lipid fluidity might take place during the aging process, thus suggesting that a variety of membrane-linked phenomena, such as enzymatic activities or hormone responsiveness[2-4], are in some way modulated by changes of plasma membrane microenvironment.

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Morphological and histochemical demonstration of a glycocalyx on the cell surface of *Chattonella antiqua*, a 'naked flagellate'

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Summary. Electron microscopy demonstrated the presence of glycocalyx on the cell surface of *Chattonella antiqua*, which was previously believed to be 'naked'. Histochemical analysis of this structure indicates that the glycocalyx consists of at least two different types of acidic complex carbohydrates, sulfated and nonsulfated, together with a neural carbohydrate-protein complex with 1,2-glycol groups and α-D-mannosyl and α-D-glucosyl residues.
Key words. Glycocalyx; histochemistry; flagellate; Rhaphidophyceae; *Chattonella antiqua*.

Modern microscopic techniques have disclosed a wide variety of cell surface specializations in phytoplankton. Nevertheless, there is a flagellate group called 'naked plankton'[1,2]. *Chattonella antiqua*, noted for its bloom, is a species of this group belonging to Rhaphidophyceae. The extremely delicate structure of these organisms has prevented an approach to the study of their surface morphology by conventional microscopic techniques. Thus, they have long been considered to be bounded simply by the plasma membrane directly exposed to the ambient water[3]. In animal and plant cells, however, recent studies have accumulated abundant evidence that most if not all cell surfaces have a special structure with polysaccharide-rich components[4,5]. Though the terminology of these structures has not been established, 'glycocalyx', proposed by Bennet[6] seems to be most often used in recent papers. In the present study, we attempted to elucidate the surface morphology and histochemistry of *Chattonella antiqua* at electron microscopic (EM) and light microscopic (LM) levels.

Materials and methods. Patches of *Chattonella antiqua* were obtained from Aichi Prefectural Fisheries Experimental Station. The organism was originally isolated by S. Toriumi from Mikawa Bay, Japan in 1972. *Chattonella* cells were grown in Guillard 'f' medium[7], at 20–24 °C, under 3000 lux CW fluorescent light, and a 14:10 h light:dark photoregime. For the present study, cultures in the early-exponential growth phase (at a cell density of about 500/ml) were used. For the EM study, 100 µl of a cell suspension were placed in a centrifuge tube, and 200 µl of 1% alcian blue (AB) solution (pH 2.5) were added to the tube just prior to the use of a fixative (200 µl) consisting of collidine-

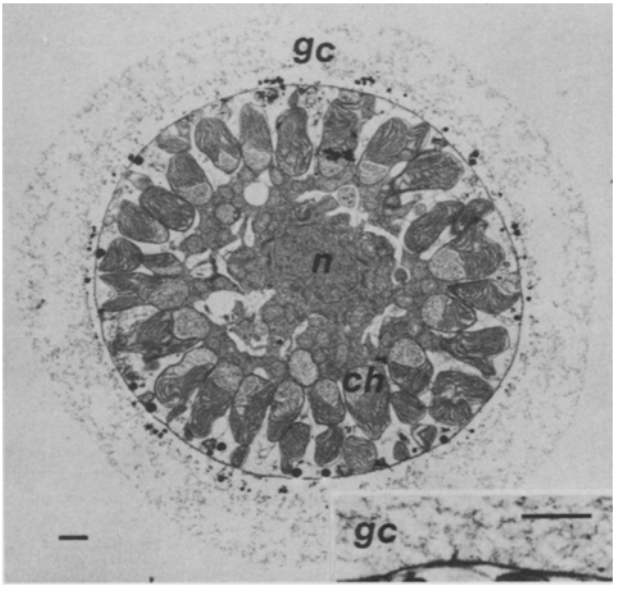

Figure 1. Electron micrograph of *Chattonella* cell (cross section) illustrating glycocalyx on the cell surface. Inset shows a part of the plasma membrane from another cell. Fine filaments appear to be continuous through the plasma membrane. Bars denote 1 µm. gc, glycocalyx; ch, chloroplast; n, nucleus.

