The EMBO Journal vol.3 no.4 pp.739–744, 1984

Development-dependent modification of the extracellular matrix by a sulphated glycoprotein in *Volvox carteri*

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Communicated by D. Oesterhelt

We report the chemical characterization of the highly sulphated glycoprotein SSG 185 from *Volvox carteri*. SSG 185 is a hydroxyproline-containing, extracellular glycoprotein. The sulphate residues are clustered within the parent saccharide structure of SSG 185, since on mercaptolysis all the sulphate residues are recovered in a small saccharide fragment containing mannose, arabinose and sulphate (in a molar ratio of 1:1:2). SSG 185 is a short-lived molecule, serving as a precursor for a high mol. wt. component of the extracellular matrix. Synthesis of SSG 185 is developmentally controlled. Different SSG 185 variants, with unknown modifications in the sulphated saccharide fragment, are synthesized at different developmental stages or under the influence of the sexual inducer. These modifications remain conserved in the aggregated state of SSG 185, indicating the development-dependent modification of the extracellular matrix.
Key words: extracellular matrix/sulphated glycoproteins/sexual inducer/*Volvox*

Introduction

The multicellular green flagellate *Volvox carteri* presents a feasible model for studying the control of embryonic development for the following reasons (Powers, 1908; Barth, 1964; Kochert, 1968; Starr, 1969, 1971; Huskey and Griffin, 1979). The asexual organism consists of only two cell types: somatic and reproductive. About 2000–4000 somatic cells are located as a single layer on the surface of a hollow sphere (spheroid); 16 reproductive cells (gonidia) are positioned within the spheroid in the posterior region. Figure 1 gives a diagrammatic scheme of the reproductive life cycle of *V. carteri*, which can be well synchronized to take exactly 48 h. During the first 12 h (phase I) of the life cycle, enlargement of the gonidia is the main developmental event. During phase II, 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. At this time the embryo enters the process of inversion, thereby turning the embryo inside-out. 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 developmental program of *Volvox* can be triggered at will to sexual reproduction by adding the sexual inducer (a glycoprotein, Starr and Jaenicke, 1974; Ely and Darden, 1972) to *Volvox* populations. This glycoprotein switches the developmental program from asexual to sexual reproduction: sperm- or egg-containing spheroids are produced during embryogenesis.

Recently, we described five different sulphated cell surface glycoproteins, all of which are synthesized at the time at which defined developmental events occur (Wenzl and Sumper, 1982). One of these glycoproteins, SSG 185, has a number of remarkable properties. Firstly, sulphate incorporation into this glycoprotein is stimulated nearly 10-fold on the initiation of embryogenesis. Secondly, the rate of sulphate incorporation during embryonic cleavages reflects the developmental program, in that maximum incorporation rates are observed during early and late embryonic cleavages, while a minimum level of incorporation marks the time at or immediately before the differentiating cell cleavage. Thirdly, the apparent mol. wt. of SSG 185 shifts towards lower values during early embryogenesis, approaching a constant value immediately before the differentiating cell cleavage. Finally, SSG 185 was shown to be a short-lived glycoprotein with a half life of ~30 min.

Here we show that SSG 185 serves as a precursor molecule for a polymeric extracellular matrix component. In addition, we show that the chemical modifications introduced into SSG 185 during development remain conserved in the polymeric

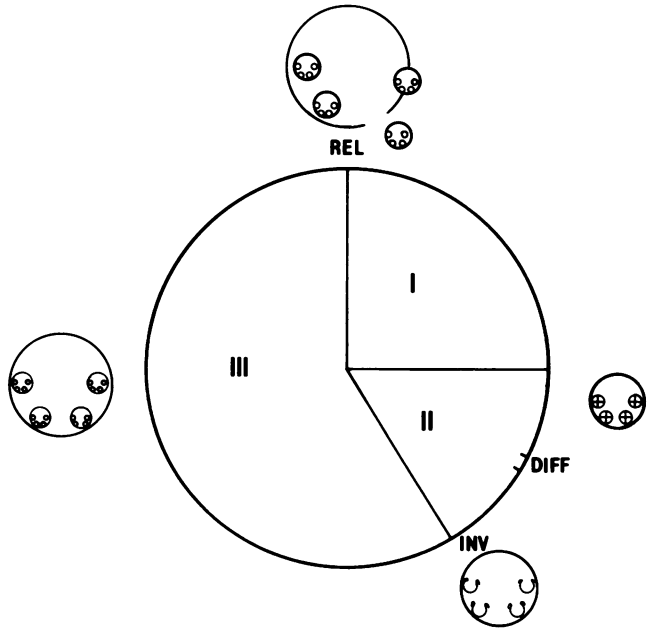

Fig. 1. Diagrammatic scheme of the life cycle of *V. carteri*. It takes exactly 48 h to complete one generation. REL: release of daughter spheroids; DIFF: differentiating cell cleavage during embryogenesis; INV: inversion of embryos.

