Identification and Further Characterization of the Specific Cell Binding Fragment from Sponge Aggregation Factor

Monika Gramzow,* Michael Bachmann,* Gerhard Uhlenbruck,‡ August Dorn,§ and Werner E. G. Müller*

*Institut für Physiologische Chemie, Universität, 6500 Mainz, Federal Republic of Germany; ‡Abteilung für Immunbiologie, Medizinische Universitätsklinik, 5000 Köln 41, Federal Republic of Germany; and §Institut für Zoologie, Universität, 6500 Mainz, Federal Republic of Germany

Abstract. Monoclonal antibodies (McAbs) were raised against the aggregation factor (AF) from the marine sponge Geodia cydonium. Two clones were identified that secrete McAbs against the cell binding protein of the AF complex. Fab fragments of McAbs: 5D2-D11 completely abolished the activity of the AF to form secondary aggregates from single cells. The McAbs were determined to react with the AF in vitro; this interaction was prevented by addition of the aggregation receptor, isolated and purified from the same species. After dissociation of the AF by sodium dodecyl sulfate and 2-mercaptoethanol, followed by electrophoretical fractionation, a 47-kD protein was identified by immunoblotting which interacted with the McAbs: 5D2-D11. During this dissociation procedure, the sunburst structure of the AF was destroyed. In a second approach, the 47-kD protein was isolated by immunoprecipitation; 12 molecules of this protein species were calculated to be associated with the intact AF particle. The 47-kD AF fragment bound to dissociated Geodia cells with a high affinity ($K_a$ of $7 \times 10^8$ M$^{-1}$) even in the absence of Ca$^{2+}$ ions; the number of binding sites was $\approx 4 \times 10^6$/cell. This interaction was prevented by addition of the aggregation receptor to the 47-kD protein in the homologous cell system. Moreover, it was established that this binding occurs species-specifically. The 47-kD fragment of the AF was localized only extracellularly by indirect immunofluorescence staining in cryostat slices. These data suggest that the 47-kD protein is the cell binding molecule of the AF from Geodia.

For almost 80 years (35) sponge systems have proven to be a useful model to study the mechanisms of cell-cell adhesion on the molecular level. In Demosponges two types of cell surface recognition processes exist: the initial primary aggregation phase, which is most likely second-order homophilic (16), and the subsequent secondary aggregation phase. The key macromolecules of the latter recognition system are the soluble aggregation factor (AF) (9, 15, 34) and the membrane-bound aggregation receptor (20, 34), hence this adhesion system is third-order heterophilic. Results from detailed biochemical studies with the AF and the aggregation receptor led to the formulation of the modulation theory of sponge cell adhesion (14, 24). The AFs from different sponges are particles of high molecular weights ($M_r$ 2.1 x 10$^7$ to 1.3 x 10$^8$) (8, 21) and are assembled from a series of proteins that are bound to each other either covalently or noncovalently (12, 21).

Misevic et al. (12) already have succeeded in the partial fragmentation of the AF from Microciona prolifera by chemical/biochemical means, which reveals that the cell binding site in AF is highly polyvalent and its structure of high molecular weight ($>1.5 \times 10^7$). In contrast to this result, earlier data (19) from studies with the AF from Geodia cydonium showed that the cell binding molecule from this particle has an $M_r$ of ~ only 20,000.

In the approach described here, we used monoclonal antibodies (McAbs), directed against the functional domain of the AF from Geodia cydonium, to identify and purify the cell binding protein from the particles. Moreover, we used the McAbs to localize the AF in tissue slices by indirect immunofluorescence staining.

