different fractions varied over 50-fold, from < 0.1 to > 5 . The purified Ins(1,4,5) P_3 -binding fraction was enriched to a similar extent (27-fold) in the putative intravesicular Ca^{2+} -storage protein calreticulin. Our results favour the concept of a distinct Ins(1,4,5) P_3 -binding, calreticulin-containing compartment (i.e. the calciosome) in HL-60 cells.

INTRODUCTION

The identity of the Ins(1,4,5) P_3 -sensitive intracellular Ca^{2+} store in non-muscle cells remains a controversial issue. Main candidates for this function are the endoplasmic reticulum and the calciosomes (for reviews see Berridge & Irvine, 1989; Krause *et al.*, 1989; Meldolesi *et al.*, 1990; Koch, 1990; Krause, 1991; Rossier & Putney, 1991; Van Delden *et al.*, 1991). Most studies attempting to identify the Ins(1,4,5) P_3 -sensitive Ca^{2+} store have used Ins(1,4,5) P_3 -induced Ca^{2+} release as a marker and correlated its distribution in subcellular fractions with the distribution of marker enzymes for various intracellular organelles (Streb *et al.*, 1984; Authi & Crawford, 1985; Clapper & Lee, 1985; Prentki *et al.*, 1986; Ueda *et al.*, 1986; Delfert *et al.*, 1986; Krause & Lew, 1987; Volpe *et al.*, 1988; Benedetti *et al.*, 1988; Dunlop & Larkins, 1988). However, this approach has a major methodological limitation: the Ins(1,4,5) P_3 -responsiveness of intracellular Ca^{2+} stores decreases during the purification procedures, most likely owing to the loss of functional integrity of the responsible organelle. Thus specific enrichment in Ins(1,4,5) P_3 -sensitive Ca^{2+} stores of a given subcellular fraction cannot be assessed by measuring Ins(1,4,5) P_3 -induced Ca^{2+} release. However, without a high degree of purification and quantitative data on specific enrichment, it is impossible to decide conclusively whether a certain marker protein is associated with a specific intracellular compartment. In contrast with the Ins(1,4,5) P_3 -induced Ca^{2+} -release capacity, the Ins(1,4,5) P_3 -binding capacity of a given fraction is not lost during subcellular fractionation. Three recent subcellular-fractionation studies that used Ins(1,4,5) P_3 binding as a marker observed no correlation of Ins(1,4,5) P_3 -sensitive Ca^{2+} stores with endoplasmic-reticulum markers, but some correlation with plasma-membrane markers (Guillemette *et al.*, 1988; Rossier *et al.*, 1989, 1991).

In this study we used Ins(1,4,5) P_3 binding to monitor the purification of Ins(1,4,5) P_3 -sensitive Ca^{2+} stores from the myeloid cell line HL-60. We obtained a fraction that was enriched 26-fold in Ins(1,4,5) P_3 -binding sites. The fraction was not enriched in

plasma-membrane markers and showed no comparable enrichment (7-fold) in endoplasmic-reticulum markers. It was, however, similarly enriched (27-fold) in its content of the Ca^{2+} -binding protein calreticulin.

MATERIALS AND METHODS

Materials

The materials and their sources were as follows: Ins(1,4,5) P_3 and [3H]mannose (18.5 Ci/mmol) were from Amersham, and [^{32}P]Ins(1,4,5) P_3 (declared specific radioactivity 100–180 Ci/mmol) was from New England Nuclear. Each batch of [^{32}P]Ins(1,4,5) P_3 was checked for purity by h.p.l.c., as previously described (Pittet *et al.*, 1989). Percoll was obtained from Pharmacia LKB Biotech., and Chelex 100 was from Bio-Rad. All other biochemicals were of analytical grade and were obtained from Fluka, Merck, Amersham, Sigma or Calbiochem. All centrifuges and rotors used were from Beckman, and ultracentrifugations were performed in an L7-55 ultracentrifuge with an SW-41 rotor unless stated otherwise; average g forces are indicated.