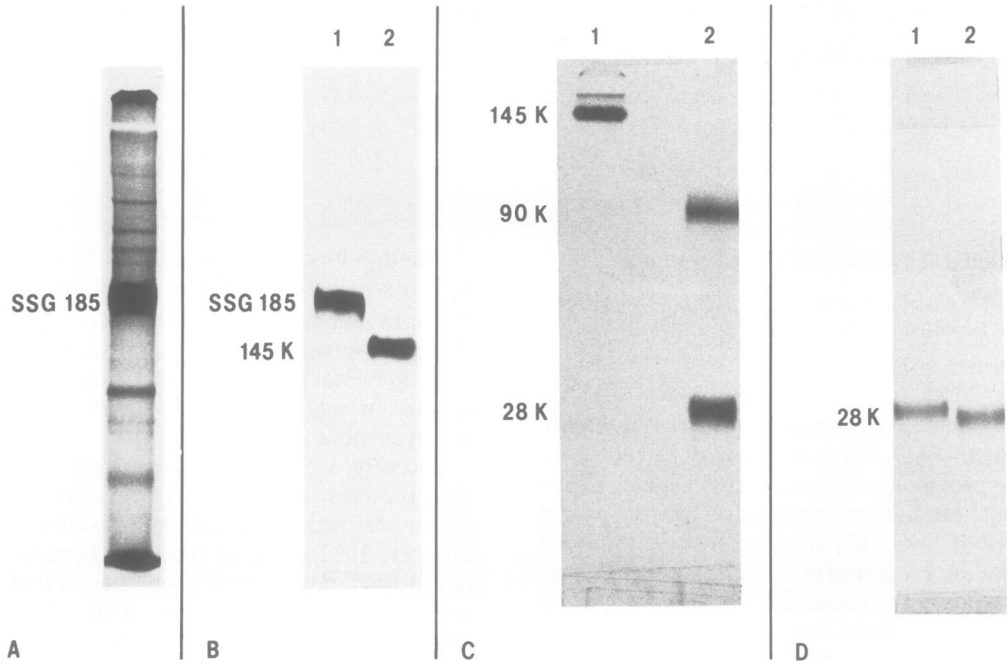

Fig. 2. Sequential degradation of SSG 185. **(A)** A crude membrane fraction of *Volvox* spheroids $^{35}SO_4^{2-}$ pulse-labelled at the time of gonidial cleavages was applied to 5% SDS polyacrylamide gel. **(B) 1**: SSG 185 purified by elution from SDS gels. **2**: As **1**, after digestion with subtilisin (50 μg subtilisin/ml, 30 min, 30°C). **(C)** ^{14}C, ^{35}S-labelled 145-K glycopeptide before **(1)** and after **(2)** mercaptolysis. Analysis was performed on a 12% SDS-polyacrylamide gel. **(D)** Mercaptolytic cleavage of [^{35}S]145-K glycopeptide pulse-labelled at the beginning **(1)** or at the end **(2)** of embryogenesis. (12% SDS polyacrylamide gel.)

product, indicating a chemical modification of the extracellular matrix during development.

Results

Chemical characterization of SSG 185

Pulse-labelling experiments with $^{35}SO_4^{2-}$ at different developmental stages revealed extensive synthesis of a sulphated glycoprotein (SSG 185) at the time of gonidial cleavage (Wenzl and Sumper, 1981). At this developmental stage ^{35}S-label is mainly incorporated into this component (Figure 2A). Partially purified SSG 185 (by elution from an SDS gel) was sensitive to protease treatment. Digestion by subtilisin lowers the apparent mol. wt. to ~145 000 daltons (Figure 2B).

Purification of SSG 185 turned out to be extremely difficult due to its high affinity for nearly all chromatographic supports. These difficulties were overcome after proteolytic digestion of a crude *Volvox* membrane fraction (containing all the extracellular matrix material). This procedure converts SSG 185 to the 145-K glycopeptide mentioned above. This derivative can easily be purified to homogeneity by anion exchange chromatography and preparative SDS-polyacrylamide gel electrophoresis. Its chemical composition is summarized in Table I. Remarkably, even after proteolytic degradation, the glycopeptide still contains ~30 mol % of amino acids, hydroxyproline being the most abundant. The neutral sugars occur in a molar ratio of ~7.6 galactose:2.4 mannose:0.5 glucose:10 arabinose:0.7 xylose.

