CHARACTERIZATION OF SPECIFIC CELL AGGREGATING MATERIALS FROM SPONGE CELLS

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Materials capable of causing selective re-aggregation of dissociated cells of marine sponges can be obtained by washing dispersed sponge cells with calcium- and magnesium-free sea water (Moscona, 1963; Humphreys, 1963). The work presented here indicates that such preparations consist largely of glycoproteins in particulate units 20 - 25 Å in diameter.

Using appropriate bioassay procedures it was previously found that preparations obtained from Microciona prolifera (to be referred to as M) and from Haliclona occulata (H) (both from Woods Hole) cause selectively the re-aggregation of cells dissociated from the corresponding species. Evidence of comparable selectivities was obtained by MacLennan (1963) using immunological techniques and other species. These findings agreed with the working assumption (Moscona, 1960; 1962; 1963) that aggregation and histological attachment of cells are mediated by specific macromolecular products functioning extracellularly, i.e., at the cell surface or between cells; and that the molecular characteristics and interactions of these cell-surface constituents are involved in contact selectivities and grouping properties of cells. The sig-

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nificance of mechanisms involved in cell contact and cell aggregation to development and cell function in multicellular systems prompted an examination of some of the chemical aspects of these cell-aggregating preparations, as a first step towards an understanding of their properties.

"Crude extracts" with cell-aggregating activity were obtained by washing suspensions of living cells in cold sea-water without divalent cations. (For details of "crude extracts," bioassay and biological characterization procedures, see Humphreys, 1963; Moscona, 1963.) From these extracts the activity was recovered and concentrated by centrifugation for 90 min. at 110,000g at 0°C, following the addition of 0.002M $CaCl_2$ at neutral pH. The sedimented material, in the form of a viscous pellet, was washed several times in 0.002M $CaCl_2$. The M pellets were mucoidal; the H pellets had a semi-solid consistency. The yields varied from 0.5 to 0.7 mg/10 ml crude extracts.

These purified preparations, resuspended in 0.002M $CaCl_2$, were examined by electron microscopy using negative stainings (Figs. 1 and 2). Both M and

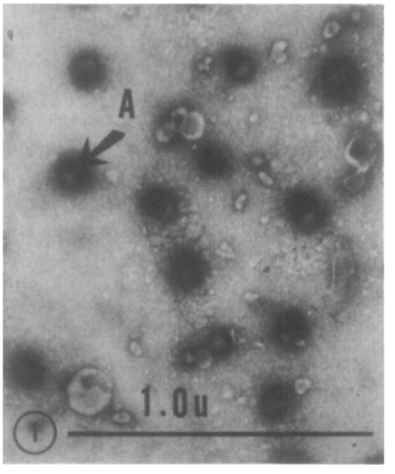

Fig. 1. Negatively stained (1% neutralized phosphotungstate) M cell aggregating preparation showing the 20 to 25 Å diameter unit particles and aggregates of particles (A). The background consists of sheets of the small units. 50,000 X.

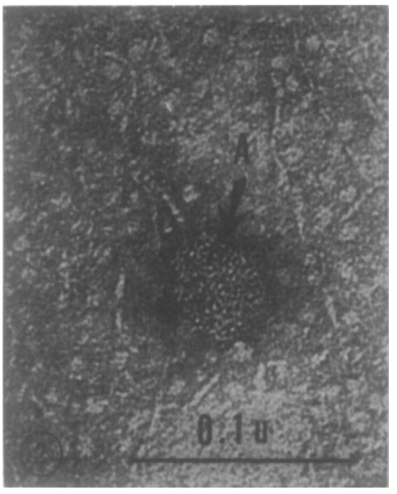

Fig. 2. Negatively stained (saturated aqueous uranyl acetate) H cell aggregating preparation showing the 20 to 25 Å units in large spherical aggregates (A), in rows and in sheets of varying thickness. 375,000 X.

