Application of a New Hydrophobic Carrier for Routine Isolation of Calmodulin and Other Proteins

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Recent investigations have unambiguously confirmed the key role of calmodulin (CaM) in the regulation of various metabolic and physiologic processes in living cells (Cheung 1981; Klee et al. 1980). During recent years, the interest in CaM function in homeostasis of living cells has increased.

So far, different methods of CaM purification have been used. The method based on the ability of CaM to bind to hydrophobic substances (i.e. phenothiazines), seems to be the most promising one. Affinity chromatography with immobilized phenothiazines has proved to be very efficient (Charbonneau and Cormier 1979; Rochette-Egly et al. 1982). Disadvantages of this procedure include temperature and light sensitivity of the affinity carriers, and toxicity of phenothiazines. These disadvantages have been abolished by using Phenyl-Sepharose Cl—4B (Pharmacia, Sweden) for the isolation (Gopalakrishna and Anderson 1982). The only disadvantage of this carrier is its relatively low flow rate as shown in the present paper; when Sepharose as a matrix of phenyl groups is replaced by microspherical cellulose, this disadvantage can be removed.

The new hydrophobic carrier is based on macroporous microspherical cellulose (Secheza, Lovosice), consisting of small beads with diameters of 90—315 μm. Crosslinking of this cellulose was done using epichlorohydrin, so that one transverse binding falls to 9—10 glucosic units (Gemeiner et al. 1980). Crosslinked spherical cellulose was hydrophobized by two step reaction. In the first step the cellulose was alkylated by epichlorohydrin (Petruš and Gemeiner 1984) and in the second step the *o*-(3-chloro-2-hydroxypropyl) cellulose was alkylated by natrium phenoxid. The product Phenyl-Cellulose was washed with ethanol, diluted with HCl in 50 % ethanol and with water.

Preisolation of CaM was done according to Guerini (Guerini et al. 1984). To a partly purified CaM sample, a small amount of ^{125}I-CaM (Jørgensen and Larsen 1980) was added. Using the batch technique, the optimal sample of

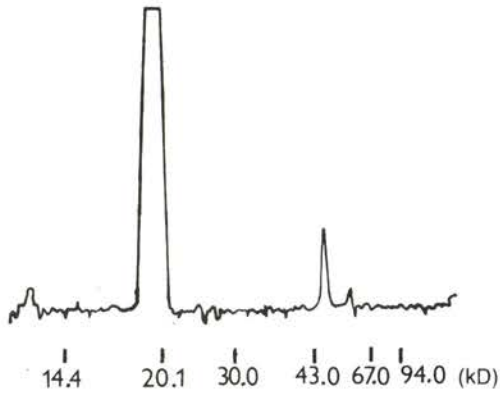

Fig. 2. Densitogram of purified CaM. Polypeptide fragments migrated with the front and second impurity had a Mw of 45 kD. The purity of isolated CaM was about 91%.

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