The occurrence of different sulphated cell surface glycoproteins correlates with defined developmental events in *Volvox*

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1. INTRODUCTION

The green alga *Volvox* has an unusual combination of attributes that make it unique for studying the biochemistry of embryonic development and cellular differentiation. The asexual organism consists of only 2 cell types: somatic and reproductive. About 2000–4000 somatic cells are located as a single layer on the surface of a hollow sphere (spheroid); 8–16 reproductive cells (gonidia) are positioned within the spheroid in the posterior region.

A new daughter spheroid is formed from each reproductive cell in a series of cleavages. During cleavage an asymmetric division (at the stage of the 32 cell embryo) delineates 16 reproductive cells (which cease division at that time) from the somatic cell initials which continue cleavage. At the termination of cell divisions, the embryo consists of thousands of somatic cell initials and 16 larger reproductive cells [1–3]. At this time the embryo enters the process of inversion, thereby turning the embryo inside-out. This inversion process bears striking resemblances to morphogenetic events such as gastrulation in vertebrates [4]. After inversion, the somatic cells begin to secrete sheath material causing each cell to move apart from its neighbours. The organism now grows in size but not cell number. When the daughter spheroids are about a quarter their final size they are released from the parent through large pores formed by enzymatic disruption of the parent sheath material.

The value of *Volvox* as a model system is enhanced by the fact that defined substances (sexual hormones) switch the developmental program from vegetative to sexual reproduction: Sperm- or egg-containing spheroids are produced during embryogenesis in the presence of the sexual inducer [5,6]. In addition, organism growth is highly synchronized enabling pulse-labeling experiments at defined developmental stages.

Here, we describe 5 different sulphated cell surface glycoproteins all of which are produced at exactly defined developmental stages. This correlation makes likely a functional involvement of these glycoproteins in the corresponding developmental event.

Abbreviations: M-SG, male-specific sulphated glycoprotein; F-SG, female-specific sulphated glycoprotein; I-SG, inversion stage-specific sulphated glycoprotein; SSG 185, sulphated surface glycoprotein, 185 000 M_r; SSG 140, sulphated surface glycoprotein, 140 000 M_r

2. MATERIALS AND METHODS

2.1. *Growth of* Volvx carteri

Volvox carteri f. *nagariensis* female strain HK 10 and male strain 69-1b from the Culture Collection of Algae of the University of Texas at Austin (R.C. Starr) were a gift from L. Jaenicke (Cologne). The organism was grown in *Volvox* medium [7] as in [6]. Illumination at 10 000 lux on a 16 h light/8 h dark cycle resulted in a synchronously growing culture.

2.2. *Pulse-labeling experiments*

Pulse-labeling with $^{35}SO_4^{2-}$ was performed as in [8]. ^{14}C-Labeled sulphated glycoproteins were

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prepared essentially by the same procedure: *Volvox* spheroids at the developmental stage in question were incubated in the presence of 100 μCi/ml [^{14}C]bicarbonate (in *Volvox* medium without glycerophosphate) for 30 min. The crude membrane fraction [8] was solubilized in Triton X-100 (1–3%) and directly or after subtilisin treatment (100 μg subtilisin/ml, 30 min, 30°C) was applied to a QAE–Sephadex colum (1 ml bed vol.). After extensive washing with 1 M NaCl, 0.5% Triton X-100, sulphated glycoconjugates were eluted with 2 M or 3 M NaCl. Final purification was achieved by SDS–polyacrylamide gel electrophoresis and elution from the gel.

2.3. *Carbohydrate analysis*

^{14}C-Labeled neutral sugars were identified as their alditol acetates by radio gas chromatography as in [8].

2.4. *Analytical criteria for sulphated cell surface glycoproteins*

The following assays were used:

(1) Covalently-linked sulphate was removed by acid hydrolysis (2 N HCl, 30 min, 80°C) and identified by polyethylene imine cellulose thin-layer chromatography.

(2) A change of the app. M_r after protease digestion as judged by SDS–polyacrylamide gel electrophoresis indicated the presence of a polypeptide.

(3) The presence of a saccharide structure in the protease-resistant core material was shown by radio gas chromatography of the corresponding [^{14}C]alditol acetates after hydrolysis (2 N HCl, 2 h, 100°C).

A sulphated glycoprotein was classified as a cell-surface component if its protease-resistant core material could be detected in the medium after short subtilisin treatment of *Volvox* spheroids (300 μg/ml, 15 min, 30°C). Under such conditions after washing with protease-free medium the viability of the cells remained unaffected.