On very mild mercaptolysis (Painter, 1969) the 145 000 dalton glycopeptide is further cleaved into a large fragment (apparent mol. wt. 90 000 daltons) and a small fragment (apparent mol. wt. 28 000 daltons), as shown in Figure 2. The large fragment contains all of the amino acids and the neutral sugars galactose and arabinose, but no sulphate. The small fragment contains no amino acids, but mannose, arabinose and sulphate in the ratio of 1:1:2. All of the sulphate residues of SSG 185 are bound to this small fragment, indicating a highly asymmetric clustering of sulphate residues within the complete structure of the parent molecule.

Mild alkali treatment, as used for β-elimination of serine-

Table I. Composition of the 145-K glycopeptide

	mol %
Total amino acids	30
Total carbohydrates	61
Total sulphate	9
Amino acid	**Residues/100**
Hyp	34
Asx	6
Thr	4
Ser	12
Glx	3
Pro	5
Gly	6
Ala	6
Val	6
Ile	2
Leu	4
Tyr	2
Phe	3
His	2
Lys	3
Arg	4

or threonine-linked saccharides causes a reduction in the apparent mol. wt. of SSG 185 of ~50 000 daltons. The same alkali-stable product is obtained if protease-treated SSG 185 (the 145 000 dalton glycopeptide) is subjected to mildly alkaline conditions: SSG 185 $\xrightarrow{\text{protease}}$ 145 K $\xrightarrow{\text{alkali}}$ 135 K.

alkali

Since both the protease-treated and the native SSG 185 yield the same product, it is very likely that these alkaline conditions remove a polypeptide chain from the sulphated SSG 185 molecule, indicating an O-glycosidic linkage to the polypeptide.

SSG 185 is the precursor of a polymeric extracellular matrix component

$^{35}SO_4^{2-}$-Pulse-chase labelling experiments demonstrated that newly synthesized SSG 185 shows turn-over with a half-life of ~30 min (Sumper and Wenzl, 1980; Wenzl and Sumper, 1981). However, during the chase period, no ^{35}S-labelled material compensating for the loss of radioactivity in the SSG 185 glycoprotein appeared in the SDS-polyacrylamide gel patterns. To account for this, we considered the existence of a specific sulphatase acting on the SSG 185 molecule. However, in an intensive search, no evidence could be obtained for the existence of such an enzymatic activity. If the loss of ^{35}S-radioactivity in the SSG 185 material cannot be accounted for by degradation to inorganic sulphate (or another low mol. wt. derivative), the only alternative is the conversion of SSG 185 to a polymer with extremely high molecular weight. In that case, the corresponding ^{35}S-radioactivity would also escape detection, even in a large pore SDS-polyacrylamide gel, because it would not be able to penetrate into the gel. In the experiment of Figure 3, *Volvox* spheroids were pulse labelled with $^{35}SO_4^{2-}$ and a crude extract applied to a 6% SDS-polyacrylamide gel. SSG 185 was the main radioactive component (lane 1). Another aliquot of the pulse-labelled *Volvox* population was further incubated under chase conditions. At the end of the chase period (90 min), the radioactivity of the SSG 185 was neither detectable in the SSG 185 molecule itself, nor in any other position in the gel pattern (lane 2). The extracts of both the pulsed and the chased *Volvox* populations were then subjected to mild alkali treatment, then again submitted to electrophoresis (lanes 1′ and 2′). As described above, the radioactive SSG 185 of the pulse labelling experiment was quantitatively converted to a sulphated derivative with an apparent mol. wt. of 135 000 daltons (lane 1′). Most surprisingly, alkali treatment of the chase extract yielded the same sulphated saccharide, and this contained all the radioactivity that had been incorporated into SSG 185 during the pulse (lane 2′). Clearly, during the chase period, SSG 185 is converted into a polymeric substance, which does not even enter the stacking polyacrylamide gel, and thus escapes detection.