buffered 1% osmic acid, 0.2% glutaraldehyde, and 5% $CaCl_2$. The organisms were fixed at 4 °C for 30 min, and then concentrated by gentle centrifugation (800 rpm) with the aid of a hand centrifuge. After discarding the fixative, the organisms were resuspended in distilled water in the tube and thoroughly washed twice. After final centrifugation, distilled water was removed and a small drop of 2% agarose (Sigma Chem. Co. Ltd., U.S.A,. Type VII) was added to the tube. The solidified agarose was cut into small cubes, dehydrated in ascending concentrations of ethanol and embedded in Quetol 651[8].

For the LM study, a centrifuge tube with 1 ml of fixative consisting of 35% formalin, 5% Ficoll (Pharmacia Fine Chem., Sweden), 20% ethanol, and 4% of $CaCl_2$ was kept at 4 °C and 100 µl of a cell suspension was added to the tube. The organisms were fixed and washed as described above. After removal of excess water, they were subjected to histochemical analysis. In some cases, agarose was added to a clot of the fixed organisms in the tube, and the mixture was smeared on glass slides and prepared for study. Histochemical methods are described in table 1. Enzymes used were the following: α-amylase (Wako Pure Chem. Indust. Ltd., Japan), sialidase (Nakarai Chem. Ltd., Japan), testicular hyaluronidase (Sigma Chem. Co. Ltd., U.S.A.), *Streptomyces* hyaluronidase (Amano Pharmaceut Co. Ltd., Japan), and chondroitinases ABC and AC (Seikagaku Chem. Indust. Ltd., Japan).

Results. An improvement in the use of cationic dye allowed satisfactory preservation of extracellular components for EM study. Thus, the glycocalyx was clearly demonstrated on the cell surface of *Chattonella antiqua*. The fine structure of the glycocalyx had the appearance of a branching network of fine filaments extending outward from the plasma membrane (fig. 1). Though this fuzzy coat was found to be easily dissociated from the cell surface during preparative process, fine filaments weaving a meshwork appeared to be intimately associated or continuous with the plasma membrane in well-preserved specimens (fig. 1, inset).

A variety of LM histochemical stains for carbohydrates distinctly aided visualization of the glycocalyx (fig. 2). The glycocalyx positively reacted to staining for acidic complex carbohydrates such as AB (pH 1.0), AB (pH 2.5), AF, DI-FCY, LID and HID. Furthermore, active methylation thoroughly suppressed both AB (pH 1.0) and AB (pH 2.5) reactivities of the structure. Saponification following active methylation, however, restored in part AB (pH 2.5) reactivity. Besides these staining reactions for acidic complex carbohydrates, the glycocalyx exhibited amylase-resistant PAS reactivity and sialidase resistant AB (pH 2.5) reactivity. Moreover, the structure showed a positive reaction to Con A-PO-DAB and PA-AP-FBK methods. In experiments using enzyme digestion, treatment with testicular hyaluronidase abolished the alcianophilia (pH 1.0 and 2.5) of the glycocalyx. Likewise, digestion with *Streptomyces* hyaluronidase and chondroitinases ABC and AC induced a notable decline in the intensity of its AB (pH 2.5) reactivity. In parallel with these reactions for complex carbohydrates, the glycocalyx was found to be colored by CT and NIN-S for proteins. Prior deamination thoroughly blocked NIN-S reactivity of the structure.

