ACTA SOCIETATIS
BOTANICORUM POLONIAE
Vol. 48, nr 4: 531-537
1979

Isolation of 'mating fraction' from *Chlamydomonas reinhardtii* gametic flagellum membranes by affinity chromatography

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(Received: May 30, 1979)

Abstract

Solubilized with Triton X-100 strain 137+ gametic flagellum membrane material was bound to CNBr-activated Sepharose 4B, and used for affinity chromatography of labeled with ^{125}I 89 gametic flagellum membrane soluble in Triton X-100. A single peak containing 2.7-4.7% of total radioactivity was obtained upon pH change of the eluting buffer. Upon re-chromatography 50-70% of this material was adsorbed and eluted. The complex was found to be stable at pH 7.0-8.0 in the presence of divalent cations (Ca^{+2} and Mg^{+2}).

INTRODUCTION

Mating in *Chlamydomonas* represents an excellent system for the study of cell recognition and specific adhesion (Snell, 1976a, b). Molecules responsible for the adhesion have not yet been isolated and characterized.

Crandall et al. (1974) isolated complementary glycoproteins from yeast responsible for the adhesion of opposite mating type gametes. Lloyd and Cook (1974, 1975) observed that desialilated fibroblast membrane glycoproteins stimulated aggregation of fibroblast cells. Jamskova et al. (1977) purified glycoprotein adhesive factors from rat liver and lung tissues. Vacquier et al. (1977), Glabe and Vacquier (1977) and Bellet et al. (1977) isolated insoluble protein 'bindin' from sea urchin sperm acrosome granule which appeared to be responsible for recognition and adhesion of sperm to the egg surface.

In this communication we present an affinity chromatography method for isolation of the factor from the flagellum membrane, involved in gamete recognition in *Chlamydomonas*.

MATERIAL AND METHODS

Chlamydomonas reinhardtii strain 137^+ mating with strain 89 were a gift of dr R. F. Jones of SUNY, Stony Brook, Biol. Dept. Both strains were cultured and harvested as described previously (S i k o r s k i, 1979). Flagella, flagellum membrane isolation and labeling with ^{125}I (carrier--free, 490 mCi/ml New England Nuclear) using lactoperoxidase (Sigma) were also described previously (S i k o r s k i, 1979).

All buffers contained 0.5% Triton X-100 (Serva) and 1.0 M NaCl.

Flagellum membranes of 89 gametic cells were treated with 0.5% Triton X-100 in 1.0 M NaCl; 0.05 M $CaCl_2$; 0.1 M Tris-HCl, pH 7.5. Flagellum membranes of 137^+ gametes were solubilized in 0.5% Triton X-100 in 1.0 M NaCl; 0.05 M $CaCl_2$; 0.1 M borate buffer, pH 8.0. Both solubilizations were carried out at room temperature for 12 hours with gentle shaking. The samples were then centrifuged at 130 000 $\times$ g for 2 hours and supernatants were used for experiments.

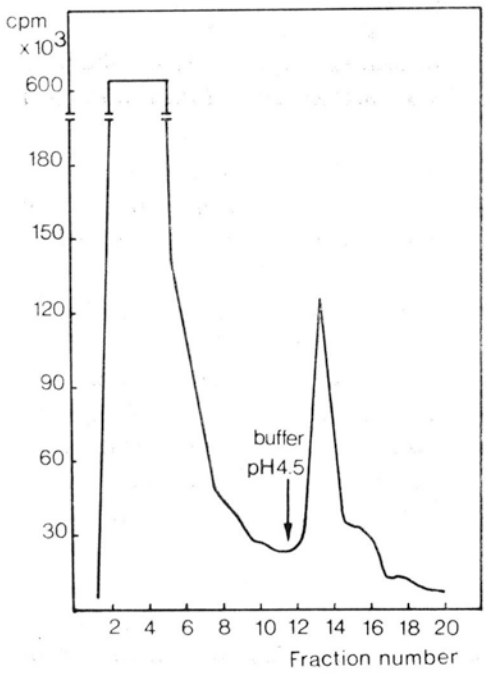
Affinity chromatography: Triton X-100 supernatant of 137^+ gametic flagellum membranes (1.0-2.0 mg of protein in 5.0 ml) was added to 1.0 g of swelled CNBr-activated Sepharose 4B (Pharmacia Fine Chem.) and kept at room temperature with continuous shaking for 12 hours. Then the suspension was transferred into the column (1.0$\times$4.0 cm) and washed with borate buffer, pH 8.0, subsequently with 0.1 M Tris-HCl buffer, pH 8.0 (overnight), 0.1 M acetate buffer, pH 4.5 (2 hours), and again with Tris-HCl, pH 8.0. The column was equilibrated with phosphate buffer containing: 8.3 mM K_2HPO_4; 5.3 mM KH_2PO_4; 0.07 mM $CaCl_2$; 0.08 mM $MgSO_4$; 1.0 M NaCl; and 0.5% Triton X-100, pH 7.0. Samples which were to be subjected to column chromatography were equilibrated with the same buffer.