While monomeric SSG 185 is completely soluble in SDS solutions, its aggregated form remains insoluble even in hot SDS-containing solutions. Thus, low speed centrifugation of a crude cell extract in the presence of 3% SDS allows selective purification of polymeric SSG 185 material. Alkali treatment converts this insoluble radioactive material quantitatively into the alkali-stable core structure (135 K) of the SSG 185 molecule. Protease (subtilisin) treatment converts this aggregate into the protease-resistant core structure of SSG 185, the 145-K fragment. The kinetics of this proteolytic degradation reveals that depolymerization of the aggregate yields oligomeric intermediates (notably dimers, trimers and tetramers) (Figure 4).

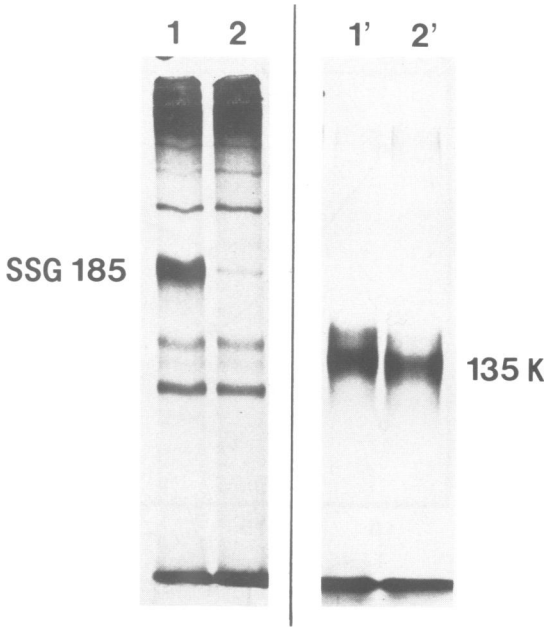

Fig. 3. Gel electrophoresis of the products of an *in vivo* $^{35}SO_4^{2-}$ pulse (**1**) and chase (**2**) labelling experiment carried out with *Volvox* spheroids at the stage of early embryogenesis. Aliquots of the extracts applied to **lanes 1** and **2** were treated with 1 N NaOH (1 h, 56°C) and re-submitted to electrophoresis (**lanes 1′** and **2′**).

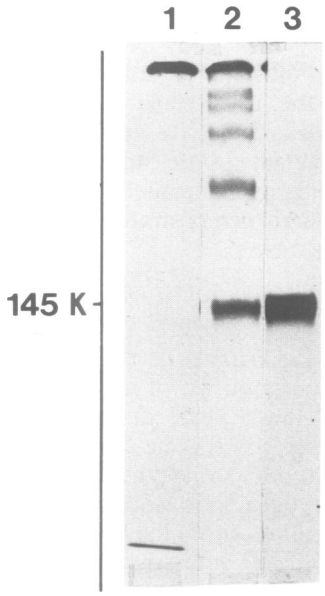

Fig. 4. Intermediates in proteolytic degradation of polymeric SSG 185, as analyzed by SDS-gel electrophoresis. Samples of polymeric [^{35}S]SSG 185 were incubated with 10 μg/ml subtilisin at 30°C for the times indicated. (**1**) Control without protease; (**2**) incubation for 3 min; (**3**) incubation for 60 min.

Boiling of intact *Volvox* spheroids in 3% SDS solubilizes most intracellular components but leaves intact the extracellular matrix structure (Kirk and Kirk, 1983). Thus, colourless *Volvox* 'ghosts' are obtained. While SSG 185 is quantitatively extracted by this procedure, its polymerization product remains associated with the extracellular matrix. However, alkali treatment of the ghosts again converts the high mol. wt. form to the soluble alkali-stable core material (cf. Figure 3).

