Original article

Calcium-dependent self-aggregation of toposome, a sea urchin embryo cell adhesion molecule

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Summary — Sea urchin embryos can be easily dissociated into single cells by exposure to Ca^{2+}- and Mg^{2+}-free seawater. When transferred back to normal seawater, isolated cells spontaneously form aggregates capable of development. Here, the Ca^{2+}-dependent self-aggregation of toposome, a 22S glycoprotein complex which mediates cell-cell adhesion in sea urchin embryos, has been investigated using the purified molecule. Results show that the 22S complex is completely converted to 15S particles by sedimentation on sucrose isokinetic gradients in the presence of EDTA. Reconstitution of the 22S complex is achieved by readdition of Ca^{2+}. We propose that the 15S particle constitutes the toposome functional unit on the cell surface.

Ca^{2+}-dependent CAM / sea urchin embryo / sucrose isokinetic gradient / glycoprotein complex

Introduction

Cell-cell adhesion is a fundamental phenomenon that is thought to control and stabilize precise interactions between cells, both in the adult and developing embryo. It has therefore become important to understand the molecular basis of differential cell adhesion and its influence on the developing embryo. A large number of cell adhesion molecules (CAMs) have been identified [3], to which more are certain to be added. In general, CAMs can be divided into two classes: those that require the presence of Ca^{2+} in order to mediate the adhesion between cells and those that do not [19]. The important role of Ca^{2+} in cell adhesion is exemplified by the fact that its withdrawal leads to dissociation of both tissues and embryos. In addition, it is generally accepted that the presence of the ion protects Ca^{2+}-dependent CAMs from enzymatic cleavage [19].

Although the presence of CAMs in vertebrate species is well established [10, 16] very little is known about their presence in invertebrate species. Among invertebrates, sea urchin embryos are uniquely suited for the study of cell-cell interactions during embryogenesis because they can be easily dissociated into single cells by removal of Ca^{2+} and Mg^{2+} from seawater [5, 7]. This treatment, in contrast with other methods that use proteolytic enzymes, does not remove CAMs from the cell surface. In fact, upon restoration of Ca^{2+} and Mg^{2+}, dissociated cells spontaneously aggregate and form embryoids able to develop into pluteus-like larvae [5]. The reversible cellular dissociation and aggregation observed in living embryos and cells respectively, can be explained assuming that Ca^{2+} mediates the interaction between adhesion molecules of adjacent cells.

We previously reported the isolation of a genus-specific 22S particle, which was extracted from cells dissociated from sea urchin embryos at the mesenchyme-blastula stage [14]. The particle is a glycoprotein complex that is involved in Ca^{2+}-dependent cell-cell adhesion. Cells lacking the complex are not able to aggregate, even in the presence of Ca^{2+} and Mg^{2+}. We named the glycoprotein complex toposome because of its putative role in position-dependent cell-cell interactions during embryogenesis [14]. More recently the role of toposome in mediating cell-substrate adhesion has been demonstrated *in vitro* using a gravitational binding assay [13].

Since toposome is extracted from dissociated cells in the presence of Ca^{2+} and Mg^{2+}, the 22S particle that we purified could result from the self-aggregation of two functional units.

In this report, we used purified 22S complex to study its Ca^{2+}-dependent self-aggregation, and therefore reproduce at the molecular level the reversible cellular dissociation and aggregation observed in living embryos and cells, respectively.

Materials and methods

Toposome purification from mesenchyme-blastula cells

Large-scale preparations of toposome were prepared from *Paracentrotus lividus* embryos according to Cervello and Matranga [2], with some minor changes. Briefly, a butanol extract was prepared by non-cytolytic treatment of dissociated mesenchyme-blastula cells with 10 volumes (w/v) of 2.5% *n*-butanol in Millipore-filtered seawater. Extract was dialyzed, concentrated and applied to a 80 × 2.5-cm S-400 Sephacryl column. The column was eluted with PBS containing Ca^{2+} and Mg^{2+}. To prevent partial proteolysis 0.5 mM phenylmethylsulphonyl fluoride was added to PBS. The 22S complex emerged as a sharp peak after two broader peaks. Purified material was dialyzed against distilled water, lyophilized by speed vacuum centrifugation and stored at $-20°C$.

Sucrose isokinetic gradients

We used the procedure described by Cervello and Matranga [2] with some minor changes. Briefly, 100 μg of lyophilized 22S

complex was dissolved in artificial seawater (ASW) or in ethylenediaminetetraacetic acid (EDTA) and loaded on top of 4.2 ml sucrose isokinetic gradients (top concentration = 5% w/w; [15]). Gradients, prepared with ASW pH 8.1 or with 0.2 mM EDTA pH 10, were centrifuged at 50000 rpm for 3 h at 15°C in a Beckman SW 56 rotor. The absorbance at 220 nm was monitored continuously and fractions of about 400 μl each, collected from the bottom, were dialyzed and lyophilized.