Materials and Methods

Materials

The following materials were used. Class and subclass specific goat anti–mouse antisera were obtained from Tago Inc. (Burlingame, CA); protein A-Sepharose and Ficoll 400 from Deutsche Pharmacia (Freiburg, Germany); nitrocellulose sheets (BA; 0.45 μm; No. 40 1180) from Schleicher & Schüll (Dassel, Germany); 125I-sodium iodine (carrier free) from The Radiochemical Centre (Amersham, England); Dowex AG1-X2 and Bio-Gel P-300 from Bio-Rad Laboratories (Richmond, CA); pathotrol from Dade (Miami, FL); anti–mouse (whole molecule) peroxide conjugate (A-2028) and anti–mouse IgG (whole molecule)

1 Abbreviations used in this paper: AF, aggregation factor; ASW, artificial seawater; CMFSW, Ca$^{2+}$- and Mg$^{2+}$-free seawater; CMFSW-E, Ca$^{2+}$- and Mg$^{2+}$-free seawater containing EDTA; McAbs, monoclonal antibodies; TRITC, tetramethylrhodamine isothiocyanate.
tetramethylrhodamine isothiocyanate (TRITC) conjugate was used to label the AF. The Fab fragments of the McAbs were prepared by papain digestion (31). The Fab fragments of IgGs were removed by washing three times with 200 µl of blocking solution, using the 4-chloro-1-naphthol/hydrogen peroxide procedure (27). To rule out non-specific staining, controls with IgG from non-immunized mice were run in parallel.

**Immunohistochemical Procedures**

Fresh sponge cubes (2 x 2 x 2 mm) were fixed in 4% paraformaldehyde (in ASW) for 30 min (4°C) and then washed in PBS. Then the tissue was embedded in Tissue Tek and incubated overnight in a vacuum desiccator under reduced pressure. 2-µm cryostat sections were obtained at -25°C with a Dittes-Duspiva (Dittes, Heidelberg, FRG) cryostat. The sections were washed with PBS and then incubated in a 1:200 diluted preparation of the 5D2-D11 McAbs (overnight; 4°C) in a humid chamber. After a further wash with PBS, the slides were incubated with TRITC-conjugated anti-mouse IgG (2 h; 20°C). The excess antiserum was washed off with PBS, and the sections were examined light microscopically. An Osram XBO 140 xenon burner served as light source; an exciter interference filter at 556 nm was used. A heat absorbing filter, Schott BG38, was mounted between light source and exciter filter, a cut off filter.
Leitz K580 was inserted into the microscope tube. For antibody absorption, the McAbs were mixed with 200 µg of purified AF. The mixture was centrifuged (200,000 g; 2 h; 2°C) to remove the AF-IgG complex, and the supernatant was used as control antibodies. Protein was determined by the Fluoram method (33) using pathotrol as a standard. The AF was visualized electron microscopically as described (15).

Results

Antibody Characterization

82 cell hybrids were raised that produced antibodies against the AF (ELISA system). The Fab fragments of the two IgG preparations (coded 5D2-D11 and 7B10-D11) were found to inhibit AF-mediated cell–cell interaction (Table I). Using the Ouchterlony test procedure the 5D2-D11 antibodies were characterized as IgG2b and the 7B10-D11 as IgG1. The IgGs produced by clone 5D2-D11 displayed a stronger neutralizing effect on the AF activity than the preparation from 7B10-D11 and were therefore used for the subsequent experiments. An amount of 10 µg of IgGs from clone 5D2-D11 abolished the AF activity (30 µg) completely and reduced the size of the aggregates from 2,150 µm (secondary aggregates) to 245 µm (primary aggregates; reference 16) (Table I).

The McAbs were determined to interact with the AF. As shown in Fig. 1, the IgGs from clone 5D2-D11 bound to the high molecular weight AF. This complex was visualized by TRITC-conjugated anti–mouse IgGs; it eluted from the P-300 column within the $V_e/V_o$ (5) range 1.0–1.5. The IgGs that had not reacted with the AF eluted with a $V_e/V_o$ above 2.7. Co-incubation of the AF and the McAbs with the homologous aggregation receptor almost completely suppressed the antibody–antigen reaction, indicating that the IgGs from 5D2-D11 compete with the aggregation receptor for the identical or a spatially adjacent binding site at the AF. The electron microscopical analyses of the fractions 10–14 (Fig. 1) revealed that in both assays (in the presence or absence of the aggregation receptor) 90% of the visible structures had the typical “sunburst” appearance (Fig. 2a).