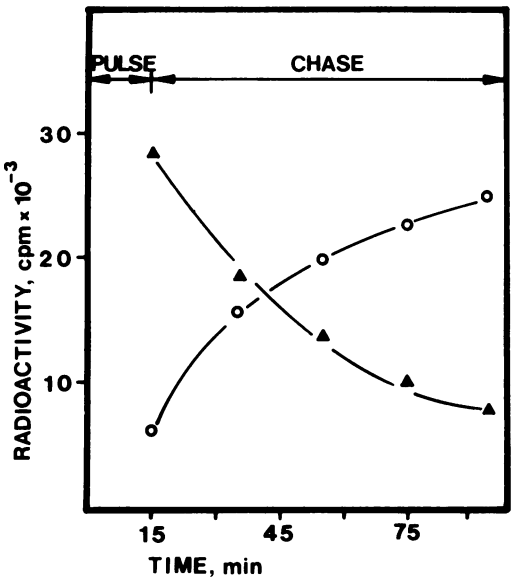

Fig. 5. Kinetics of the aggregation of SSG 185 into the polymeric state *in vivo*. Aliquots of $^{35}SO_4^{2-}$ pulse-labelled *Volvox* spheroids were taken during the chase period and analyzed for monomeric and aggregated SSG 185 as follows. Monomeric SSG 185 (▲—▲) was extracted with 2% SDS/1 M NaCl solution at 95°C for 10 min and determined by scintillation counting after gel electrophoresis. The extracted *Volvox* ghosts were then treated with 1 N NaOH (56°C, 1 h) to degrade polymeric SSG 185 (○—○) and radioactivity originally present in aggregated SSG 185 was determined after gel electrophoresis (of the product of alkali-treatment).

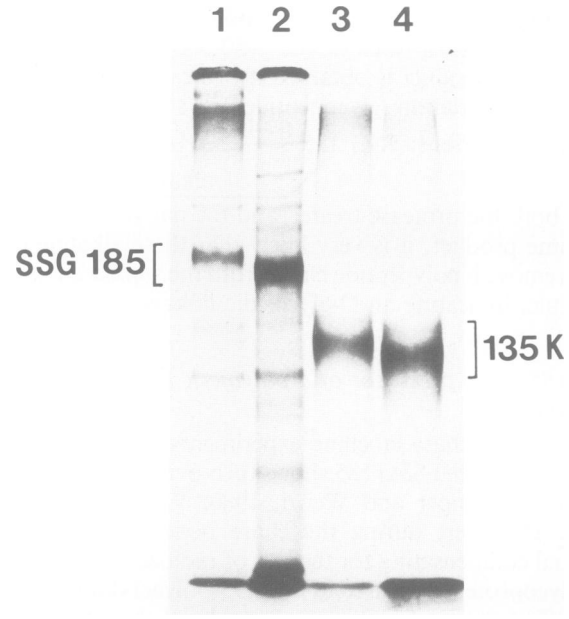

Fig. 6. The development-dependent modification of SSG 185 remains conserved in the polymeric state. *Volvox* spheroids were pulse labelled with $^{35}SO_4^{2-}$ at the beginning (**1**) and at the end (**2**) of embryogenesis. Lysates of aliquots were analyzed by SDS-gel electrophoresis. The pulsed *Volvox* populations were further incubated under chase conditions to convert SSG 185 into the polymeric form (90 min). Polymeric SSG 185 produced during early (**3**) and late (**4**) embryogenesis was extracted and degraded by 1 N NaOH and analyzed by SDS-gel electrophoresis.

This observation indicates a function for the SSG 185 polymer within the extracellular matrix. Using this experimental approach, the *in vivo* kinetics of the conversion of SSG 185 to its aggregated state can easily be followed during pulse-chase labelling experiments. The results of this experiment (Figure 5) clearly demonstrate a precursor-product relationship.

The developmentally controlled change in mol. wt. of SSG 185 is conserved in the polymeric state

Synthesis of SSG 185 is under developmental control. In particular, the apparent mol. wt. of newly synthesized SSG 185 shifts towards a lower value during early embryogenesis (Wenzl and Sumper, 1981). The difference between the apparent mol. wts. of SSG 185 synthesized at the beginning and at the end of embryogenesis is ~8000 daltons. To localize this structural modification within the molecule, SSG 185 obtained during early and late embryogenesis was cleaved by mild mercaptolysis. As shown in Figure 2D, the structural alteration is located in the highly sulphated 28-K saccharide fragment. This structural change in the SSG 185 molecule could indicate a modification of the extracellular matrix during development, provided that this structural modification remains preserved in the polymeric state. To answer this question, samples of polymeric SSG 185 synthesized during early and late embryogenesis were submitted to alkaline degradation and compared on SDS-polyacrylamide gels. As seen in lanes 3 and 4 of Figure 6 the difference in the apparent mol. wt. had indeed been conserved in the polymeric form of SSG 185.

From $^{35}SO_4^{2-}$ labelling experiments performed under conditions which allow ^{35}S incorporation into SSG 185 only in trace amounts Kirk and Kirk (1983) deduced that SSG 185 synthesis occurs within the somatic cell matrix. Use of carrier-free sulphate and a pulse length of 60 min results in $^{35}SO_4^{2-}$ depletion within a few minutes so that initially labelled SSG 185 becomes polymerized during the prolonged incubation. Nonetheless, reproduction of their experiment under our pulse labelling conditions confirmed their conclusion. After pulse labelling, *Volvox* spheroids were mechanically separated into a matrix sheet containing all the somatic cells and into a suspension of embryos. Radioactive SSG 185 was found exclusively in the somatic cell matrix of the parent colony. Since SSG 185 synthesis perfectly reflects the developmental program of the reproductive cells (embryos), its localization in the somatic cell sheet is an unexpected result.