Buffers

The buffer termed '0.1 M-KCl/Hepes' contained 25 mM-Hepes, 0.1 M-KCl and 2 mM-KH₂PO₄ (pH 7.0). Ca^{2+} contamination of this buffer was decreased by use of the ion-exchange resin Chelex 100. The buffer termed 'binding buffer' contained 25 mM-Na₂HPO₄, 100 mM-KCl, 20 mM-NaCl and 1 mM-Na-EDTA (pH 7.4).

Preparation of subcellular fractions

HL-60 cells were incubated with the proteinase inhibitor diisopropyl fluorophosphate (5 μ M) and disrupted by nitrogen cavitation [2.3 MPa (23 bars) for 30 min] as described previously (Krause & Lew, 1987; Volpe *et al.*, 1988). The resulting preparation will be referred to as homogenate (H) throughout the text. Proteinase inhibitors in the cavitation buffer were aprotinin

§ To whom correspondence should be addressed.

(76.8 nM), benzamidine (0.83 mM), iodoacetamide (1 mM), leupeptin (1.1 μ M), pepstatin A (0.7 μ M) and phenylmethanesulphonyl fluoride (0.23 mM). A postnuclear supernatant (Pns), obtained by centrifugation of homogenates at 3000 *g* for 5 min, was incubated (10 min, 37 °C) with digitonin (100 μ M) to increase the density of plasma membranes, contaminating low-density fractions, and to shift them towards fractions of higher density (Amar-Costec *et al.*, 1974; Lange & Steck, 1985; Van Delden *et al.*, 1991). The postnuclear supernatant was then separated on a discontinuous Percoll gradient (19 % and 33 % Percoll made iso-osmotic with KCl/Hepes buffer) by centrifuging at 160 000 *g* for 10 min. Three fractions (F_1 , F_2 , F_3 in order of decreasing density) were obtained. Percoll was removed from the fractions by dilution and centrifugation at 60 000 *g* for 25 min (rotor 60 TI). The light-density fraction of the Percoll gradient (F_3) was resuspended in binding buffer at a protein concentration of 3–5 mg/ml and was mixed with a 2 M-sucrose solution to a final sucrose concentration of 1.2 M. A discontinuous sucrose gradient was then formed, composed of 3 ml of 1 M-sucrose solution (top of the gradient), 2.5 ml of 1.2 M-sucrose mixture (containing the sample), 3 ml of 1.3 M-sucrose solution and 2 ml of 1.5 M-sucrose solution (bottom of the gradient). The concentration of all sucrose solutions was checked before use by refractometry. The gradient was centrifuged at 200 000 *g* for 180 min, and three bands of decreasing densities were identified: heavy-density fraction (interface 1.5 M- and 1.3 M-sucrose, S_1), intermediate-density fraction (interface 1.3 M- and 1.2 M-sucrose, S_2) and low-density fraction (interface 1.2 M- and 1 M-sucrose, S_3). To remove sucrose, the collected fractions were diluted in binding buffer and centrifuged at 210 000 *g* for 55 min. The pellets from this centrifugation were resuspended in binding buffer (protein concn. 0.9–2.5 mg/ml), frozen in liquid nitrogen and stored at –70 °C.

All steps were performed at 4 °C, and all solutions contained phenylmethanesulphonyl fluoride (58 μ M) and benzamidine (0.83 mM), if not otherwise stated.

Assays for marker enzymes and proteins

Activities of marker enzymes were determined as described for alkaline phosphatase (DeChatelet & Cooper, 1970), galactosyl-transferase (Bretz & Staubli, 1977), β -glucuronidase (Dewald *et al.*, 1982), sulphatase-C (Canonica *et al.*, 1978) and NADPH-cytochrome *c* reductase (Sottocasa *et al.*, 1967). Protein concentrations were determined in the presence of 1 % SDS by a colorimetric assay as described by Peterson (1977).