Identification of the Cell Binding Domain of an AF

As known from previous studies (3, 12) the sponge AF is composed of different proteins that are noncovalently or covalently bound to each other (19). The functional domain of the AF was identified after the disintegration of the particle in the presence of sodium dodecyl sulfate and a subsequent separation by polyacrylamide gel electrophoresis (see Materials and Methods; Fig. 3a). After the transfer of the proteins to nitrocellulose, the monospecific antibody (5D2-D11), which was found to suppress secondary aggregation (Table I), reacted only with one protein species characterized by a molecular weight of 47,000 (Fig. 3b). As a control, the polyacrylamide gel was stained for protein after the blotting procedure. No bands became visible (Fig. 3c), indicating that all of the proteins had been transferred on the blot. During the treatment with sodium dodecyl sulfate and 2-mercaptoethanol the sunburst structure of the AF (Fig. 2a) appeared to be dissociated into the central ring and the free arms (Fig. 2b).

Table I. Influence of McAbs against Geodia AF on the Secondary Aggregation of Dissociated Homologous Cells

| McAbs added during preincubation with the AF | Size of the secondary aggregates µm |
|---------------------------------------------|-------------------------------------|
| None                                        | 2,150 ± 470                         |
| 5D2-D11                                     |                                     |
| 1 µg                                        | 1,210 ± 255                         |
| 3 µg                                        | 430 ± 85                            |
| 10 µg                                       | 245 ± 50                            |
| 7B10-D11                                    |                                     |
| 1 µg                                        | 1,840 ± 405                         |
| 3 µg                                        | 1,090 ± 205                         |
| 10 µg                                       | 685 ± 150                           |

Where indicated, Fab fragments of two IgG preparations were preincubated with 30 µg of AF as described in Materials and Methods. Incubation in the presence of cells was performed in the standard assay. The results are from five parallel determinations; the SDs are given.
domain of the AF. Purified AF was electrophoresed on a 12% polyacrylamide gel under denaturing conditions and either directly stained with Coomassie Brilliant Blue (a) or the proteins were transferred to a nitrocellulose filter and incubated with McAbs (5D2-D11) to identify the binding protein as described under Materials and Methods. (b, c) Polyacrylamide gel of AF (as in a) after blotting procedure and staining with Coomassie Brilliant Blue. (d and e) Sodium dodecyl sulfate polyacrylamide gel electrophoresis of the radiolabeled binding protein in the complex AF; visualization was performed by autoradiography (d) or by silver staining (e). The binding protein was purified by immunoprecipitation. Molecular weight standards were (1) bovine serum albumin (Mr 66,000), (2) ovalbumin (45,000), (3) carbonic anhydrase (31,000), (4) soybean trypsin inhibitor (21,500), and (5) lysozyme (14,400).

Figure 3. Identification of the binding protein in the aggregation factor complex. (a, b, and c) Protein blot analysis of the functional domain of the AF. Purified AF was electrophoresed on a 12% polyacrylamide gel under denaturing conditions and either directly stained with Coomassie Brilliant Blue (a) or the proteins were transferred to a nitrocellulose filter and incubated with McAbs (5D2-D11) to identify the binding protein as described under Materials and Methods (b). (c) Polyacrylamide gel of AF (as in a) after blotting procedure and staining with Coomassie Brilliant Blue. (d and e) Sodium dodecyl sulfate polyacrylamide gel electrophoresis of the radiolabeled binding protein in the complex AF; visualization was performed by autoradiography (d) or by silver staining (e). The binding protein was purified by immunoprecipitation. Molecular weight standards were (1) bovine serum albumin (Mr 66,000), (2) ovalbumin (45,000), (3) carbonic anhydrase (31,000), (4) soybean trypsin inhibitor (21,500), and (5) lysozyme (14,400).

the cells, was isolated by an immunoprecipitation procedure (see under Materials and Methods). The protein was analyzed by polyacrylamide gel electrophoresis and subsequent autoradiography or silver staining. The results showed (Fig. 3, d and e) that only one protein with a molecular weight of 47,000 D became visible. This value is identical with the molecular weight of that protein in the complex AF, identified by the described blotting procedure using the same McAbs.