The sexual inducer triggers a modification of the extracellular matrix

The sexual inducer, if applied at least 6–8 h before the initiation of embryogenesis, triggers development of sexual rather than asexual embryos. The molecular mechanism switching the developmental program to egg (or sperm) differentiation is unknown. Recently, we showed that the sexual inducer triggers the synthesis of a new sulphated extracellular glycoprotein with an apparent mol. wt. of 280 000 daltons, denoted as F-SG (Wenzl and Sumper, 1982). Originally, we reported that synthesis of F-SG occurs in female strains. Upon re-examination, however, F-SG synthesis turned out to take place in male strains as well. Thus synthesis of F-SG is a general response to the application of the sexual inducer. Short pulse labelling (5 min) experiments revealed that F-SG synthesis became detectable after a lag period of only 25 min.

At the time of sensitivity to the sexual hormone (~20–5 h before initiation of embryogenesis), only a low level of [^{35}S]-sulphate incorporation into SSG 185 is detectable in the somatic cell matrix of the young spheroids. Furthermore, in the absence of the sexual inducer, the apparent mol. wt. of

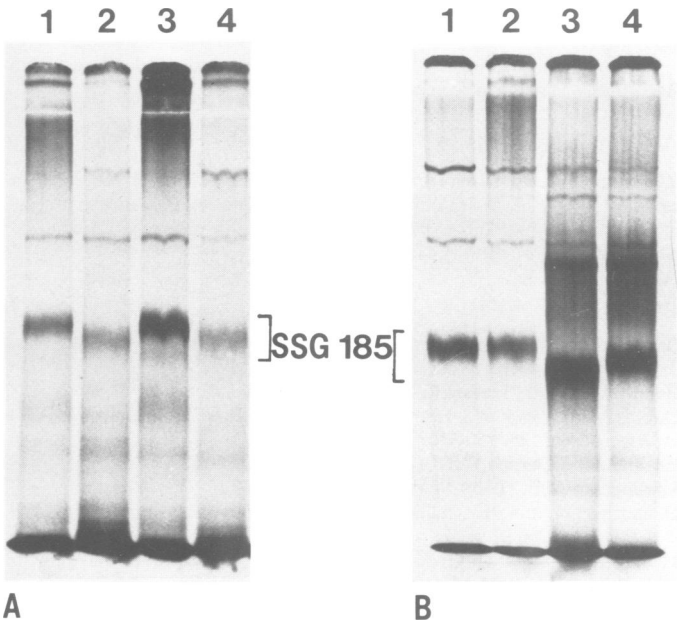

Fig. 7. The sexual inducer affects SSG 185 synthesis. **(A)** *Volvox* spheroids were pulse-labelled with $^{35}SO_4^{2-}$ 12 h before initiation of embryogenesis and the crude membrane fraction analyzed by SDS-gel electrophoresis. **(1,2)** Membrane fraction from female strain incubated, respectively, in the absence and presence of the sexual inducer; **(3,4)** membrane fraction from male strain incubated in the absence and presence of the sexual inducer respectively. **(B)** $^{35}SO_4^{2-}$ pulse labelling of sexually induced *Volvox* spheroids at early and late embryogenesis. **(1,2)** Female and male strains respectively at the beginning of embryogenesis; **(3,4)** female and male strains respectively, at the end of embryogeneis.

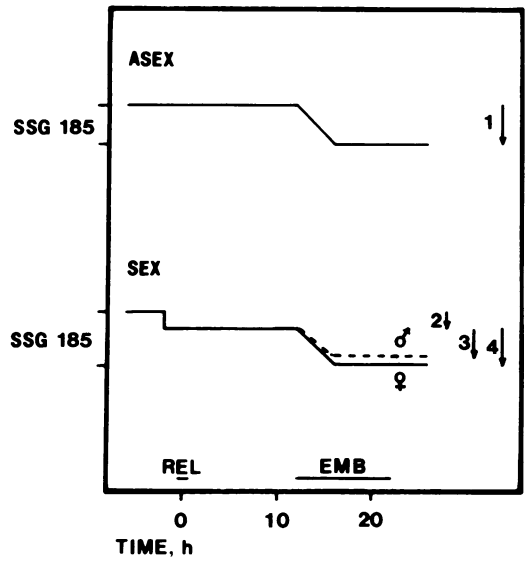

Fig. 8. Scheme summarizing the shifts of the apparent mol. wt. of SSG 185 during development. **(1)** Shift during embryogenesis of asexual growing colonies; **(2)** shift after addition of the sexual inducer; **(3 and 4)** shift during embryogenesis of sexual, growing colonies from the male **(3)** and female **(4)** strain. REL, stage of release; EMB, stage of embryogenesis.