Binding studies

Subcellular fractions (80–350 μ g of protein, depending on the expected binding activity) were incubated for 10 min at 4 °C in a total volume of 250 μ l of binding buffer containing 1 mg of BSA/ml, 10 nCi of [32 P]Ins(1,4,5) P_3 and 0.1 μ Ci of [3 H]mannose. By h.p.l.c. analysis we could not detect any Ins(1,4,5) P_3 metabolites at 4 °C after 15 min. Bound ligand was separated from unbound by centrifugation through oil as described previously (Spät *et al.*, 1986). Samples were centrifuged at 46 000 *g* for 40 min (rotor JA 21) through 600 μ l of an oil mixture composed of dibutyl phthalate and bis-(3,5,5-trimethylhexyl)-phthalate (1.49:1, w/w). The pellets were dissolved in 30 μ l of Triton X-100 (2 %) and transferred to scintillation vials. Non-specific binding was determined by including 5 μ M-Ins(1,4,5) P_3 in the incubation medium. Binding values were corrected for extravascular volume on the basis of [3 H]mannose contamination (Spät *et al.*, 1986). This correction never exceeded 1 % of the total 3 H added and correlated closely with non-specific-binding values, which were less than 10 % of total binding. We chose the oil-centrifugation method rather than filtration in order to avoid

the rapid dissociation of Ins(1,4,5) P_3 from low-affinity binding sites (Spät *et al.*, 1986; Worley *et al.*, 1987).

SDS/PAGE and immunochemical analysis of HL-60 proteins

The analysis of proteins by SDS/PAGE (5–16 %-acrylamide gradient), staining with Coomassie Blue and with the cationic carbocyanine dye Stains-all, and electrophoretic transfer to Immobilon poly(vinylidene difluoride) membranes were performed by standard methods, as described previously (Krause *et al.*, 1990). Antibodies against human calreticulin were produced by immunization of rabbits with synthetic peptides coupled to keyhole-lympet haemocyanin. The 20 N-terminal amino acids of human calreticulin were deduced from protein sequencing and cDNA cloning (Krause *et al.*, 1990; McCauliffe *et al.*, 1990). Peptides were synthesized by using a Labortech SP 650 peptide synthesizer in combination with a Tecan RSP 5052 laboratory robot using 9-fluorenylmethyloxycarbonyl-protected amino acids and a Wang resin (Bachem). For immunoblotting the transfers were blocked with phosphate-buffered saline, 0.05 % Tween-20 and 0.5–1 % non-fat dry milk before incubation with the primary antibody. 35 S-Protein A was used instead of a secondary antibody to develop immunoblots. To analyse the calreticulin content of subcellular fractions quantitatively, at least three different amounts of protein were tested. The amount of bound 35 S-Protein A was detected by autoradiography by using a storage phosphor screen and quantified with the PhosphorImager (Molecular Dynamics). The slope of the relationship between the protein amount and the pixel values was used to calculate the calreticulin enrichment of a given fraction with respect to the calreticulin content of the homogenate.

RESULTS AND DISCUSSION

The present study was designed to obtain subcellular fractions highly enriched in Ins(1,4,5) P_3 binding. The purified fractions were analysed for specific enrichment in marker enzymes of the endoplasmic reticulum and the plasma membrane, as well as in the Ca^{2+} -binding protein calreticulin, a putative Ca^{2+} -storage protein of Ins(1,4,5) P_3 -sensitive Ca^{2+} stores (Hashimoto *et al.*, 1988; Pozzan *et al.*, 1988; Volpe *et al.*, 1988; Krause *et al.*, 1989, 1990). The initial steps of the procedure (nitrogen cavitation, digitonin preincubation and discontinuous Percoll gradient) are similar to a previously described fractionation procedure (Krause & Lew, 1987; Volpe *et al.*, 1988). The light-density fraction of the Percoll gradient was enriched 6.1-fold in Ins(1,4,5) P_3 binding compared with the homogenate (Fig. 1a). Analysis of the distribution of markers of other organelles in the Percoll gradient (fractions F_1 , F_2 and F_3) showed only minor enrichments in the light-density fraction (F_3) for endoplasmic-reticulum markers (sulphatase-C and NADPH-cytochrome *c* reductase; 2.7-fold, Fig. 1b) and plasma-membrane markers (alkaline phosphatase; 1.6-fold, Fig. 1c). Similarly this fraction was not markedly enriched (1.4-fold) in the Golgi-apparatus marker enzyme galactosyltransferase and showed no enrichment in the granule marker enzyme β -glucuronidase [results not shown; see also Volpe *et al.* (1988), Krause *et al.* (1989) and Van Delden *et al.* (1991)]. In order to purify the Ins(1,4,5) P_3 -binding membranes further, we next separated the light-density fraction of the Percoll gradient (F_3) on a discontinuous sucrose gradient. Three fractions were obtained (S_1 , S_2 , S_3 , in order of decreasing density). The intermediate-density fraction (S_2) showed a 26-fold enrichment in Ins(1,4,5) P_3 binding (Fig. 1d). Again, no enrichment of plasma-membrane markers (Fig. 1f) and only a comparatively moderate enrichment (7-fold) of endoplasmic-reticulum markers (Fig. 1e) was observed.