Electrophoretic analysis

Gradient fractions were analyzed by 6% SDS-PAGE [11] under reducing conditions. Protein standards (Pharmacia) were ferritin (220 kDa), phosphorylase *b* (94 kDa), and lactate dehydrogenase (36 kDa). Gels were stained with Coomassie blue.

Results

If we assume that the 22S complex is dissociated into halves by Ca^{2+} withdrawal, according to our theoretical calculations [14, 15], a sedimentation coefficient of 15S can be expected for each unit. In order to verify this hypothesis we initially attempted to dialyze toposomes against Ca^{2+}- and Mg^{2+}-free seawater (CMFSW) at pH 8.1, or EDTA (from 2 mM to 20 mM) at pH 8.1, for different periods of time (from 12 to 6 h), and at different temperatures (25 and 4°C). Preparations were then analyzed by sedimentation on sucrose isokinetic gradients prepared with CMFSW or with EDTA (from 2 mM to 20 mM). Results indicated that from 50 to 80% of toposome remained in the 22S aggregate conformation, with the corresponding appearance of a 15S peak (not shown). For this reason, we dissolved lyophilized molecules with increasing concentrations of EDTA solutions, whose pH was increased to 10 in order to optimize the chelating capacity of EDTA. Samples were then analyzed by sedimentation on sucrose isokinetic gradients, prepared with 0.2 mM EDTA, pH 10. In support of our hypothesis, we found that increasing the EDTA concentration led to an increase in the 15S peak, at expense of the 22S peak (fig 1).

We have already reported that reducing SDS-PAGE of the 22S complex showed that it consists of 6 polypeptides ranging from 60 to 160 kDa [14]. In order to determine if the 15S peak had the same polypeptide composition of the 22S complex, fractions from the gradient shown in figure 1b were analyzed by SDS-PAGE under reducing conditions. As expected, the two conformations of the molecule (15S and 22S) exhibited the same electrophoretic pattern (fig 2).

Moreover, as for dissociated cells, we were able to reverse at the molecular level the dissociation phenomenon by adding back the withdrawn ions. In fact, 15S particles completely aggregate giving rise to a single 22S peak in the presence of Ca^{2+} (fig 3c), whereas in the absence of Ca^{2+} they remained in the dissociated configuration of 15S (fig 3b).