3. RESULTS AND DISCUSSION

Recently, we presented evidence for the involvement of sulphated cell surface glycoproteins in the control of cellular differentiation during embryogenesis of *Volvox*. In particular, attention was directed towards a sulphated cell surface glycoprotein with app. M_r 185 000 (denoted as SSG 185): Production of this component was shown to be perfectly correlated with the developmental program [8]. $^{35}SO_4^{2-}$ Pulse-labeling experiments performed over the whole life cycle of both vegetative and sexually induced organisms revealed the developmentally controlled production of at least 4 additional sulphated glycoproteins. Fig.1 gives a brief survey of these glycoproteins: $^{35}SO_4^{2-}$ pulse-labeling experiments at 4 selected developmental stages each produces a defined set of sulphated glycoproteins, which will be described below. The individual substances will be denoted in the following as M-SG (male-specific sulphated glycoprotein), F-SG (female-specific sulphated glycoprotein), I-SG (inversion stage-specific sulphated glycoprotein), SSG 185 (sulphated surface glycoprotein, app. M_r 185 000) and SSG 140 (sulphated surface glycoprotein, app. M_r 140 000).

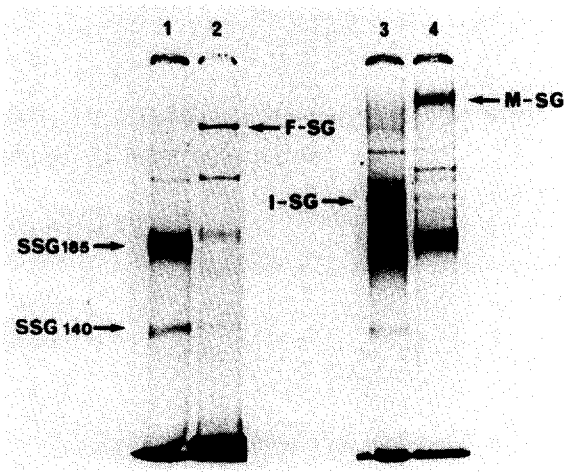

Fig.1. Fluorograms of SDS–polyacrylamide gels (5%) loaded with membrane fractions from *Volvox* colonies after 30 min $^{35}SO_4^{2-}$ pulse-labeling at defined developmental stages: (1) vegetatively growing female strain (HK 10) during early embryogenesis; (2) female strain (HK 10) at the stage of release, 60 min after the addition of the sexual hormone; (3) vegetatively-growing organisms (HK 10) at the stage of embryonic inversion; (4) sexually-induced males (69-1b) containing sperm packets.

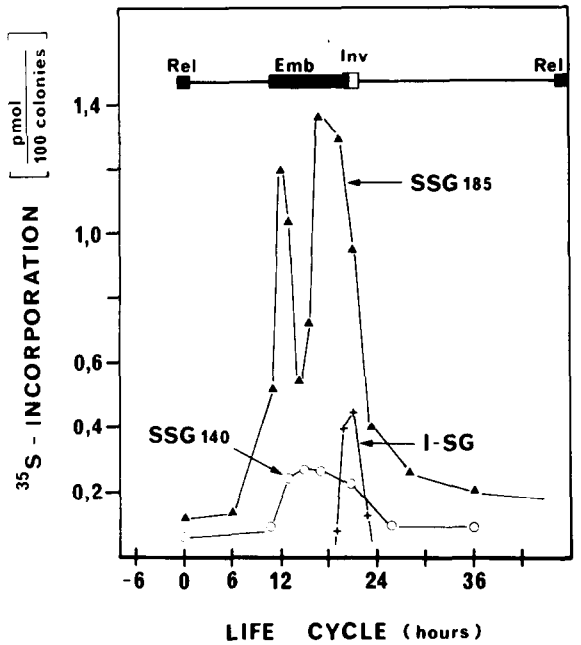

Fig.2. Developmentally-controlled synthesis of sulphated glycoproteins: $^{35}SO_4^{2-}$ pulse-labeling (30 min) of vegetatively-growing *Volvox* HK 10 at different times over the whole period of its life cycle. The respective membrane fractions were applied to SDS–polyacrylamide (5%) gels. After fluorography [10] the incorporated radioactivity was determined by liquid scintillation counting of the excized material. The insert shows the developmental program: Rel, release of daughter spheroids; Emb, embryogenesis; Inv, inversion of embryos