To analyse in more detail the relationship between the

Fig. 1. Distribution of Ins(1,4,5) P_3 -binding membranes, endoplasmic-reticulum and plasma-membrane marker enzymes in subcellular fractions of HL-60 cells

HL-60 cells were disrupted by nitrogen cavitation and separated on a discontinuous Percoll gradient (first gradient) that yielded three subcellular fractions (F_1 , F_2 , F_3 , in order of decreasing density). The light-density fraction of the Percoll gradient [F_3 , enriched 6-fold in Ins(1,4,5) P_3 binding] was then separated on a discontinuous sucrose gradient (second gradient) that yielded three subcellular fractions (S_1 , S_2 , S_3 , in order of decreasing density). [^{32}P]Ins(1,4,5) P_3 binding (panels *a* and *d*), endoplasmic-reticulum (E.R.) markers [panels *b* and *e*, NADPH-cytochrome *c* reductase (▨) and sulphatase-C (■)] and the plasma-membrane marker alkaline phosphatase (panels *c* and *f*) were determined in all fractions. Values are given as enrichment, i.e. fold increase in specific activity compared with the homogenate. Values are means $\pm$ S.E.M. of 3–8 separate experiments, each performed in duplicate.

Fig. 2. Ratio of enrichment of Ins(1,4,5) P_3 binding and endoplasmic-reticulum markers in various subcellular fractions

The ratio of enrichment of Ins(1,4,5) P_3 binding and the endoplasmic-reticulum marker sulphatase-C (■) or of the two endoplasmic-reticulum markers sulphatase-C and NADPH-cytochrome *c* reductase (▨) was determined. In contrast with the constant ratio of approx. 1 observed for the two endoplasmic-reticulum markers, the ratio between Ins(1,4,5) P_3 binding and endoplasmic-reticulum markers varied more than 50-fold. It was < 0.1 in fraction F_1 and > 5 in fraction S_1 (for details of the fractions, see Fig. 1).

fraction of the sucrose gradient (S_1), and < 0.1 in the heavy-density fraction of the Percoll gradient (F_1), where Ins(1,4,5) P_3 binding was only one-tenth of the binding found in the homogenate while endoplasmic-reticulum markers were even slightly enriched. In contrast, the ratio of enrichment between the two endoplasmic-reticulum markers sulphatase-C and NADPH-cytochrome *c* reductase (Fig. 2, hatched bars) was invariably about 1, as expected for two proteins that are part of the same intracellular compartment.