Discussion. In eukaryotic algal cells, extracellular polysaccharide was found in *Porphyridium aerugineum*, a red alga[20], and the presence of a surface coat consisting largely of glycoprotein with some neuraminic acid residues was described for *Dunaliella tertiolecta*, Chlorophyceae[21]. Very recently Willey[22] reported a carbohydrate-containing glycocalyx covering the cell surface of *Colacium calvum*, Euglenophyceae. In these studies, however,

Histochemical reactions of the glycocalyx on the cell surface of *Chattonella antiqua*

Staining procedures	Reactions
1) For complex carbohydrates	
Periodic acid-Schiff (PAS)[9]	2–3M
AB (pH 1.0)[10]	1–2B
AB (pH 2.5)[10]	2–3B
Aldehyde fuchsin (AF)[9]	2–3PR
Dialyzed iron-ferrocyanide (DI-FCY)[9]	3–4B
Low iron diamine (LID)[11]	1–2Bl
High iron diamine (HID)[11]	± –1Bl
AB (pH 2.5)-PAS[12]	2–3MP
AF-AB (pH 2.5)[13]	2–3PB
Concanavalin A-peroxidase-diaminobenzidine-periodic acid-*m*-aminophenol-Fast Black salt K (Con A-PO-DAB-PA-AP-FBK)[14]	2–3BrP
Mild methylation-AB (pH 1.0)[15]	± –1B
Mild methylation-AB (pH 2.5)[15]	1–2B
Active methylation-saponification-AB (pH 1.0)[15]	0– ± B
Active methylation-saponification-AB (pH 2.5)[15]	1–2B
α-Amylase-PAS[16]	2–3M
Sialidase-AB (pH 2.5)[9]	2–3B
Testicular hyaluronidase-AB (pH 1.0)[17]	0– ± B
Testicular hyaluronidase-AB (pH 2.5)[17]	0– ± B
Streptomyces hyaluronidase-AB (pH 1.0)[18]	± –1B
Streptomyces hyaluronidase-AB (pH 2.5)[18]	± –1B
Chondroitinases ABC-AB (pH 1.0)[19]	± –1B
Chondroitinases ABC-AB (pH 2.5)[19]	± –1B
Chondroitinases AC-AB (pH 1.0)[19]	± –1B
Chondroitinases AC-AB (pH 2.5)[19]	± –1B
2) For proteins and amino acids	
Coupled tetrazonium (CT)[10]	1–2Br
Ninhydrin-Schiff (NIN-S)[10]	± –1M
Deamination-NIN-S[10]	0

Abbreviations: M = magenta; B = purple; R = red; Bl = black; Br = brown; 0 = negative; ± = a weak reaction; 1-n = number being proportional to the intensity of reactions.

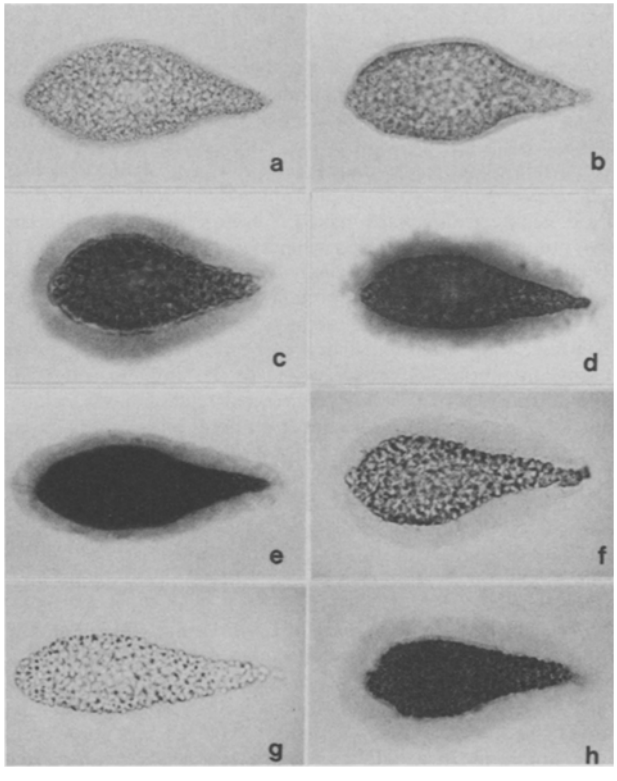

Figure 2. Light micrographs of *Chattonella* cells stained by *a* AB (pH 1.0), *b* AB (pH 2.5), *c* PAS, *d* DI-FCY, *e* AB (pH 2.5)-PAS, *f* active methylation-saponification-AB (pH 2.5), *g* *Streptomyces* hyaluronidase-AB (pH 2.5) and *h* TZ. × 320.

the precise nature of the polysaccharides remained unclear because of the use of limited experimental methods.