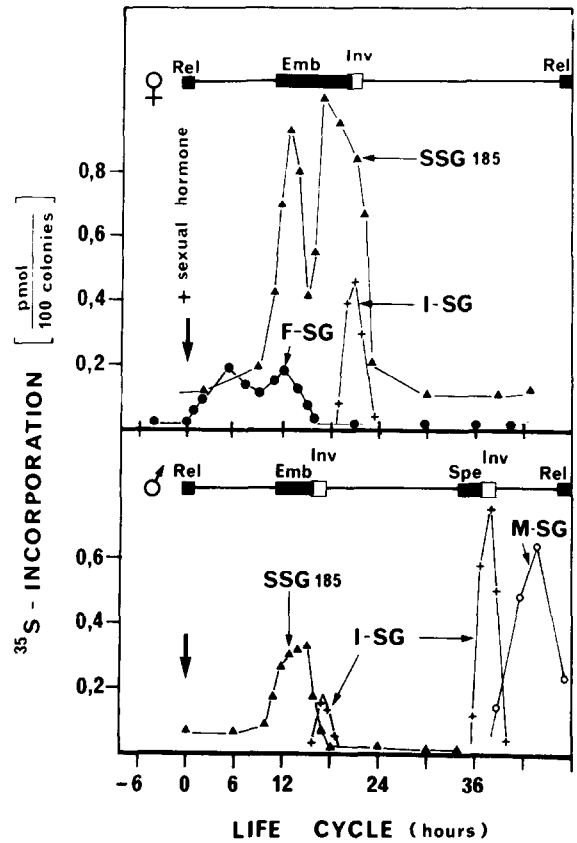

Fig.3. Developmentally-controlled synthesis of sulphated glycoproteins: $^{35}SO_4^{2-}$ pulse-labeling (30 min) of sexually-induced female (upper panel) and male (lower panel) *Volvox* colonies at different times over the whole period of their life cycles. For details see legend to fig.2. The inserts show the developmental programs of the sexually-induced organisms: Rel, release of daughter spheroids; Emb, embryogenesis; Inv, inversion of embryos and sperm packets, respectively; Spe, spermatogenesis

3.1. *Developmentally controlled sulphated glycoproteins in the asexual life cycle*

3.1.1. SSG 185

In the experiment of fig.2 net ^{35}S-incorporation into sulphated glycoproteins was quantitatively measured as a function of the developmental stage: ^{35}S-incorporation into SSG 185 steeply increases at the initiation of embryogenesis, reaches a maximum level during the early cleavage stages (2 and 4 cell embryos) and drops to a minimum immediately before the differentiating cell division (32–64 cell embryo). Thereafter a high level of incorporation is restored until the end of cell cleavage. At all other developmental stages incorporation remains at a low level.

3.1.2. SSG 140

By the criteria given in section 2 this molecule is a sulphated cell-surface glycoprotein with saccharides being composed of the neutral sugars arabinose, galactose and glucose (~2:1:0.3). SSG 140, as shown in fig.2 is produced during the whole period of embryogenesis at a fairly constant rate. Before and after embryonic cleavages synthesis of SSG 140 is found to be depressed.

3.1.3. I-SG

The $^{35}SO_4^{2-}$ pulse-labeling experiments revealed the appearance of an additional sulphated component (estimated app. M_r 200 000) exactly at the time of embryonic inversion. Production of this I-SG is strictly limited to this short period of the life cycle (fig.2). Embryonic inversion in an individual embryo is completed within ~30 min; however, even in optimally synchronized populations invesion of all embryos takes ~90 min. From this slight asynchrony it follows, that synthesis of I-SG is even more limited in time than indicated by the experimental data in fig.2.

By the criteria summarized in section 2, I-SG is a sulphated glycoprotein with saccharides being composed of the neutral sugars arabinose, galactose and xylose (~2:1:0.2).

3.1.4. M-SG and F-SG

The appearance of both M-SG and F-SG is restricted to the sexual life cycle. During vegetative growth these glycoproteins are either not detectable (M-SG) or, at best, in trace amounts (F-SG).

3.2. *Developmentally controlled sulphated glycoproteins in the sexually-induced life cycle*

3.2.1. SSG 185

Production of SSG 185 is also observed both in sexually-induced male and female strains (fig.3) and again correlates with the developmental program [8].

3.2.2. SSG 140

However, detectable amounts of SSG 140 are only produced in vegetatively growing *Volvox* populations. In sexually-induced male as well as female strains, production of SSG 140 is depressed.

3.2.3. I-SG

Production of I-SG is also observed in sexually-induced male and female strains (fig.3) exactly at the developmental stage of embryonic inversion.