SSG 185 does not change until the initiation of embryogenesis. In sharp contrast, only 10 min after the addition of the sexual inducer, the apparent mol. wt. of newly synthesized SSG 185 drops by ~5000 daltons (Figure 7A). In addition, net incorporation of $^{35}SO_4^{2-}$ into this SSG 185 variant decreases. During subsequent embryogenesis an additional drop of the apparent mol. wt. of SSG 185 is observed, analogous to that described for the asexual development. Due to the hormone-induced shift in mol. wt., sexual development produces a significantly smaller SSG 185 variant at the end of embryogenesis as compared to the asexual control.

Both the male and female strains respond to the sexual inducer with the production of a SSG 185 variant, and these are indistinguishable with respect to mol. wt. However, during subsequent sexual embryogenesis, the modulation of SSG 185 synthesis described reveals sex-specificity. The shifts in the apparent mol. wt. of SSG 185 are significantly different in both sexes, as documented in Figure 7B. All the observed variations of SSG 185 synthesis are schematically summarized in Figure 8. It should be stressed that all of the different variants of SSG 185 described yield identical cleavage patterns after mercaptolysis or alkali treatment.

Discussion

The present results demonstrate that SSG 185 is the monomeric precursor of aggregated extracellular material. Disaggregation of this polymer by proteolysis yields dimeric, trimeric and higher assemblies as intermediates. However, it is not known whether the assembly reaction includes the formation of covalent cross-links between SSG 185 monomers. Since the intact SSG 185 behaves as a highly 'sticky' substance adsorbed to most chromatographic supports, aggregation by non-covalent interactions or via link proteins as found for animal glycosaminoglycans is a conceivable alternative (Heinegård and Hascall, 1974; Hardingham, 1979).

SSG 185 is a developmentally regulated molecule. In particular, its apparent mol. wt. changes in a defined manner during defined developmental events. During early embryogenesis the mol. wt. is shifted towards a lower value. In addition, SSG 185 appears to be involved in the process of sexual induction. Only a few minutes after the application of the sexual hormone, the somatic cells respond with the synthesis of a different SSG 185 variant. Moreover, this SSG 185 variant shows sex-specificity, i.e., SSG 185 molecules from induced male and female strains are significantly different. Remarkably, these structural variations of the SSG 185 molecule remain conserved in the polymer, indicating a defined modulation of the extracellular matrix at defined developmental stages. Chemical modification of the extracellular matrix seems to be involved in the process of sexual induction, since at least two sulphated glycoproteins (F-SG and the SSG 185 variant) are specifically produced in response to the sexual inducer.

After mechanical disruption of *Volvox* spheroids, polymeric SSG 185 is associated with the extracellular sheath material containing the somatic cells and is not found within the developing embryos. On the other hand, its synthesis and chemical variation perfectly reflects the developmental program of the embryo. This fact strongly suggests some sort of communication between the somatic and the reproductive cells. Alternatively, an endogenous program operating in somatic cells may cause the variations in SSG 185 synthesis which only coincidentally correlate with the developmental program of the reproductive cells. However, a role of SSG 185 in mediating embryonic cell-to-cell contacts, as we proposed earlier, becomes very unlikely in the light of its association with the somatic cell sheet. Therefore our interest will be focused on the localization of SSG 185 by means of immune fluorescence studies of intact *Volvox* spheroids to exclude the

possibility that SSG 185 is redistributed during the process of mechanical disintegration of somatic and reproductive cells.

Materials and methods

Culture conditions

V. carteri f. *nagariensis*, strains HK 10 (female) and 69-1 b (male) from the Culture Collection of Algae at the University of Texas at Austin were a gift from L. Jaenicke (Cologne). Synchronous cultures were grown in *Volvox* medium (Provasoli and Pintner, 1959) at 28°C in an 8 h dark/16 h light (10 000 lux) cycle (Starr and Jaenicke, 1974).