In Rhaphidophycean algal cells, the presence of a glycocalyx was first demonstrated at EM level in the present study. In addition, the histochemical nature of this extracellular structure was made clear for the first time by the use of a wide range of current methods for LM carbohydrate histochemistry. The staining reactions of the glycocalyx imply that the structure contains acidic complex carbohydrates. In line with this, chemical modification of the reactive components clearly indicates that ester sulfate and carboxyl groups are responsible for the above reactions. Digestion experiments with amylase and sialidase show that the glycocalyx contains a neutral carbohydrate-protein complex besides acidic complex carbohydrates. Further, reaction with Con A-PO-DAB-PA-AP-FBK demonstrates the existence of 1,2-glycol and α-*D*-mannosyl and α-D-glucosyl residues[15]. The substrate specificities of hyaluronidases[17,18] and chondroitinases[19] and the staining selectivities of the AB (pH 2.5) reactions indicate that a particular moiety involved in the acidic complex carbohydrates of the glycocalyx may be hyaluronic acid or at least a closely related substance.

Demonstration of functional roles for complex carbohydrates involved in the extracellular structure of phytoplankton has long been lacking. However, recent knowledge on the subject in animal and plant cells[4,5,23] suggests that these substances may perform physiologically important functions in plankton. Our present findings may provide a histochemical basis for assigning a valid function for the flagellate glycocalyx.

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Adrenocortical metabolism and plasma corticosterone in soman intoxicated rabbits[1]

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31 October 1984

Summary. The organophosphate neurotoxin soman produced impairments in adrenocortical RNA and protein metabolism. Fasciculate and reticular cell RNA and protein contents were suppressed with sublethal to acutely lethal dosages (20, 30 and 40 µg/kg, s.c.) during the acute excitatory phase of intoxication and at 6–8 h post injection. All three dosages produced ca 90% inactivation of plasma cholinesterase. A transient elevation of plasma corticosterone occurred with 20 µg/kg soman whereas there was a protracted increase with 30 µg/kg. Corticosterone was not significantly elevated with 40 µg/kg, but death occurred at 13 ± 4 min. Thus, the magnitude and/or nature of soman-induced metabolic impairments does not appear to prevent adrenal activation.
Key words. Adrenocortical RNA and protein; plasma corticosterone levels; organophosphate-intoxicated rabbits; quantitative cytophotometry.

The role of adrenal glucocorticoids in eliciting adaptive and homeostatic adjustments occurring with diverse forms of stress, including exposure to toxic chemicals, is well documented. It might therefore be expected that organophosphates (OP) cause adrenal activation and enhanced corticoid secretion. This has indeed been shown following the administration of sublethal dosages of OP insecticides[2,3]. However, several OP insecticides have also been shown to impair adrenal steroidogenesis both in vitro and in vivo. Impairments are evident as: 1) reduced endogenous corticoid synthesis with diminished in vitro corticosteroidogenesis in response to ACTH or cAMP stimulation[4]; and 2) reduced in vivo capacity for steroid output in response to subsequent cold stress[2]. Presently, alterations in adrenal metabolism and function occurring with related but more highly toxic OP, generally classified as chemical warfare agents, have not been ascertained.

The current investigation was conducted to determine dose- and time-dependent effects of the potent OP-neurotoxin, soman (pinacolyl methylphosphonofluoridate), on rabbit adrenocortical metabolism and accompanying alterations in plasma corticosterone levels. Dosages used were previously determined to be: sublethal but producing overt toxic symptoms (20 µg/kg); lethal producing delayed death (30 µg/kg); and lethal during the acute excitatory period (40 µg/kg)[5]. Quantitative azure B-RNA and mercuric bromophenol blue-protein cytophotometry was used to monitor responses of individual fasciculate and reticular