The use of Ins(1,4,5) P_3 binding as a marker for Ins(1,4,5) P_3 -sensitive Ca^{2+} stores is based on the assumption that the observed binding is to the Ins(1,4,5) P_3 -sensitive Ca^{2+} channel. However, enzymes of Ins(1,4,5) P_3 metabolism might also bind Ins(1,4,5) P_3 . Although we cannot entirely exclude some binding to enzymes of Ins(1,4,5) P_3 metabolism in our fractions, the latter possibility is probably without quantitative importance, as: (1) in HL-60 cells, virtually all Ins(1,4,5) P_3 3-kinase activity was found in the cytosol; (2) the particulate Ins(1,4,5) P_3 5-phosphatase did not co-purify with Ins(1,4,5) P_3 binding in the above-described subcellular fractions; (3) the affinity for Ins(1,4,5) P_3 of the Ins(1,4,5) P_3 -binding membranes was unaffected by the purification procedures (thus the observed enrichment is not due to a change in the K_m) and was approximately three orders of magnitude higher than the affinity of the particular Ins(1,4,5) P_3 5-phosphatase (C. Van Delden, D. Pittet, K. H. Krause & D. P. Lew, unpublished work). The latter observations are in accordance with results obtained in other cell types (Downes *et al.*, 1982; Connolly *et al.*, 1985; Hansen *et al.*, 1987). Thus Ins(1,4,5) P_3 binding is presumably a specific marker for the Ins(1,4,5) P_3 -sensitive intracellular organelle.

The analysis of Ins(1,4,5) P_3 binding and marker enzymes shows that the Ins(1,4,5) P_3 -sensitive organelle of HL-60 cells is not identical with the plasma membrane, which could be shifted

Ins(1,4,5) P_3 -binding membranes and the endoplasmic reticulum, we calculated the ratio between the specific enrichments of Ins(1,4,5) P_3 -binding sites and endoplasmic-reticulum markers (Fig. 2). This ratio (Fig. 2, black bars) varied over 50-fold in the fractions described above. It was > 5 in the heavy-density

Fig. 3. SDS/PAGE and immunochemical analysis of subcellular fractions

Subcellular fractions of HL-60 cells (70 μ g of protein/lane) were separated by gel electrophoresis and stained with Coomassie Blue (a) or Stains-all (b), or transferred to nitrocellulose and subjected to immunoblotting using anti-calreticulin antibodies and 35 S-Protein A (c). A 60 kDa protein that stained metachromatically blue with Stains-all was recognized by the anti-calreticulin antibodies and showed a subcellular distribution similar to that of Ins(1,4,5) P_3 binding (see Fig. 1).

from low-density fractions towards fractions of higher densities by digitonin preincubation (Van Delden *et al.*, 1991). Results in other experimental systems (Rossier *et al.*, 1991) suggest a cytochalasin B-sensitive attachment of Ins(1,4,5) P_3 -sensitive Ca^{2+} stores to the plasma membrane. Our data do not exclude such an attachment in intact HL-60 cells. However, the lack of co-purification of plasma membrane and Ins(1,4,5) P_3 -binding sites suggests that, after nitrogen cavitation of HL-60 cells, this putative association is not preserved.

The results also clearly indicate a lack of correlation between Ins(1,4,5) P_3 binding and endoplasmic-reticulum markers in the various subcellular fractions. This is compatible with the calciosome theory, i.e. the concept of a specialized, Ins(1,4,5) P_3 -sensitive, intracellular Ca^{2+} store that may support Ca^{2+} storage and release even in cells almost devoid of endoplasmic reticulum, such as neutrophils (Volpe *et al.*, 1988; Krause *et al.*, 1989).

There is, however, some enrichment of endoplasmic-reticulum markers in the purest Ins(1,4,5) P_3 -binding fraction. This might be explained by co-purification of distinct Ins(1,4,5) P_3 -binding membranes with light-density endoplasmic-reticulum vesicles. Alternatively, this observation is compatible with the hypothesis that the Ins(1,4,5) P_3 -sensitive Ca^{2+} pool is a specialized portion of the endoplasmic reticulum. If the latter hypothesis is correct, the distribution of endoplasmic-reticulum markers and Ins(1,4,5) P_3 binding and their differential enrichments found in our study would predict that approx. 80 % of the endoplasmic reticulum does not contain appreciable amounts of Ins(1,4,5) P_3 -binding sites and that the binding sites are concentrated in 20 % of the total endoplasmic reticulum. In this respect it is noteworthy that recent electron-microscopy studies in Purkinje neurons (Ross *et al.*, 1989; Sato *et al.*, 1990; Otsu *et al.*, 1990) suggest such a concentration of Ins(1,4,5) P_3 -binding sites in specialized cisternae of the smooth endoplasmic reticulum. However, this does not necessarily apply to other cell types, as Purkinje cells are particular cells concerning the characteristics and number of Ins(1,4,5) P_3 -binding sites.