Discussion

In this report we have shown that the 22S glycoprotein complex, named toposome, isolated from sea urchin embryonic cells, can undergo a Ca^{2+}-dependent self-aggregation and dissociation when analyzed onto sucrose isokinetic gradients in the absence or presence of chelating agents respectively. The fundamental unit of the molecule is therefore a 15S particle that is resolved in SDS-
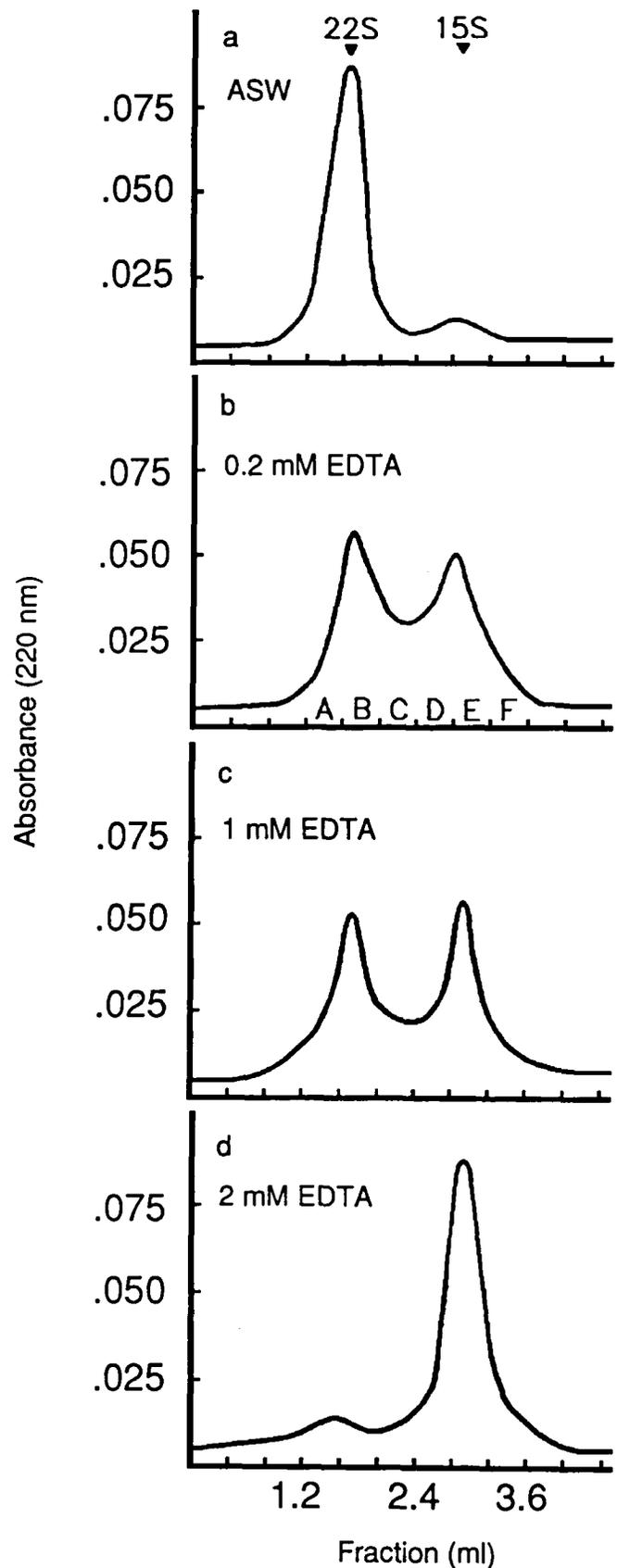
Fig 1. Dissociation of 22S complex by Ca^{2+} withdrawal. Sucrose isokinetic gradients were prepared with ASW pH 8.1 (**a**) or 0.2 mM EDTA pH 10 (**b** – **d**). Lyophilized toposome were dissolved as indicated.
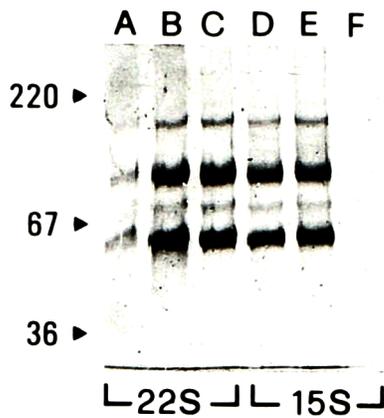
Fig 2. SDS-PAGE of 22S and 15S peaks. Fractions A – F from gradient b in figure 1 analyzed on a 6% gel under reducing conditions. Molecular weight markers are indicated in kDa.

PAGE in a number of polypeptides ranging from 60 to 160 kDa.

It has been previously reported that the 22S complex purified from eggs is constituted of six polypeptides, having a molecular mass of 180 kDa for the *Paracentrotus lividus* species [2], that are proteolytically cleaved during development. At the blastula stage, when the processing is complete, the molecule does not change its sedimentation value because the lower molecular weight polypeptides, generated by limited proteolysis, remain assembled in the complex [2, 9, 14, 22].

Similarly, we propose that the 15S monomeric unit, having a molecular mass of about 500 kDa, obtained from blastula toposome, consists of three 160-kDa polypeptides that are held together because of stabilization by non-covalent bonds and intrachain S-S links.

Results reported here demonstrate that the 15S particle forms dimers in the presence of Ca^{2+} ions, and this dissociation-aggregation transition correlates with the experimental observations obtained with living embryos and cells [5]. Taking into consideration the present results and previous reports, we propose that neighbouring cells are held together by the Ca^{2+}-dependent interaction of two 15S particles that constitute the functional unit of toposome on the cell surface.

The fact that severe conditions are required in order to dissociate purified toposome might be explained by conceiving that once the 22S dimer is formed, during the isolation procedure, the complex is further stabilized by interactions involving other regions of the molecule. This is not surprising since, for example, it has been reported that the homophilic binding of the *Dictyostelium discoideum* gp80 cell adhesion molecule is further stabilized by hydrophobic interactions, after the initial ionic binding in *in vitro* experiments [8]. The recent discovery of an E-cadherin-like molecule in sea urchin embryos [4], indicates that more than one Ca^{2+}-dependent adhesion system exists in the sea urchin embryo. We do not have any evidence to say whether toposome belongs to the cadherin family or not. Nevertheless, since Ca^{2+} is required to maintain toposome in a trypsin-resistant conformation [14], at least a similar behaviour of the molecule can be assumed.

