CHEMICAL ANALYSIS OF A MUCOPOLYMER COMPONENT IN CELL WALLS OF THE BLUE-GREEN ALGA PHORMIDIUM UNCINATUM.

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Recent progress in the elucidation of the chemical nature of the cell walls of bacteria and the discovery of a few new compounds, such as α - ε - diaminopimelic acid and muramic as unique building units, present only in the wall fraction of the bacterial cell (for review see: Salton 1960), have focused attention on the possibility to utilize the specific chemical characteristics of the cell wall for the purposes of bacterial taxonomy (Cummins and Harris, 1956). Work and Dewey (1953) have extended the search for diaminopimelic acid beyond the bacteria proper to other microorganisms and have found it in only one other group, the blue green algae. A taxonomic relationship between bacteria and cyanophyceae has long been suspected although the evidence for it rested largely and exclusively on morphological data (Pringsheim, 1949). Obviously, the demonstration of a chemical identity between the macromolecular cell wall structures in bacteria and in blue green algae would be a weighty argument in support of the morphological evidence. Therefore a quantitative chemical study of isolated cell walls from the blue green alga Phormidium uncinatum was undertaken.

Materials and Methods.

Phormidium uncinatum was grown by daylight in stationary liquid culture. Cell walls were isolated from the harvested algae by applying the conventional Mickle technique (Salton and Horne, 1951) and separating walls and cell content by differential centrifugation. The purity of the final wall preparation was verified by electron microscopy.

Quantitative analysis of amino acids and amino sugars was carried out by ion exchange chromatography on an automatic amino acid analyzer (Hannig, 1959). Samples were hydrolyzed in 4 N HCl for 14 hours at 100°C in a nitrogen atmosphere.

Reducing sugars were identified by paper chromatography of wall samples, hydrolyzed in 1 N HCl for 2 hours at 100°C, using aniline phosphate as spraying agent.

Results

a) Amino acids and amino sugars.

The mean values of three quantitive analysis are summarized in table 1. Data on mucopolymer membranes from two gram-negative bacteria, E.coli B and Spirillum sp. are included for comparison (Martin and Frank, 1962).

In Phormidium ninhydrinpositive compounds constitute 55 % of the cell wall dry weight. 95 % of these or 52 % of the total wall are contributed by two amino sugars, glucosamine and muramic acid and by three amino acids, alanine, glutamic acid and diaminopimelic acid.

b) Reducing sugars.

2 mgs of hydrolyzed dry cell wall were chromatographed side by side with a standard mixture conlaining 20 µg each of galacturonic acid, galactose, glucose, arabinose, xylose and rhamnose. No trace of neutral reducing sugars or uronic acids could be found in Phormidium walls under these conditions.

Table 1

Quantitative amino acid and amino sugar composition of isolated cell walls from Phormidium uncinatum and of mucopolymer membranes from cell walls of gram-negative bacteria.

Anhydro-amino acids and amino sugars	Cell Walls of Phormidium uncinatum		Mucopolymer Membranes (Martin and Frank, 1962)	
	%	Molar ratio	E.coli B	Spirillum sp.
			Molar ratio	
muramic acid	10,2	0,63	1	1
glucosamine	11,5	1,0	1	1
diaminopimelic acid	12,0	1,0	1	1,2
glutamic acid	8,2	0,94	1,1	1,2
alanine	10,0	2,0	1,8	2,6
Total Mucopolymer components	51,9			
lysine	-	-	0,25	-
arginine	-	-	0,1	-
aspartic acid	0,94	0,04	-	-
threonine	0,29	0,04	-	-
serine	0,45	0,075	0,15	-
glycine	0,43	0,11	-	-
isoleucine	0,44	0,056	-	-
leucine	0,55	0,07	-	-
Total	54,55			

Discussion

In their qualitative and quantitative amino acid and amino sugar content cell walls from Phormidium uncinatum show a remarkable similarity to mucopolymer membranes from cell walls of gram-negative bacteria. In bacteria mucopolymer layers of this typical composition have been shown to form the rigid backbone to which the cell wall owes its definite shape and most of its mechanical strength (Weidel and Primosigh, 1957;

Weidel et al., 1960). It appears safe to conclude now that a mucopolymer with comparable properties and functions is also present in the cell walls of blue-green algae. No explanation is available for the low values for muramic acid in Phormidium walls (ratio glucosamine : muramic acid = 1 : 0.63). Preferential destruction of muramic acid was not encountered before under our conditions of hydrolysis and further investigation might reveal a structural pecularity of algal mucopolymer in that respect. - For the time being, the chemical nature of the non-mucopolymer part of our Phormidium cell wall preparation remains unknown. Neutral or acidic polysaccharides do not seem to be present and the pectin-containing cell wall layer which has been observed previously in other blue-green algae (Metzner, 1955), is evidently not a constituent of the rigid part of the Phormidium cell wall isolated by the Mickle procedure. A detailed report of this work, including morphological and chemical data, is in preparation.

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