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AGGREGATION OF SPONGE CELLS

XX. SELF-AGGREGATION OF THE CIRCULAR PROTEID PARTICLE

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Summary

In the extracellular space of the tissue of the sponge *Geodia cydonium*, circular proteid particles are found which carry as subunits the aggregation factor and a series of glycosyltransferases.

Using the technique of velocity sucrose gradient centrifugation, the sedimentation coefficient ($s^0_{20,w}$) of the particle-monosomes was determined to be 90. By means of the Svedberg equation a molecular weight of $1.3 \cdot 10^8$ daltons could be estimated. The monosomes aggregate in the presence of Ca^{2+} to higher complexes via disomes, trisomes, and pentasomes. The complexes can be redissociated by dodecyl sulfate but not by EDTA. During the Ca^{2+}-mediated self-aggregation, the particles lose their biological activity with respect to their aggregation promoting function.

Introduction

Moscona [1] isolated an "extra-cellularly functioning material" from sponges which is found to promote attachment and organization of cells. This "material" which was termed the aggregation factor was purified and characterized from the sponge *Geodia cydonium* [2,3] and from the sponge *Microciona parthena* [4]. The aggregation factor from *Geodia* is associated with large, spheroidal particles (circular proteid particles) which contain a filamentous circular core structure [2,5]. The particles consist of 74% protein, 5% lipid and 10% carbohydrate [6]; they act multifunctionally. The first subunit identi-

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fied was the aggregation factor [2,3], later a series of glycosyltransferases was identified as subunits of the *Geodia* particle [7,8].

The circular proteid particles do not only provoke cell-cell aggregation via membrane-bound aggregation receptors [9] but they also clump together in the presence of Ca^{2+} and Mg^{2+} even if cells are absent. This latter characteristic is described in the present study in some detail with the main focus on the alteration both of the sedimentation coefficient and of the biological activity of the particles during Ca^{2+}- and Mg^{2+}-mediated self-aggregation.

Materials and Methods

Source of the materials: Sepharose 4B and Ficoll from Deutsche Pharmacia, Freiburg (F.R.G.); CM-cellulose from Serva, Heidelberg (F.R.G.).

Animals. The siliceous sponge *Geodia cydonium* Jam. (Tetractinellida) was collected in the vicinity of Rovinj (Yugoslavia).

Solutions. The calcium- and magnesium-free artificial sea water and calcium- and magnesium-containing artificial sea water were composed as described [7].

Isolation of the aggregation factor. The purification procedure of the aggregation factor from *Geodia cydonium* was performed as described [3]. The purified fraction contained 50 μg protein/ml and had a specific activity of $6.1 \cdot 10^7$ aggregation units/mg protein. The aggregation activity is expressed in aggregation units as described [2]. The purified aggregation factor was dissolved in calcium- and magnesium-free artificial sea water.

Isolation of archaeocytes. The sponge tissue was dissociated into separate cells as described [2]. For isolating the archaeocytes from the cell suspension which contained different cell types, the discontinuous gradient method with Ficoll was applied [10,11].

Aggregation assay. In the standard assay, 3 ml of a suspension with $25 \pm 5 \cdot 10^6$ single archaeocytes/ml calcium- and magnesium-free artificial sea water was placed in 20-ml glass tubes and rollered at 35 rev./min at 20° C as described [2]. Some assays were supplemented with 10 mM $CaCl_2$ and 50 mM $MgCl_2$ (corresponding to the concentrations present in calcium- and magnesium-containing artificial sea water) and 10 μg of particles (based on protein content). The diameter of the aggregates was determined as described [7].

Polyribosomes. Polyribosomes were isolated from fresh oviduct homogenates [12,13].

Sucrose density gradients. Particles as well as polyribosomes were isolated on sucrose gradients (0.5—1.5 M) [12,13]. Centrifugation conditions were as described [12]. For separation of polyribosomes, the sucrose was dissolved in the described medium [12]. In the case of the analysis of the particles, the sucrose gradients were prepared with calcium- and magnesium-free artificial sea water containing 0, 0.3 or 1 mM of Ca^{2+}. The number of polyribosomal units in a polyribosomal fraction was determined by electron microscopy as described earlier [14].

For protein determination the method of Lowry et al. [15] was used. The sedimentation constant ($s^0_{20,w}$) was determined by isokinetic sucrose gradients as described [16].

Results and Discussion

Sedimentation of the circular proteid particle-monosomes

In the absence of Ca^{2+}, the particles sediment in an isokinetic sucrose density gradient as one homogeneous band (Fig. 1). Electronmicrographs were taken from each fraction and more than 85% of the total number of the particles were detected in fractions 5—9. 95% of the particles were found to be in the native state [5]. This finding is a direct proof that the observed homogeneous band contains the particles. Calculations of the sedimentation coefficient ($s^0_{20,w}$) of the particles, monoribosomes, disomes, and trisomes up to heptasomes were used as internal markers. The $s^0_{20,w}$ values of these macromolecules are precisely known [16]. From the known $s^0_{20,w}$ of the internal markers used as a reference, the sedimentation coefficient of the particle band was calculated and found to be 90 ± 3.

In a previous study [17] the following properties of the particles were determined: intrinsic viscosity $[\eta]_{20} = 80 \pm 40$ ml/g, translational diffusion coefficient $D_{20} = 0.7 \pm 0.1 \cdot 10^{-8}$ cm²/s and the partial specific volume $\bar{v} = 0.76$ ml/g. From these data and the $s^0_{20,w}$ of the particles, molecular weights can be calculated by means of the Svedberg equation [18]. Using this method a value of $1.31 \pm 0.04 \cdot 10^8$ daltons is determined.