Affinity chromatography was carried out at flow rate 1 ml/10 min. Adsorbed radioactive material was eluted with 0.5% Triton X-100, 1.0 M NaCl in 0.1 M sodium acetate buffer, pH 4.5. Two milliliter fractions were collected. Radioactivity measurements were taken with the use of Isodyne (Nuclear Chicago) or Polon USB-2 gamma counters.

RESULTS AND DISCUSSION

^{125}I-labeled *Chlamydomonas* flagellum membranes were solubilized with 0.5% Triton X-100 in borate or Tris buffer containing 1.0 M NaCl. As estimated on the base of I^{125} radioactivity, 70-78% of membrane protein material was soluble.

Labeled with ^{125}I 137^+ gametic flagellum membrane proteins soluble in 0.5% Triton X-100 were bound to CNBr-activated Sepharose 4B. It was found, that about 75% of membrane material was bound to activated CNBr-Sepharose under the conditions applied.



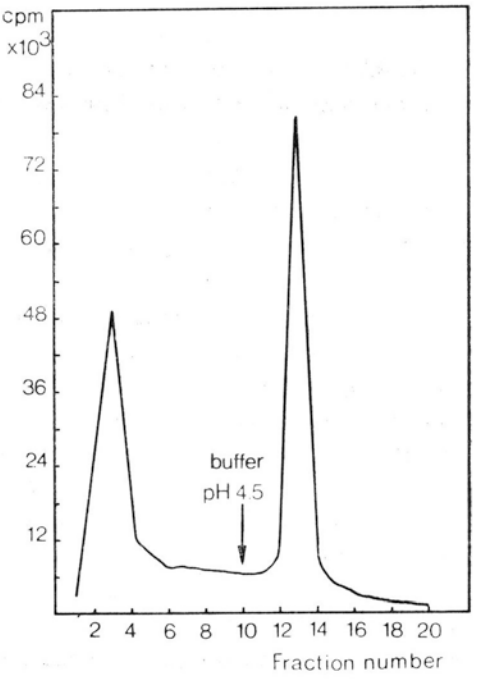


Fig. 1

Fig. 2

Fig. 1. Chromatography of ^{125}I labeled 89 gametic flagellum membrane Triton X-100 supernatant on affinity column (1.0×4.0 cm) containing 137+ gametic flagellum membrane material soluble in Triton X-100 bound to CNBr-activated Sepharose 4B. Adsorption was performed in 0.5% Triton X-100 in phosphate buffer, pH 7.0, containing: 8.3 mM K_2HPO_4; 5.3 mM KH_2PO_4; 0.07 mM $CaCl_2$; 0.08 mM $MgSO_4$ and 1.0 M NaCl. Elution was carried out with 0.5% Triton X-100; 1.0 M NaCl in 0.1 M acetate buffer, pH 4.5. For other details see Material and methods.

Fig. 2. Re-chromatography of ^{125}I labeled 89 gametic flagellum membrane adhesive material eluted from affinity column (fractions 12-14, Fig. 1) on the same column. Prior to chromatography the pH of the solution was adjusted to 7.0 with 0.83 M K_2HPO_4. Other conditions as in Fig. 1.

For affinity chromatography unlabeled 137^+ gametic flagellum membrane proteins were bound to CNBr-activated Sepharose 4B in the same conditions. On the column formed of this material an aliquot of '89' gametic flagellum membranes labeled with ^{125}I was applied. After washing with phosphate buffer, the column was eluted with acetate buffer, pH 4.5. In separate experiments single peaks containing from 2.7 to 4.7% of initial radioactivity were obtained (Fig. 1). After washing with acetate (pH 4.5) and Tris-HCl (pH 8.0) buffer the column was used for chromatography repeatedly.

Material obtained in the peak (Fig. 1) in the first chromatography after pH adjustment to 7.0 with K_2HPO_4 was rechromatographed. Now 50-70% of radioactivity was adsorbed and eluted with acetate buffer (Fig. 2).

Three control experiments were performed. Active groups of CNBr--Sepharose were blocked by washing with 0.1 M Tris-HCl buffer, pH 8.0 containing 0.5% Triton X-100 and 1.0 M NaCl overnight. Then the column was washed with acetate and equilibrated with phosphate buffers. Triton X-100 soluble labeled material of 89 gametic flagellum membranes was applied on such a column. None of the membrane material was found to be adsorbed on the column containing no 137^+ gametic membranes.

On the column packed with 137^+ gametic membrane proteins bound to Sepharose, Triton X-100 supernatant of the labeled 89 vegetative flagellum membrane was chromatographed. Only 0.4% of initial radioactivity was eluted with acetate buffer.

To test whether all the adhesive activity was adsorbed in the single experiment, the nonadsorbed material of 89 gametic membranes (Fig. 1) was rechromatographed. Upon pH change only 0.4% of the applied radioactivity was eluted.