Interaction of the Functional Domain of the AF with Cells

A quantitative evaluation revealed that 423 ng of the 47-kD protein was obtained from 100 μg of purified AF (Mr 1.3 × 10^12; reference 21), using the described immunoprecipitation procedure. On a stoichiometric basis, ~12 molecules of 47-kD protein were calculated to be associated with the AF complex.

The 47-kD AF fragment bound with a high affinity to homologous cells (Table II). The affinity constants (K_a) as well as the maximal numbers of specific binding sites per cell did not vary remarkably in dependence on the presence (ASW) or absence of Ca^{2+} ions (CMFSW or CMFSW-E); the K_a values were determined to be ~7 × 10^6 M^{-1} and the binding sites per cell to be ~4 × 10^6. On the other hand, heterologous cells from sponges of the subclasses Tetractinomorpha (Tethya lyneurium) and Ceratinomorpha (Mycale massa, Spongia officinalis) as well as of the class Calcispongia (Clathrina coriacea) did not bind measurable amounts of the AF fragment. As a further control to support the specificity of the binding of the AF fragment to homologous cells, the soluble and purified aggregation receptor from Geodia cydonium was added to incubation reaction assay (Table II). The results revealed that in the presence of 2.5 μg of aggregation receptor (Mr 18,000, reference 20; corresponding to 8.4 × 10^{13} molecules), no binding of 47-kD protein to homologous cells could be measured; in this experiment 100 ng of the AF fragment (1.3 × 10^{12} molecules) was added to 2.5 × 10^7 cells. These findings, together with the above-mentioned immunological data (Fig. 1), strongly suggest that the 47-kD fragment of the AF is that protein which interacts with the aggregation receptor, irrespectively of whether the receptor is in the membrane bound or the solubilized state.

Table II. Binding of 47-kD Protein, Which Carries the Biologically Active Domain, to Sponge Cells under Different Incubation Conditions

| Cells from | Incubation conditions | K_a | Number of binding sites per cell |
|-----------|-----------------------|-----|---------------------------------|
| Geodia cydonium | CMFSW | 71.3 | 4.2 |
|             | CMFSW-E | 68.0 | 4.1 |
|             | ASW | 74.7 | 4.2 |
|             | CMFSW + aggregation receptor | 0.05 | 0.08 |
| Clathrina coriacea | CMFSW | <0.05 | <0.08 |
| Tethya lyneurium | CMFSW | <0.05 | <0.08 |
| Mycale massa | CMFSW | <0.05 | <0.08 |
| Spongia officinalis | CMFSW | <0.05 | <0.08 |

Homologous or heterologous cells were incubated in the presence of radiolabeled 47-kD AF fragment as described under Materials and Methods. The binding parameters (association constants and maximal number of specific binding sites per cell) were determined. In one experiment 2.5 μg of aggregation receptor (Geodia cydonium) was added to the incubation mixture.

Figure 4. Localization of the AF in sections of Geodia cydonium. (a) Indirect immunofluorescence staining of a section through a cell cluster in the cortex region with McAbs directed against the functional domain of the AF. (b) Staining with the same McAbs adsorbed with AF.

Figure 4. Localization of the AF in sections of Geodia cydonium. (a) Indirect immunofluorescence staining of a section through a cell cluster in the cortex region with McAbs directed against the functional domain of the AF. (b) Staining with the same McAbs adsorbed with AF.

The AF was localized immunohistochemically in cryostat slices using McAbs specifically directed against the biologically active domain (47-kD protein) of this proteoglycan complex. The antibody-antigen complex was visualized by the indirect fluorescence technique (Fig. 4a). With this method the antigen was detectable only extracellularly and was found to be localized on the surfaces of the cells as apparently continuous layers. Adsorption of the McAbs from AF eliminated the specific antibody staining (Fig. 4b).