Due to the fact that both the particle preparation used is homogeneous [17] and that the particles appear as spheroidal structures (as visualized electron microscopically [5]) it is now possible to estimate from the molecular weight of the particles and their buoyant density (1.31 g/cm³) [3] the weight as well as the volume of one particle. Such calculations revealed the following values: $2.17 \cdot 10^{-16}$ g and $1.65 \cdot 10^{-4}$ μm^3, respectively. The spheroidal particles con-

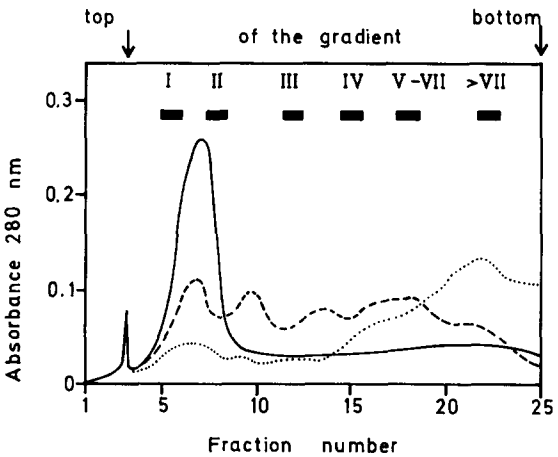

Fig. 1. Effect of calcium ion concentration on the sedimentation of circular proteid particles in the sucrose gradient. 1 ml of the particle preparation (dissolved in calcium- and magnesium-free artificial sea water and containing 1.7 mg protein) was supplemented with 0, 0.3, and 1 mM Ca^{2+} and incubated for 30 min at 20°C; then the preparations were layered onto sucrose gradients containing calcium- and magnesium-free artificial sea water and the corresponding calcium ion concentration. Pattern of the particle preparation treated with 0 (———); 0.3 (— — —); and 1 mM Ca (· · · · · ·). Polysomes were extracted as described under Methods; the profile of the extract was determined in a separate run. The bars with the Roman numerals indicate the number of ribosomal units in the respective polyribosomal fraction.

sist of a core structure with a central circle of a diameter of 1125 Å [5]. Assuming that this central circle describes the outer boundary of the major axis of a spheroid the minor axis of this figure is calculated to be 248 Å. Thus the radius of the central axis of the spheroid is 563 Å and that of the minor axis is 124 Å.

Aggregation of the particles in the presence of Ca^{2+}

The particle-monosomes aggregate in the presence of Ca^{2+} to larger entities. After incubation of the monosomes with 0.3 mM Ca^{2+} the size of the 90 S peak is considerably reduced, while four additional peaks with higher sedimentation coefficients (130, 160, 220, >260) are observed (Fig. 1). Under incubation conditions with 1 mM Ca^{2+} the bulk of the particles are found at an $s^0_{20,w}$ of higher than 260.

Mg^{2+} (up to 50 mM) does not cause self-aggregation of monosomes to larger entities. This divalent cation exerts also no enhancing effect on the Ca^{2+}-mediated aggregation of monosomes. After aggregation of the particles in the presence of Ca^{2+}, the large complex cannot be dissociated by EDTA. For example, if particle-aggregates, which are formed by incubation with 1 mM Ca^{2+} are treated (2 h at 15° C) with 20 mM EDTA and subsequently analyzed by sucrose density gradient centrifugation an almost identical sedimentation pattern is observed similar to that obtained after analysis of particle-aggregates not treated with EDTA (data not shown).

The particle-aggregates formed in the presence of Ca^{2+} can be dissociated back into monosomes by incubation in 3% (w/v) sodium dodecyl sulfate. The analysis of the sodium dodecyl sulfate-treated monosomes by sucrose density gradient centrifugation revealed a sedimentation coefficient of 25 S. This means that after treatment of the particle-aggregates with this detergent, the particle-complex is dissociated into monosomes characterized by a lower $s^0_{20,w}$ value. To prove the assumption whether the native spheroidal particles are disintegrated during the sodium dodecyl sulfate treatment, the particles were examined electron microscopically. This study revealed that almost 100% of the particles were converted from their native form to the core structure during the treatment with the detergent. The morphology of the core structure obtained was found to be identical with the one described earlier [17,5]; it consists of a central circle and 25 radially-arranged filaments.

Biological activity of particle-monosomes and particle-aggregates

The data mentioned concerning the physico-chemical properties of the particles revealed, that these particles are provided with the potency to aggregate with each other to larger entities. In a series of studies the biological activity of the monosomes was compared with the one of the particle-aggregates in the cell-aggregation assay. Single archaeocytes have a diameter of about 8 μm, in the absence of Ca^{2+} and Mg^{2+} only small cell clumps of a diameter of 30 μm are formed; addition of 10 mM Ca^{2+} and 50 mM Mg^{2+} causes formation of somewhat larger aggregates of 80 μm. Addition of a particle-preparation (10 μg protein/3 ml) (carrying the aggregation promoting activity; see refs. 2 and 3) results in a formation of large cell aggregates with an average diameter of 2300 μm. In case of a preincubation of the same amount of a particle preparation

with 10 mM Ca^{2+} and 50 mM Mg^{2+} and a subsequent incubation of the Ca^{2+}- and Mg^{2+}-pretreated preparation with single cells, the size of the aggregates formed was determined to be 280 μm. This means that after preincubation of the particle-preparation with Ca^{2+} and Mg^{2+} its aggregation promoting activity is reduced by 88%.

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