The stability of the complex was shown to be affected by the presence of divalent ions (Ca^{+2} and Mg^{+2}). Fig. 3 shows the result of the experiment, where elution with phosphate buffer without $CaCl_2$ and $MgSO_4$ containing 0.001 M EDTA was performed.

The pH stability of the complex was studied by elution of adsorbed material from the column with phosphate buffers of pH in the range from 5.0 to 8.5. The complex was found to be stable in a pH range from 7.0 to 8.0 (Fig. 4).

Ashwell and Morell (1977) suggested a lectin-like mechanism of interaction of membrane surface molecules with other ligands. Lectins of cellular slime mould were supposed to be responsible for cell adhesion in these organisms (Rosen et al., 1977). The formation of the complex of opposite mating type flagellum membranes might be also of lectin--polysaccharide type. Preliminary results, however, showed that its

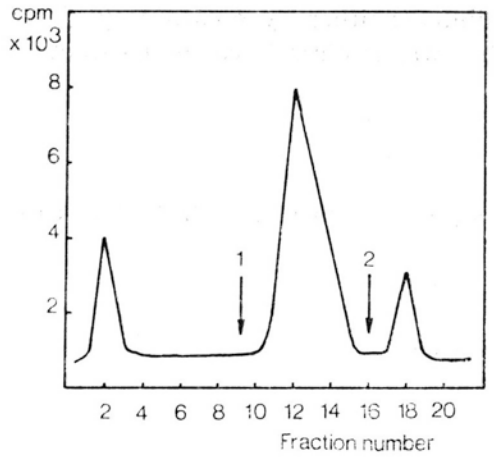


Fig. 3. Effect of divalent cations removal on the stability of the complex. Adhesive 89 gametic flagellum membrane material obtained by affinity chromatography was re-adsorbed on the same column (as in Fig. 2). First ('1') elution was carried out with phosphate buffer, pH 7.0 in whih $CaCl_2$ and $MgSO_4$ were omitted and EDTA was added to the final concentration of 0.001 M. The remaining material was eluted ('2') with acetate buffer, pH 4.5, as in Fig. 1.

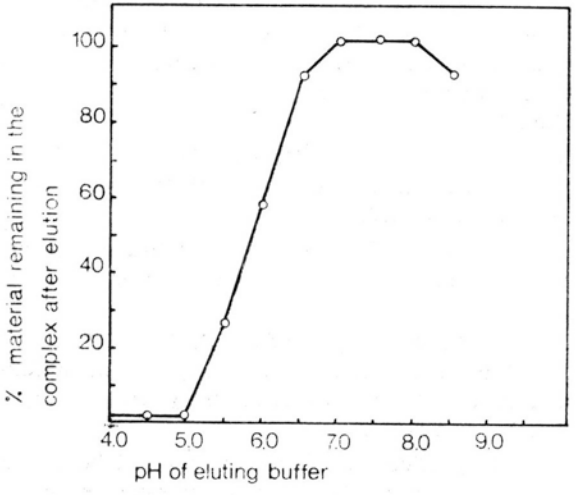


Fig. 4. Effect of pH on the stability of the complex. Adhesive 89 gametic flagellum membrane material obtained by affinity chromatography (Fig. 1) was re-adsorbed on the same column (as in Fig. 2). The column was then washed with phosphate buffers of pH ranging from 5.0 to 8.5 containing Triton X-100, $MgSO_4$, $CaCl_2$ and NaCl in concentrations indicated in legend to Fig. 1. The pH was adjusted by changing the proportions of phosphates. The remaining material was eluted with acetate buffer, pH 4.5, as in Fig. 1.

formation was inhibited neither by orosomucoid (human) nor by asialo-orosomucoid. Neither was it dissociated by α-D-methylmannoside.

Acknowledgments

This research was performed in part at dr R. F. Jones Laboratory at Stony Brook (State University of New York) which oportunity A. F. S. appreciates very much.

Thanks go to Professor W. Dobryszycka and dr M. Woźniak for making available to the author the radioisotope laboratory at the Medical Academy, Wrocław, Poland.

This study was supported by a INT Grant No. 76/22205.

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Izolacja frakcji odpowiedzialnej za swoistą adhezję gamet Chlamydomonas reinhardtii metodą chromatografii swoistej sorpcji

Streszczenie

Białka błon wici gamet szczepu 137+ rozpuszczalne w Tritonie X-100 związano z aktywowaną CNBr Sefarozą 4B. Tak przygotowane złoże użyto do chromatografii znakowanych J^{125} białek błon wici gamet szczepu 89 rozpuszczalnych w Tritonie X-100. Po zmianie pH elucji uzyskiwano pojedynczy szczyt zawierający 2.7-4.7% radioaktywności wyjściowej. Materiał uzyskiwany w szczycie poddany rechromatografii na tej samej kolumnie adsorbował się w 50-70%. Stwierdzono, że kompleks ten jest trwały w zakresie pH 7.0-8.0 w obecności kationów dwuwartościowych (Ca^{+2} i Mg^{+2}).