In contrast to the development of asexual *Volvox* colonies, the developmental program of sexually-induced male embryos includes an additional morphogenetic process: In the first inversion process the male embryo turns inside out. Afterwards each reproductive cell (androgonidia) undergoes a series of cleavages to form a packet of sperm cells.

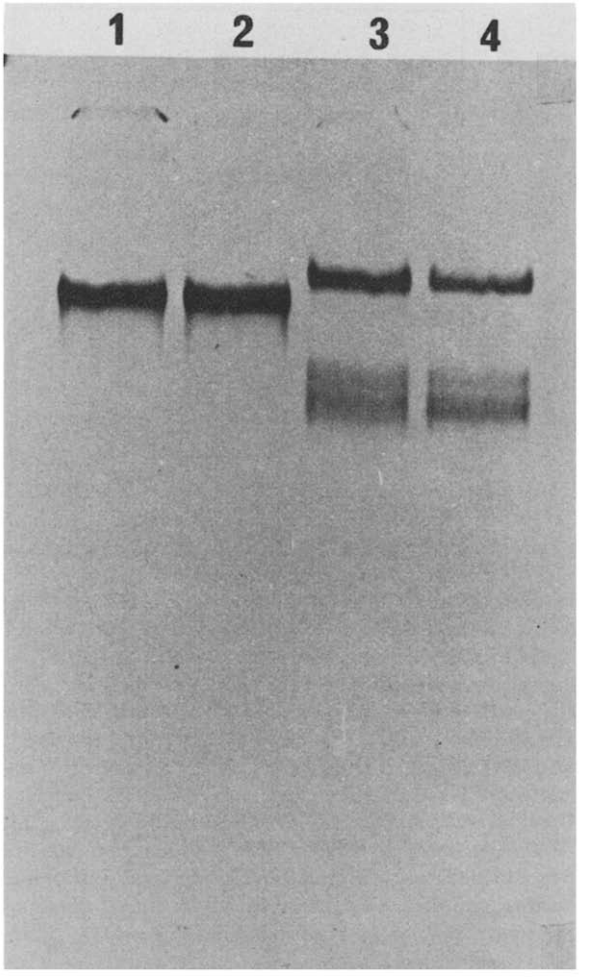

Fig.4. Identity of the I-SGs isolated from sexually induced males after $^{35}SO_4^{2-}$ pulse-labeling at the first (lane 1) and second (lane 2) inversion stage. Lanes 3 and 4 show the samples after proteinase K treatment according to [11]. Patterns were obtained on SDS–polyacrylamide (6%) gels and visualized by fluorography.

This bowl-shaped sperm cell mass again performs a morphogenetic process (second inversion) to form a sperm bundle which is convex on its anterior side. A functional role of I-SG in the inversion process would become highly probable if its synthesis is re-initiated at the time of this second inversion. The $^{35}SO_4^{2-}$ pulse-labeling experiments over the whole life cycle of sexually-induced male colonies clearly show, that I-SG is indeed synthesized during both the first and second inversion period (fig.3). Identity

of the I-SGs synthesized during the first and second inversion was proven by their protease cleavage patterns on SDS–polyacrylamide gels (fig.4).

3.2.4. F-SG

Synthesis of this high-M_r (app. M_r ~280 000) in sexually-induced female strains. By the criteria summarized in section 2 this molecule is a sulphated cell surface glycoprotein: Arabinose, xylose, mannose and galactose (~4.5:0.2:0.2:1.0) are the predominant neutral sugars as analyzed by radio gas chromatography. Interestingly, synthesis of this glycoprotein is initiated within 20–30 min after the addition of the sexual hormone inducer (fig.3). This is the first early biochemical event detected to be involved in the sexual induction process. F-SG is continued to be synthesized until the early stages of embryogenesis. Immediately before the differentiating cell division (64–124 cell embryo) production of F-SG sharply drops to a very low level. These observations suggest a functional role of F-SG in the process of egg cell differentiation.

3.2.5. M-SG

Production of another high-M_r glycoprotein (app. M_r ~320 000) is under strict developmental control: M-SG is only produced in fully developed sperm packets for a limited period after the second inversion. By the criteria given in section 2 this component again is a sulphated cell surface glycoprotein with glycoconjugate(s) being composed of the following neutral sugars: arabinose, xylose, mannose, galactose and glucose (~10:1:1:6:2).

Since M-SG is synthesized only in fully differentiated sperm cells, a role of this cell surface component in the sperm–egg recognition mechanism seems likely. Sea urchin eggs contain a fucose sulphate polysaccaride in the extracellular matrix shown to be responsible for inducing the acrosome reaction [9]

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