H materials consisted of roughly spherical unit particles 20 - 25 Å in diameter. These were in spherical aggregates ranging from 200 to 2000 Å in diameter, with a preponderance in the 500 to 1000 Å range; or packed in irregular sheets; or in rows (Fig. 2). The size and frequency of occurrence of the large aggregates varied from preparation to preparation, in agreement with the ultracentrifugal patterns referred to below.

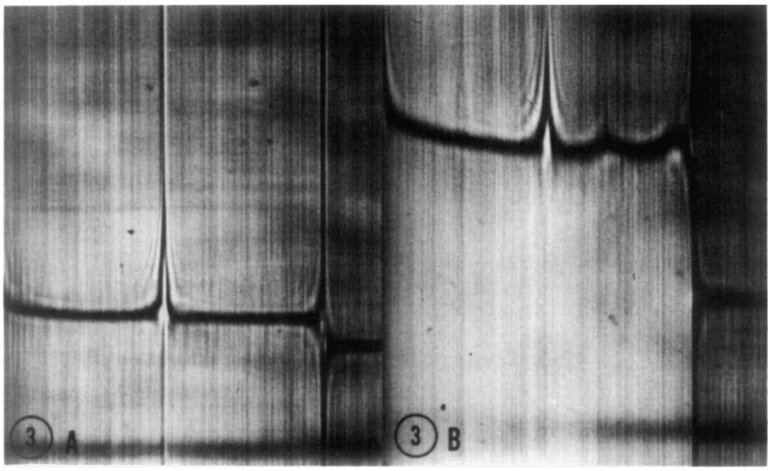

Fig. 3. Ultracentrifugal sedimentation patterns of M cell aggregating preparation. A: Fresh suspension in 0.002M calcium chloride. Picture taken at 55° phase angle, 51 min. after attaining a speed of 29,500 RPM. B: Same suspension dialyzed 18 hours against calcium-free 0.9% sodium chloride solution at 4°. Picture taken at 50° phase angle, 17 min. after attaining a speed of 59,700 RPM. In both pictures sedimentation is from right to left.

Examined in the ultracentrifuge the majority of M preparations formed a sharp boundary sedimenting at S_{20} values of 25.0 (Fig. 3); the others were polydisperse. The H preparations were always polydisperse. After overnight dialysis against calcium-free 0.1 M sodium chloride solution, monodisperse M preparations also became polydisperse with boundaries sedimenting at S_{20} values of 25.3, 14.2 and 2.3, respectively (Fig. 3). The estimated molecular weight of the slowest sedimenting component was within 15,000 to 20,000.

Table I shows that the amino acid compositions of the M and H preparations are essentially similar. Calculated on the basis of single residues of histidine and methionine, the M and H preparations contain 72 - 75 and 51 - 54 residues, respectively. The only carbohydrates identified in both preparations were those listed in Table I. No significant amounts of sialic and uronic acids were detected. As with the amino acids, the differences in the carbohydrate components were quantitative: H preparations contained smaller amounts of the monosacharides present also in M preparations. Semiquantitative estimation of sulfate content indicated at least 3 moles sulfate per mole of glycoprotein, possibly considerably more. The variations in glucose content of individual preparations have not, so far, been explained. The species differences in sugar content did not appear to result from different degrees of artifactual degradation of possibly identical carbohydrate moieties during the preparation of the extracts, since the total amounts of carbohydrates present in the crude extracts and in pellets of H preparations did not amount to more than 1/3 of that found in pellets of M preparations.

Analyses for ribose and deoxyribose indicated that the total amount of nucleic acid was less than five percent. The quantities of lipids detected were below one mole per mole of protein and probably represented residual contamination with sponge pigments. (Fatty acids and steroids were determined by gas chromatography, following hydrolysis in 2 N sodium hydroxide at 98° for 16 hours and separation of the saponifiable and non-saponifiable lipid fractions.)

TABLE I

Composition of cell aggregating preparations from Microciona prolifera (M) and Haliclona occulata (H), expressed in moles per mole protein, assuming the protein to contain single histidyl and methionyl residues.