Pulse-chase labelling experiments

Pulse-labelling with $^{35}SO_4^{2-}$ was performed as described by Wenzl and Sumper (1981). After a 30 min pulse period, the labelled colonies were washed with sulphate-containing *Volvox* medium and resuspended in the same medium for the subsequent chase period. The suspension was treated in the same manner as under pulse conditions. Usually, the chase period lasted for 90 min.

Labelling of SSG 185 with ^{14}C was carried out in essentially the same way, using 100 μCi/ml $[^{14}C]HCO_3^-$.

Crude membrane fractions were prepared as described (Wenzl and Sumper, 1981).

Preparation of the 145-K fragment from SSG 185

A (pulse-labelled) crude membrane fraction in 50 mM Tris-HCl pH 8.0 was treated with 50 μg/ml subtilisin for 30 min at 30°C. The solution was then brought to 5% Triton X-100 and 1 M NaCl, and boiled for 2 min.

After low speed centrifugation, the sample was applied to a QAE-Sephadex A-25 column equilibrated with 1 M NaCl in 50 mM Tris-HCl pH 8.0. After the column was washed with five volumes of the starting buffer, the 145-K fragment was eluted with 4 M NaCl in 50 mM Tris-HCl (or, in preparative work, with a linear gradient of 1–4 M NaCl). Radioactive fractions were pooled. After dialysis and lyophilization the material was applied to a preparative 5% SDS-polyacrylamide gel. After autoradiography the 145-K fragment was eluted with water, dialysed and lyophilized.

Alkali treatment

Typical β-elimination conditions (0.1 M KOH, 1 M $NaBH_4$, 6 h at 37°C) yielded the same reaction products as the more rapid alkali treatment described here. SSG 185 or crude membrane fractions were incubated in 1 M NaOH for 90 min at 56°C. The reaction was stopped by neutralization with 5 M acetic acid. Aliquots were mixed with an equal volume of SDS sample buffer (Laemmli, 1970) and heated for 2 min at 95°C. SDS-PAGE was performed as described by Laemmli (1970) using 5% polyacrylamide in the separation gel. Fluorography was performed according to Bonner and Laskey (1974).

Mercaptolysis

Mercaptolysis was performed according to Painter (1960) with the following modifications. Radioactive SSG 185 or its 145-K fragment were lyophilized to dryness. 50 μl of an ice cold 2.3 M solution of mercaptoethanol in 12 M HCl were added and the sample was incubated for 30 min at 0°C. The reaction was stopped by neutralization with Tris base, and after dialysis and lyophilization, the cleavage products were analyzed on SDS-PAGE using 12% acrylamide in the separation gel.

SDS extraction of intact Volvox spheroids

To separate SSG 185 from insoluble extracellular material, aliquots of pulse- (or pulse-chase-) labelled spheroids were diluted with two volumes of a 3% SDS solution containing 1 M NaCl and boiled for 10 min at 95°C. Low speed centrifugation pelleted the *Volvox* ghosts containing the extracellular matrix. SSG 185 is quantitatively found in the supernatant. The ghosts were disintegrated by ultrasonic treatment.

Preparation of the sexual inducer

Sexual inducer was prepared according to Starr and Jaenicke (1974). More highly purified inducer preparations (by ConA-Sepharose chromatography, hydroxyapatite chromatography and preparative polyacrylamide gel electrophoresis) elicited identical effects with respect to SSG 185 synthesis. To exclude the action of a contaminating protease or glycosidase on SSG 185, labelled SSG 185 was incubated *in vitro* in the presence of increasing amounts of inducer. No change of the apparent mol. wt. of SSG 185 could be observed in these controls.

Analytical determinations

145-K glycoprotein was hydrolyzed in 6 N HCl for 24 h at 105°C under nitrogen and analyzed in an automatic Biotronic LC 5000 amino acid analyzer. Total carbohydrate content was determined according to Dubois *et al.* (1956), sugars were analyzed as their alditol acetates by gas liquid chromatography according to Laine *et al.* (1972). Sulphate was determined using the sodium rhodizonate method according to Terho and Hartiala (1971).

Apparent mol. wts. were estimated using protein standards purchased from Boehringer, Mannheim (combithek).

Acknowledgements

We would like to thank Brigitte Seidel and Ulrike Stöckl for excellent technical assistance. We also thank Dr. Peter Orlean for assistance in preparing the manuscript. This work was supported by the Deutsche Forschungsgemeinschaft through SFB 43.

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Received on 28 November 1983; revised on 19 January 1984