Having purified Ins(1,4,5) P_3 -binding membranes and analysed their relationship to other organelles, we next investigated the protein composition of the Ins(1,4,5) P_3 -binding fractions by SDS/PAGE and immunoblotting. Fig. 3(a) shows Coomassie-Blue-stained gels of a homogenate (H), a light-density fraction of the Percoll gradient [F_3 , enriched 6-fold in Ins(1,4,5) P_3 binding] and the intermediate-density fraction of the sucrose gradient [S_2 , enriched 26-fold in Ins(1,4,5) P_3 binding]. Analysis of the same fractions by Stains-all staining, known to stain certain Ca^{2+} -binding proteins metachromatically blue (Campbell *et al.*, 1983) (Fig. 3b) showed the progressive enrichment of two blue-staining bands with molecular masses of 60 and 100 kDa. We have not yet identified the 100 kDa putative Ca^{2+} -binding protein of HL-60 cells. We have, however, previously purified the 60 kDa Ca^{2+} -binding protein and shown that it is calreticulin (Krause *et al.*, 1990). We therefore screened all fractions shown in Fig. 1 by immunoblotting with anti-calreticulin antibodies. These results (Fig. 3c) confirm that the 60 kDa protein is calreticulin and, importantly, demonstrate that the subcellular distribution of calreticulin is identical with that of Ins(1,4,5) P_3 -binding sites.

To analyse quantitatively the relationship between Ins(1,4,5) P_3 binding and calreticulin, we performed Ins(1,4,5) P_3 binding and calreticulin immunoblots in the fractions of interest, over a range of protein amounts (Fig. 4).

Ins(1,4,5) P_3 binding was linear over a wide range of protein amounts (Fig. 4a). Immunoblotting for calreticulin was initially tested by using alkaline-phosphatase-coupled secondary antibodies. This method was found to be unsuitable for quantitative analysis (results not shown). In contrast, immunoblots yielded linear results when 35 S-labelled Protein A was used instead of a secondary antibody and quantitative analysis was performed with a storage phosphor screen and the PhosphorImager (Fig. 4b).

To calculate the enrichment of Ins(1,4,5) P_3 binding and calreticulin with respect to the homogenate, the slope of the signal/protein relationship of a subcellular fraction was divided by the slope for the homogenate. This analysis indicated the following enrichment of Ins(1,4,5) P_3 -binding sites and calreticulin: 6.1 ± 1.0 -fold and 6.9 ± 0.7 -fold respectively in the light-density fraction of the Percoll gradient (F_3), and 25.8 ± 2.7 -fold and 27.0 ± 3.9 -fold respectively in the intermediate-density fraction of the sucrose gradient (S_2) (values are means $\pm$ s.d. of two independent experiments performed in duplicate).

These results strongly support the concept that in myeloid cells the Ins(1,4,5) P_3 -sensitive Ca^{2+} channel and calreticulin are localized within the same subcellular compartment and suggest, albeit

Fig. 4. Signal/protein relationship of Ins(1,4,5) P_3 binding and calreticulin immunoblots in homogenates and subcellular fractions of HL-60 cells

HL-60 cell homogenates (H) and subcellular fractions (F₃, S₂) were obtained by nitrogen cavitation and Percoll- and sucrose-density-gradient separation. Ins(1,4,5) P_3 binding was assessed by the oil-centrifugation method. Calreticulin content was assessed by incubation of transfers from SDS/PAGE with rabbit anti-calreticulin antibodies, followed by incubation with ^{35}S -Protein A and quantitative analysis using a storage phosphor screen and the PhosphorImager. The calreticulin content was derived from pixel values detected by the PhosphorImager. The relationship between the amount of protein in the assay and the amount of bound [^{32}P]Ins(1,4,5) P_3 (a) or of calreticulin (b) was linear. Results shown are typical experiments performed twice in duplicate.

indirectly, a role for calreticulin as Ca^{2+} -storage protein of the Ins(1,4,5) P_3 -sensitive Ca^{2+} pool.