Gromnow et al. Cell Binding Protein from Sponge AF
**Discussion**

The sponge AFs, which are large glycoprotein complexes (M, \(2.1 \times 10^{6} - 1.3 \times 10^{5}\)), are provided with a series of functional subunits: (a) \(Ca^{++}\) binding site(s) (10, 29), which control the stability of the particle as well as its cell binding function; (b) the lectin binding site (6), which determines the functional role of the AF in the cell–cell and the cell–substrate interactions; (c) two glycosyl transferases (24), which modulate the strength of the interactions between AF and aggregation receptor; and (d) the cell binding site. The latter was assumed to interact with the membrane-bound aggregation receptor (20, 34).

Applying immunobiochemical and immunohistochemical techniques, we succeeded in identifying and partially characterizing that fragment of the AF from *Geodia cydonium* that exhibits the cell binding function. After disintegration of the AF in the presence of sodium dodecyl sulfate, a 47-kD protein could be identified by immunoblotting which reacted with a selected McAb. AF in the presence of sodium dodecyl sulfate, a 47-kD protein characterizing that fragment of the AF from *Geodia cydonium* that exhibits the cell binding function. After disintegration of the AF in the presence of sodium dodecyl sulfate, a 47-kD protein could be identified by immunoblotting which reacted with a selected McAb. AF in the presence of sodium dodecyl sulfate, a 47-kD protein was characterized by immunoblotting which reacted with a selected McAb. AF in the presence of sodium dodecyl sulfate, a 47-kD protein could be identified by immunoblotting which reacted with a selected McAb. AF in the presence of sodium dodecyl sulfate, a 47-kD protein could be identified by immunoblotting which reacted with a selected McAb.

After purification of the 47-kD protein by immunoprecipitation and its application for binding studies with homologous cells revealed that approximately 12, 47-kD protein molecules are associated with one AF particle. Hence the *Geodia* AF is highly polyvalent with respect to its number of cell binding sites. The affinity of the isolated cell binding protein to homologous cells is high and independent of the presence of \(Ca^{++}\) ions. This result is a further evidence for the presence of at least one separate \(Ca^{++}\) binding molecular species in the sponge AFs, as already suggested earlier (10). Concerning the *Geodia* AF, 820 mol \(Ca^{++}\) was determined to be bound per 1 mol AF (unpublished observations). It is well established that \(Ca^{++}\) ions play essential roles in AF-mediated cell–cell interactions (35), however, the molecular aspect of this event is not understood. Since our earlier studies (22), which were confirmed later (1), it is well established that macromolecular polycations, but not monovalent cations, can substitute for \(Ca^{++}\). At least one possibility that appears to be ruled out by our presented data is that \(Ca^{++}\) ions are involved in the species-specific restriction mechanism of the *Geodia* AF, since the binding of the 47-kD protein to cells occurred only in the homologous system.

A further outcome of the binding studies with the 47-kD protein and the homologous *Geodia* cells was the determination of the number of binding sites per cell. Competition experiments revealed that the binding sites on the plasma membrane are identical with the isolated aggregation receptor. Using this approach, \(4 \times 10^{6}\) binding sites were determined to be available at one *Geodia* archaeocyte. This figure is close to that determined already earlier (23) using a different, solely biochemical procedure.

On the basis of biochemical (19, 34) and first electron microscopical data (17) it was strongly suggested that (a) the sponge AF exists extracellularly and (b) its functions there as a bridge-like molecule, linking the cells together. Now, by application of an indirect immunofluorescence staining method using the McAbs directed against the cell binding protein, the first direct proof for such functions of the AF is given. The staining at the cell–cell contact zones was continuous, which indicates a high density of AF molecules between cells.

We want to thank Dr. J. Conrad for helpful discussions, Ms. V. Pondeljak and R. Steffen as well as Mr. R. Beyer for skillful biochemical preparations, and Ms. K. Bartelt for typing the manuscript.

