Isolation and Preliminary Chemical Analysis of the Cyst Wall of the Amoeba-Flagellate Naegleria grüberi

J. M. WERTH1 AND A. J. KAHN
Department of Zoology, Syracuse University, Syracuse, New York 13210

Received for publication 26 July 1967

Investigations of the chemical composition of protozoan cyst walls have been hampered by the difficulty in obtaining purified wall preparations. Recently some of the technical difficulties have been resolved and preliminary chemical analyses have been undertaken (G. Tomlinson and E. A. Jones, Biochim. Biophys. Acta 63:194, 1962; R. J. Neff, W. F. Benton, and R. H. Neff, J. Cell Biol.

The present communication describes a method for the isolation and purification of cyst walls from the amoeba-flagellate, Naegleria grüberi, and reports the results of certain preliminary analyses of purified wall preparations.

Cysts (Fig. 1) were harvested after 14 days of incubation, and isolated and purified by the methods outlined in Fig. 2. Unless otherwise specified, all reagents were sterilized and all operations were performed at 4 C. The effect of the initial isolation procedures was followed microscopically by staining preparations with Lugol's iodine. Lugol's iodine stains cytoplasm, but not walls, reddish-brown. Purified prepara-

FIG. 1. Unstained whole cysts of Naegleria grüberi under phase contrast. X ~700.

1 Present address: Department of Bacteriology and Botany, Syracuse University, Syracuse, N.Y.

1272
Cysts Collected $\rightarrow$ Washed (0.9% NaCl) $\rightarrow$ Pressure Disrupted

Suspended in Buffer, pH 7.0 $\leftarrow$ Sonically Disrupted $\leftarrow$ Wall Fragments Disrupted $\rightarrow$ Washed (0.9% NaCl)

Trypsin (100 μg/ml, 37°C, 6 Hrs, pH 7.6) $\rightarrow$ Washed $\rightarrow$ RNase (100 μg/ml, 37°C, 6 Hrs, pH 7.6) $\rightarrow$ Lyophilized $\&$ Stored, -12°C $\leftarrow$ Washed & Suspended in Distilled Water

Fig. 2. Isolation and purification sequence for cyst walls. Cysts were disrupted in a French Pressure Cell at 10,000 to 15,000 lb/in². Washing and collecting of samples was accomplished with a Sorvall SS-1 centrifuge. The buffer in all cases was 0.1 M phosphate buffer. Wall fragments were sonic treated in an MSE Ultrasonic Power Unit (~1.5 a).

remained constant. Figure 3 shows a purified, enzyme-treated cyst wall preparation.

Protein determinations through use of a modification of the method of O. H. Lowry et al. (J. Biol. Chem. 193:265, 1951) indicated that the cyst walls contained 40% (dry weight) protein.

Purified walls were hydrolyzed in 6 N HCl for periods of 16 to 24 hr at 100°C. Amino acids in the hydrolysate were separated by two-dimensional paper chromatography by use of n-butanol-acetic acid-water (4:1:5) for 24 hr in the first direction and n-butanol-methyl ethyl ketone-water-17 N ammonia (5:3:1:1) for 48 hr in the second direction (M. Wolfe, Biochim. Biophys. Acta 23: 186, 1957). After spraying the dried chromatograms with ninhydrin, the following amino acids were identified: cysteic acid, aspartic acid, glutamic acid, lysine, arginine, glycine, histidine, serine, alanine, proline, tyrosine, valine, threonine, isoleucine, leucine, phenylalanine, and an unidentifed component.


Alkaline hydrolysis of purified walls in 60% KOH for 3 hr at 160°C (G. Tomlinson and E. A. Jones, Biochim. Biophys. Acta 63:194, 1962) resulted in the appearance of an alkali-insoluble component which had the solubility and staining properties of cellulose. This tentative identification was supported by the results of one-dimensional paper chromatography [by use of n-butanol-pyridine-water (6:4:3)] of 2 N H₂SO₄ hydrolysates which revealed the presence of only one monosaccharide, glucose. It is interesting that the cyst wall did not exhibit a positive reaction with cellulose staining reagents (J. Brontë Gatenby and H. W. Beams, The Microtomist's Vade-Mecum, p. 648, The Blakiston Company, Philadelphia, 1950), but that positive results were obtained with the isolated alkali-insoluble component. This apparent "masking effect" of cellulose was also noted by Tomlinson and Jones in the cyst walls of Acanthamoeba sp.

Lipids were extracted by the method of M. J. R. Salton (Biochim. Biophys. Acta 10:512, 1953). Of a 13% total, approximately 2% was readily extractable and 11% was tightly bound.
Our results indicate certain similarities between the cyst wall of *N. grüberi* and that of another soil amoeba, *Acanthamoeba* sp. (R. J. Neff, W. F. Benton, and R. H. Neff, J. Cell Biol. 23:66A, 1964). The walls of both organisms have a relatively high protein content, readily extractable and tightly bound lipid components, and an alkali-insoluble material that appears to be cellulose. The high lipid and protein content and masking of the cellulose-staining properties in the unhydrolyzed cyst wall suggests that the cellulose may be bound to a lipoprotein complex.

This investigation was supported by grant GB-4281 from the National Science Foundation to A. J. Kahn.