	M	H
Lysine	1.93	2.75
Histidine	0.88	0.98
Arginine	2.51	2.18
Aspartic acid	9.34	6.17
Threonine	5.20	3.26
Serine	4.29	3.80
Glutamic acid	8.06	5.31
Proline	3.87	2.38
Glycine	8.51	4.17
Alanine	5.09	3.02
Valine	6.91	3.85
Methionine	1.12	1.02
Isoleucine	3.84	3.17
Leucine	5.82	4.50
Tyrosine	2.15	1.69
Phenylalanine	4.31	3.88
Glucosamine	2.6	2.7
Fucose	5.0	2.0
Mannose	2.2	1.2
Galactose	5.6	1.6
Glucose	17.3 - 40.6	trace - 9.8

The amino acid composition was determined using a Spinco-Beckman amino-acid analyzer (Spackman et al., 1958), following hydrolysis in 6 N hydrochloric acid in vacuo for 40 hours at 105°. Glucosamine was similarly determined in 1 N hydrochloric acid hydrolysates, carried out at 98° for 2, 5, 9, and 14 hours. Sugars were estimated by gas-liquid column chromatography, using a microadaptation of the method of Sweeley et al. (1963) following methanolysis at 80°, in vacuo for 16 hours. Monosaccharides were also identified by paper chromatography following hydrolysis in 0.1 N hydrochloric acid at 98° for 16 hours. The solvents used were ethyl acetate-acetic acid-water (44:20:10 vols.) and n-butanol-pyridine-water (6:4:3 vols.); the chromatograms were developed with an alkaline silver nitrate reagent. Estimates for glucose were checked by direct determination with glucose oxidase ("glucostat"; Worthington Biochemical Corp.)

The amino acid and sugar compositions are averages of determinations on three and five different batches, respectively. Cysteine and tryptophan were not determined. The amounts of glucose varied over too wide a range to give significant average values.

The minimal equivalent weight of the M material calculated from the amino acid and carbohydrate analyses, assuming the presence of single histidyl and methionyl residues in each molecule, is of the order of 13,000. This is not far from the roughly estimated molecular weight of the slowest sedimenting component of the polydisperse preparations, and fits within the size limits of

the smallest particulate units visualized by electron microscopy. Therefore, these units could not consist of more than a few molecules, possibly as little as one.

The analytical data suggest that the particulate material consists predominantly of glycoprotein. That the carbohydrate might indeed be bound to the protein is indicated by the lack of separation of the two when chromatographed on Sephadex gel columns; furthermore, following removal of about 80% of the protein by proteolysis the carbohydrate moiety remained attached to a residual peptide.

The homogeneity and purity of the unit particles has been demonstrated by the method of purification, their ultracentrifugal behavior and by electron microscopy. Nevertheless, no definitive proof is available, at present, that the particles of the glycoprotein are the active substance, or that the preparation is "pure" in a molecular sense. However, the high biological activity of the preparation makes it very unlikely that activity resides in an as yet undetected "impurity." Assuming that the biological activity of these preparations is, indeed, a function of the glycoprotein particles, it becomes of interest to examine their molecular structure, manner of uptake and attachment to the cell surface, functional dependence on calcium, and relation of specificity to the protein or carbohydrate moiety. Work along these lines is in progress.

REFERENCES

Humphreys, T., Developtl. Biology, 8, 27 (1963).
MacLennan, A. P., Biochem. J., 89, 99 p. (1963).
Moscona, A. A., in Developing Cell Systems and Their Control, The Ronald Press Company, p. 45 (1960).
Moscona, A. A., J. Cell. Comp. Physiol., 60, suppl. 1, 65 (1962).
Moscona, A. A., Proc. Natl. Acad. Sci. U.S., 49, 742 (1963).
Spackman, D. H., Stein, W. H. and Moore, S., Anal. Chem., 30, 1190 (1958).
Sweeley, C. C., Bentley, R., Makita, M. and Wells, W. W., J. Am. Chem. Soc., 85, 2497 (1963).