This research was supported by grants from the Deutsche Forschungsgemeinschaft (Mu 348/8-1) and from the Academy of Science and Literature, Mainz.

Received for publication 16 July 1985, and in revised form 13 September 1985.

**References**

1. Burkart, W., and M. M. Burger. 1981. The contribution of the calcium-dependent interaction of aggregation factor molecules to recognition: a system providing additional specificity forces? J. Supramol. Struct. Cell Biochem. 16:179–192.

2. Clemens, M. J. 1984. Translation of eukaryotic messenger RNA in cell-free extracts. In Transcription and Translation. B. D. Hames and S. J. Higgins, editors. IRL Press, Oxford. 231–270.

3. Conrad, J., G. Uhlenbruck, R. K. Zahn, B. Kurelec, B. Jerievic, and W. E. G. Müller. 1984. The role of lectin I and of glycoconjugates in recognition of cells from the siliceous sponge *Geodia cydonium*. Biol. Cell. 51:287–294.

4. Cuello, A. C., C. Milstein, and G. Galfi. 1983. Preparation and application of monoclonal antibodies for immunohistochemistry and immunocytochemistry. In Immunohistochemistry. A. C. Cuello, editor. John Wiley & Sons, New York. 214–226.

5. Determann, H. 1969. Gel Chromatography. Springer-Verlag, Berlin. 202 pp.

6. Diehl-Seifert, B. G. Uhlenbruck, M. Geisert, R. K. Zahn, and W. E. G. Müller. 1985. Physicochemical and functional characterization of the polymerization process of the *Geodia cydonium* lectin. Eur. J. Biochem. 147:517–523.

7. Ey, P. L., S. J. Prowse, and C. R. Jenkin. 1978. Isolation of pure IgG1 and IgG2b immunoglobulins from mouse serum using protein A-Sepharose. Immunchemistry. 15:429–436.

8. Henkart, P., S. Humphreys, and T. Humphreys. 1973. Characterization of sponge aggregation factor. A unique proteoglycan complex. Biochemistry. 12:3045–3050.

9. Humphreys, T. 1963. Chemical dissolution and in vitro reconstitution of sponge cell adhesion. J. Cell Biol. 8:27–47.

10. Jumblatt, J. E., V. Schlup, and M. M. Burger. 1980. Cell–cell recognition: specific binding of Microciona sponge aggregation factor to homotypic cells and the role of calcium ions. Biochemistry. 19:1038–1042.

11. Junqua, S., M. Lemonnier, and L. Robert. 1981. Glycoconjugates from *Spongilla officinalis* (phyllum Porifera). Isolation, fractionation by affinity chromatography on lectins and partial characterization. Comp. Biochem. Physiol. 69B:445–453.

12. Misievic, G. N., J. E. Jumblatt, and M. M. Burger. 1982. Cell binding fragments from a sponge proteoglycan-like aggregation factor. J. Biol. Chem. 257:6931–6936.

13. Moscona, A. A. 1963. Studies on cell aggregation: demonstration of materials with selective cell-binding activity. Proc. Natl. Acad. Sci. USA. 49:742–747.

14. Müller, W. E. G. 1985. Cell–cell recognition in lower multicellular eukaryotes. Models supporting the modulation theory of adhesion. Proc. Subcell. Biol. 9:156–175.

15. Müller, W. E. G., and R. K. Zahn. 1973. Purification and characterization of a species-specific aggregation factor in sponges. Exp. Cell Res. 80:95–104.

16. Müller, W. E. G., J. Conrad, R. K. Zahn, M. Gramzow, B. Kurelec, and G. Uhlenbruck. 1985. Identification and isolation of the primary aggregation factor from the cell membrane of the sponge *Geodia cydonium*. Mol. Cell. Biochem. 67:55–64.