At present, we cannot say how toposome is anchored
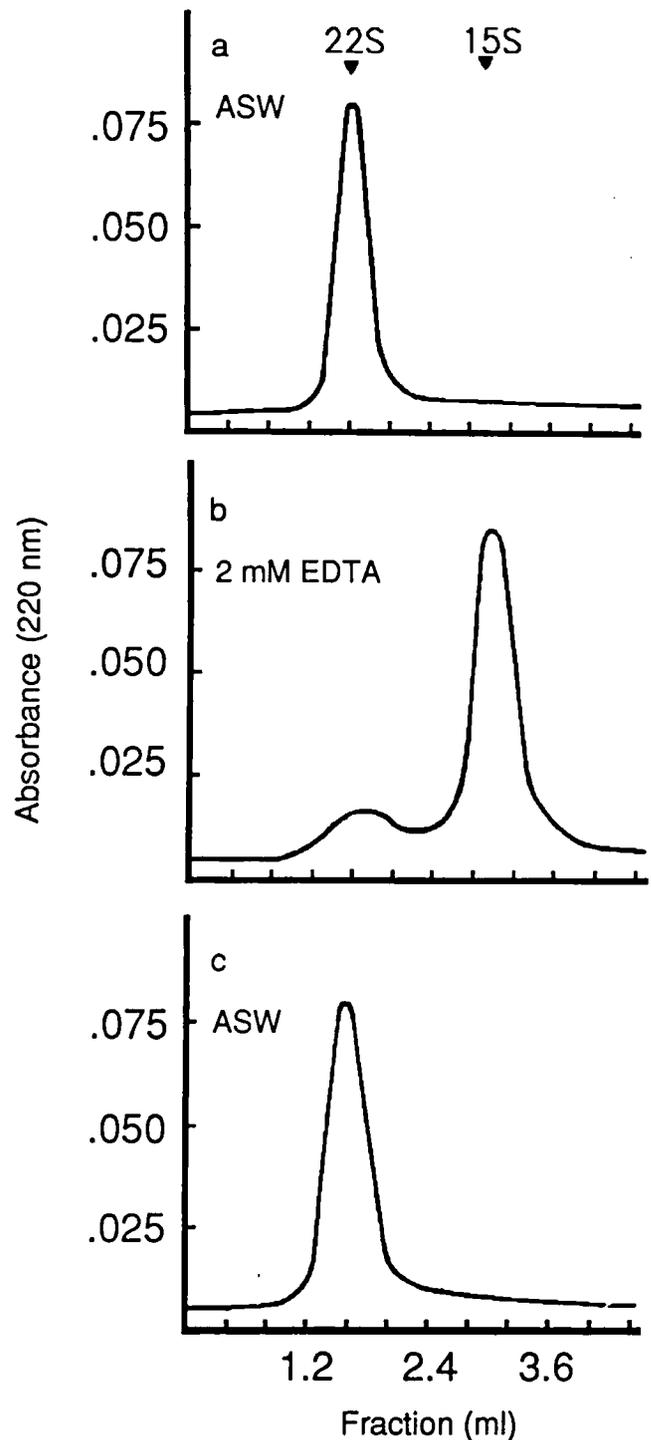
Fig 3. Calcium-induced aggregation of 15S particles. Sucrose isokinetic gradients were prepared with ASW pH 8.1 (a,c) or 0.2 mM EDTA pH 10 (b). 22S complex dissolved in ASW (a). 15S peaks dissolved as indicated (b,c).

to the cell surface, but different hypotheses can be made. These include: a) the presence of a specific receptor, as reported for the adhesion factor in the sponge system [20]; b) a phosphatidylinositol anchorage, as for the neural cell recognition molecule F11 [21] and for NCAM-120 [6]; and c) an insertion of toposome into the lipid bilayer, as for most CAMs [10, 16].

The presence of oligomeric complexes, involved in cell-cell and cell-substrate adhesion, is not restricted to the sea urchin embryo. Among vertebrates, particles of a sedimentation coefficient of 16S and 12S, named adherons, have been isolated from the media of myoblast and embryonic neural retina cell cultures, respectively [17, 18]. With striking similarity to the sea urchin 15S particle, also in this system calcium induces aggregation of 16S particles from myoblastic cells [17].

The similarity in the structure and function among glycoprotein complexes found in organisms so spread in the evolutionary scale would suggest that the association of polypeptides in complexes could have an important significance to be conserved during evolution.

In principe it is possible that within the complex each polypeptide, or their different combination, could allow for a limited number of biological functions. For example, the cell-cell or the cell-substrate adhesion specificity, the stage-, tissue- or species-specificity, or the transduction of signals from the extracellular compartment to the cytoplasm. The fact that all these functions exist in a single structure could be of advantage for the cell since different functions could be modulated at the same time in response to a single event. Efforts should be made in order to clarify the role of each polypeptide in the complex and of the overall structure. In fact, as reported for the myoblast adheron [17] and for integrins [1], a class of extracellular matrix molecules receptors constituted of two subunits, the oligomeric integrity of complexes is necessary in order to maintain their biological activities.

The availability of a simple model for studies on cell adhesion, as the sea urchin embryo system, gives us the opportunity to contribute in the understanding of the matter.

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