In conclusion, we describe a subcellular-fractionation procedure that yields highly purified Ins(1,4,5) P_3 -binding intracellular membranes. Quantitative analysis of these fractions demonstrated co-purification of Ins(1,4,5) P_3 binding with calreticulin, but not with enzyme markers of the endoplasmic reticulum and the plasma membrane. These results are in favour of the concept of a specialized Ins(1,4,5) P_3 -sensitive, calreticulin-containing, intracellular Ca^{2+} -storage and -release compartment, i.e. the calciosome, in HL-60 cells. This compartment is not identical with the endoplasmic reticulum; however, further studies will be necessary to establish the precise relationship between calciosomes and endoplasmic reticulum in HL-60 cells and other cell types.

We thank Dr. R. A. Clark, Dr. W. Schlegel and Dr. M. Prentki for helpful discussions, and A. Monod and J. Gil for technical assistance.

This research was supported by grants 32-30161.90 and 70 UP-029786 from the Swiss National Foundation. C.V.D. is supported by the De Reuter Foundation, Geneva, and by the Dr. Henry Dubois-Ferrière-Dinu Lipatti Foundation, Geneva.

REFERENCES

- Amar-Costec, A., Wibo, M., Thines-Sempoux, D., Beaufay, H. & Berthet, J. (1974) *J. Cell Biol.* **62**, 717–745
- Authi, K. S. & Crawford, N. (1985) *Biochem. J.* **230**, 247–253
- Benedetti, A., Fulceri, R., Romani, A. & Comporti, M. (1988) *J. Biol. Chem.* **263**, 3466–3473
- Berridge, M. J. & Irvine, R. F. (1989) *Nature (London)* **341**, 197–205
- Bretz, R. & Staubli, W. (1977) *Eur. J. Biochem.* **77**, 181–192
- Campbell, K. P., MacLennan, D. H. & Jorgensen, A. O. (1983) *J. Biol. Chem.* **258**, 11267–11273
- Canonica, P. G., Beaufay, H. & Nyssens-Jadin, M. (1978) *J. Reticuloendothel. Soc.* **24**, 115–138
- Clapper, D. L. & Lee, H. C. (1985) *J. Biol. Chem.* **260**, 13947–13954
- Connolly, T. M., Bross, T. E. & Majerus, P. W. (1985) *J. Biol. Chem.* **260**, 7868–7874
- DeChatelet, L. R. & Cooper, M. R. (1970) *Biochem. Med.* **4**, 61–68
- Delfert, D. M., Hill, S., Pershadsingh, H. A., Sherman, W. R. & McDonald, J. M. (1986) *Biochem. J.* **236**, 37–44
- Dewald, B., Bretz, U. & Baggiolini, M. (1982) *J. Clin. Invest.* **70**, 518–525
- Downes, C. P., Mussat, M. C. & Michell, R. H. (1982) *Biochem. J.* **203**, 169–177
- Dunlop, M. E. & Larkins, R. G. (1988) *Biochem. J.* **253**, 67–72
- Guillemette, G., Balla, T., Baukal, A. J. & Catt, K. J. (1988) *J. Biol. Chem.* **263**, 4541–4548
- Hansen, C. A., Johanson, R. A., Williamson, M. T. & Williamson, J. R. (1987) *J. Biol. Chem.* **262**, 17319–17326
- Hashimoto, S., Bruno, B., Lew, D. P., Pozzan, T., Volpe, P. & Meldolesi, J. (1988) *J. Cell Biol.* **107**, 2523–2531
- Koch, L. E. (1990) *BioEssays* **12**, 527–531