17. Müller, W. E. G., A. Dorn, and G. Uhlenbruck. 1985. The molecular mechanisms of the distinct calcium-dependent aggregation systems in marine sponges and corals. Acta Histochem. 31(Suppl):137–46.

18. Müller, W. E. G., I. Müller, V. Pondeljak, B. Kurelec, and R. K. Zahn. 1978. Species-specific aggregation factor in sponges; isolation, purification and characterization of the aggregation factor from *Suberites domuncula*. Differentiation. 10:45–53.

19. Müller, W. E. G., I. Müller, and R. K. Zahn. 1974. Two different aggregation principles in reaggregation process of dissociated sponge cells (*Geodia cydonium*). Experientia. 30:899–900.

20. Müller, W. E. G., I. Müller, R. K. Zahn, and B. Kurelec. 1976. Species-specific aggregation factor from sponges. VI. J. Cell Sci. 21:227–241.

21. Müller, W. E. G., R. K. Zahn, J. Arendes, B. Kurelec, R. Steffen, and I. Müller. 1979. Aggregation of sponge cells. Cell-aggregation of the circular proteoid protein. Biochim. Biophys. Acta. 551:363–367.

22. Müller, W. E. G., R. K. Zahn, B. Kurelec, and I. Müller. 1978. Aggregation of sponge cells. XIV. Exp. Cell Res. 113:409–414.
23. Müller, W. E. G., R. K. Zahn, B. Kurelec, and I. Müller. 1978. Species-specific aggregation factor in sponges; VIII. Wilhelm Roux’s Arch. Dev. Biol. 184:29-40.
24. Müller, W. E. G., R. K. Zahn, B. Kurelec, I. Müller, G. Uhlenbruck, and R. Vaith. 1979. Aggregation of sponge cells; a novel mechanism of controlled intercellular adhesion, basing on the correlation between glycosyltransferases and glycosidases. J. Biol. Chem. 254:1280-1287.
25. Müller, W. E. G., R. K. Zahn, I. Müller, B. Kurelec, G. Uhlenbruck, and P. Vaith. 1981. Cell aggregation of the marine sponge Geodia cydonium. Identification of lectin-producing cells. Eur. J. Cell Biol. 24:28-35.
26. Munson, P. J., and D. Dodbard. 1980. LIGAND: a versatile computerized approach for characterization of ligand-binding systems. Anal. Biochem. 107:220-239.
27. Nakane, P. K. 1968. Simultaneous localization of multiple tissue antigens using the peroxidase-labeled antibody: a study on pituitary glands of the rat. J. Histochem. Cytochem. 16:557-560.
28. Oakley, B. K., D. R. Kish, and N. R. Morris. 1980. A simplified ultrasensitive silver stain for detecting proteins in polyacrylamide gels. Anal. Biochem. 105:361-362.
29. Rice, D. J., and T. Humphreys. 1983. Two Ca** functions are demonstrated by the substitution of specific divalent lanthanide cations for the Ca** required by the aggregation factor complex from the marine sponge, Microciona prolifera. J. Biol. Chem. 258:6394-6399.
30. Towbin, H., T. Staehelin, and J. Gordon. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some application. Proc. Natl. Acad. Sci. USA. 76:4350-4354.
31. Utsumi, S. 1969. Stepwise cleavage of rabbit immunoglobulin G by papain and isolation of four types of biologically active Fc fragments. Biochem. J. 112:343-355.
32. Weber, K., and M. Osborn. 1969. The reliability of molecular weight determinations by dodecyl sulfate-polyacrylamide gel electrophoresis. J. Biol. Chem. 244:4406-4412.
33. Weigele, M., S. L. DeBernardo, and W. Leimgruber. 1973. Fluorometric assay of secondary amino acids. Biochim. Biophys. Res. Commun. 50:352-356.
34. Weinbaum, G., and M. M. Burger. 1973. A two-component system for surface guided reassociation of animal cells. Nature (Lond.). 244:510-512.
35. Wilson, H. V. 1907. On some phenomena of coalescence and regeneration in sponges. J. Exp. Zool. 5:245-258.