- Krause, K. H. (1991) *FEBS Lett.* **285**, 225–229
- Krause, K. H. & Lew, D. P. (1987) *J. Clin. Invest.* **80**, 107–116
- Krause, K. H., Pittet, D., Volpe, P., Meldolesi, J. & Lew, D. P. (1989) *Cell Calcium* **10**, 351–361
- Krause, K. H., Simmerman, K. B., Jones, L. R. & Campbell, K. P. (1990) *Biochem. J.* **270**, 545–548
- Lange, Y. & Steck, T. L. (1985) *J. Biol. Chem.* **260**, 15592–15597
- McCauliffe, D. P., Zappi, E., Lieu, T. S., Michalak, M., Sontheimer, R. D. & Capra, J. D. (1990) *J. Clin. Invest.* **86**, 332–335
- Meldolesi, J., Madeddu, L. & Pozzan, T. (1990) *Biochim. Biophys. Acta* **1055**, 130–140
- Otsu, H., Yamamoto, A., Maeda, N., Mikoshiba, K. & Tashiro, Y. (1990) *Cell Struct. Funct.* **15**, 163–173
- Peterson, G. L. (1977) *Anal. Biochem.* **83**, 346–356
- Pittet, D., Schlegel, W., Lew, D. P., Monod, A. & Mayr, G. W. (1989) *J. Biol. Chem.* **264**, 18489–18493
- Pozzan, T., Volpe, P., Zorzato, F., Bravin, M., Lew, D. P., Krause, K. H., Hashimoto, S., Bruno, B. & Meldolesi, J. (1988) *J. Cardiovasc. Pharmacol.* **12**, 80–84
- Prentki, M., Biden, T. J., Janjic, D., Irvine, R. F., Berridge, M. J. & Wollheim, C. B. (1986) *Nature (London)* **309**, 562–564
- Ross, C. A., Meldolesi, J., Milner, T. A., Satoh, T., Supattapone, S. & Snyder, S. H. (1989) *Nature (London)* **339**, 468–470
- Rossier, M. F. & Putney, J. W., Jr. (1991) *Trends Neurosci.*, in the press
- Rossier, M. F., Capponi, A. M. & Vallotton, M. B. (1989) *J. Biol. Chem.* **264**, 14078–14084
- Rossier, M. F., Bird, G. J. & Putney, J. W., Jr. (1991) *Biochem. J.* **274**, 643–650
- Sato, T., Ross, C. A., Villa, A., Supattapone, S., Pozzan, T., Snyder, S. H. & Meldolesi, J. (1990) *J. Cell Biol.* **111**, 615–624
- Sottocasa, G. L., Kuylentstierna, B., Ernster, L. & Bergstrand, A. (1967) *J. Cell Biol.* **32**, 415–438
- Spät, A., Bradford, P. G., McKinney, J. S., Rubin, R. P. & Putney, J. W., Jr. (1986) *Nature (London)* **319**, 514–516
- Streb, H., Bayerdörffer, E., Haase, W., Irvine, R. F. & Schulz, I. (1984) *J. Membr. Biol.* **81**, 241–253
- Ueda, T., Chueh, S. H., Noel, M. W. & Gill, D. L. (1986) *J. Biol. Chem.* **261**, 3184–3192

- Van Delden, C., Jaconi, M. E. E., Pittet, D., Demareux, N., Krause, K. H. & Lew, D. P. (1991) in *Cellular and Molecular Mechanisms of Inflammation: Signal Transduction in Inflammatory Cells I* (Cochrane, C. C. & Gimbrone, M., eds.), Academic Press, Orlando, FL, in the press
- Volpe, P., Krause, K. H., Hashimoto, S., Zorzato, F., Pozzan, T., Meldolesi, J. & Lew, D. P. (1988) *Proc. Natl. Acad. Sci. U.S.A.* **85**, 1091–1095
- Worley, P. F., Baraban, J. M., Supattapone, S., Wilson, V. S. & Snyder, S. H. (1987) *J. Biol. Chem.* **262**, 12132–12136

Received 11 July 1991/29 August 1991; accepted 4 September 1991