CELL WALL POLYPEPTIDES OF **POLYPHYSA (ACETABULARIA) CLIFTONII**: AMINO ACID COMPOSITION OF STALK AND CAP CELL WALL POLYPEPTIDES

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Summary: The amino acid composition of stalk and cap cell wall polypeptides of the unicellular alga **Polyphysa (A.) cliftonii** has been investigated. In spite of chemical and physical differences between stalk and cap cell wall polysaccharides, the amino acid composition of the cell wall polypeptides appeared qualitatively similar in both structures. however, quantitative differences have been observed. The results are discussed on the basis of a possible role of the polypeptides in the growth of the cell wall.

In plant cells growth and species-specific formation of shape depend on the directed formation of polysaccharide fibrils. Several investigations support the idea that these processes are mediated by special proteins located mainly in the cell wall and in those regions of the plasmalemma which are adjacent to the cell wall (1,2). Cell wall polypeptides, however, have been found to alterate during growth processes and to show specific patterns in their amino composition (2,3,4).

This favors the theory that cell wall polypeptides may allow the extension of the polysaccharide network after cleavage of bonds between polypeptide and polysaccharide which seems to be characteristic for cell walls elongated by multinet-growth (5,6,7).

In order to obtain further data on the polysaccharide-polypeptide interactions during growth, investigations have been undertaken on the single-celled **Polyphysa (A.) cliftonii** because the cell walls of the stalk and the cap of this organism differ in the composition and molecular architecture of their polysaccharide which is in conjunction with the differences

occuring in the shapes of both cell structures (8,9,10,11).

This study was undertaken in order to establish differences in the amino acid composition of the cell wall polysaccharide-polypeptide macromolecules because it is important to know whether possible alterations of the cell wall polysaccharides are in conjunction with differences in the chemical composition of the corresponding cell wall polypeptides.

MATERIALS AND METHODS

Polyphysa (A.) cliftonii cells were cultivated in Erd-Schreiber-medium according to the method described by Hämmerling (12). For the preparation of purified cell walls, stalks and caps were homogenized separately in 0.15 M potassium phosphate buffer, pH 7.5, in a VIRTIS homogenizer at 4° C. The cell walls were collected separately and homogenized for a second time using an aqueous 0.1 % TRITON X-100 solution as a washing medium. The detergent was removed by several centrifuge runs by 1000 rpm, and after collecting the pellet further dialysis was performed of the suspended pellet against double distilled water. Extensive purification of the cell walls were achieved by several washing steps using solutions of 0.1, 0.5, 1, and 2 M sodium chloride with following low speed centrifugation for 20 minutes until no cytoplasmic material could be detected.

The disappearance of cytoplasmic material was proved by fluorescence microscopy (LEITZ LABOR-LUX) using dansyl chloride [+)] as a fluorescent dye (13). Several aliquots of the cell walls were suspended in a 0.1 M potassium phosphate buffer, pH 8.5, and a drop of a 1 % solution of dansyl chloride in acetone were placed on a microscope slide for examination of fluorescent positive or negative material.

The cell wall suspension was further dialyzed against distilled water at 4° C in order to obtain a salt free cell wall preparation. The material obtained was lyophilized and stored in a desiccator over P_2O_5.

For determination of the amino acid composition the samples were hydrolyzed in sealed test tubes using 6 N HCl for 24 hours at 110° C. Normally 2 mg of the different cell wall material has been hydrolyzed. Separation of damaged carbohydrates from

+) 1-dimethylaminonaphthalene-5-sulfonylchloride

the free amino acids was performed using gel filtration on a SEPHADEX G 75 (0.5 x 30 cm) column with a linear gradient ranging from 0.1 M to 2.0 M sodium chloride at pH 7. Samples in acid-washed iquition tubes were evaporated to dryness with a rotary evaporator. To these tubes 10 µl of 0.1 M phenol and 0.5 ml of 6 N HCl were added and the tubes were constricted with an O_2 flame. The tubes were evacuated to below 20 µl and then sealed. Since we are comparing 24 h samples with a generally similar overall composition no corrections were made for possible destruction of serine and threonine. The hydrolysates were then analyzed for amino acid composition by the method of Spackman et al (14) on a BIOCAL amino acid analyzer. - Furthermore, fractions containing the amino acids have been proved by thin layer chromatography.

RESULTS AND DISCUSSION

This is the first time that the amino acid composition of stalk and cap cell wall polypeptides of Acetabularia have been investigated, and we provide evidence that the amino acid composition of cell wall preparations of stalk and cap which have been cleaned from cytoplasmic contaminations unequivocally show mainly quantitative rather than qualitative differences.

Figure 1 illustrates a typical amino acid composition of cell wall polypeptides of stalk and cap. It has been found that all polypeptides in stalk and cap are rich in glycine, valine, glutamic acid but particularly rich in alanine and aspartic acid. Only small amounts of methionine, proline, and hydroxyproline could be detected.

It is important to point out that the total amino acid composition of the stalk is higher than that of the cap cell walls of the same plant. Only arginine, methionine, and proline are present in the same amount in stalk and cap. Although the total amino acid composition of the stalk and the cap cell walls are different, nine amino acids - arginine, phenylalanine, tyrosine, leucine, isoleucine, valine, glutamic acid, threonine, and aspartic acid - vary relatively in the same ratio of stalk and cap cell walls. For example, leucine and isoleucine appeared constantly in a ratio of 2:1.

The most prominent quantitative differences between stalk

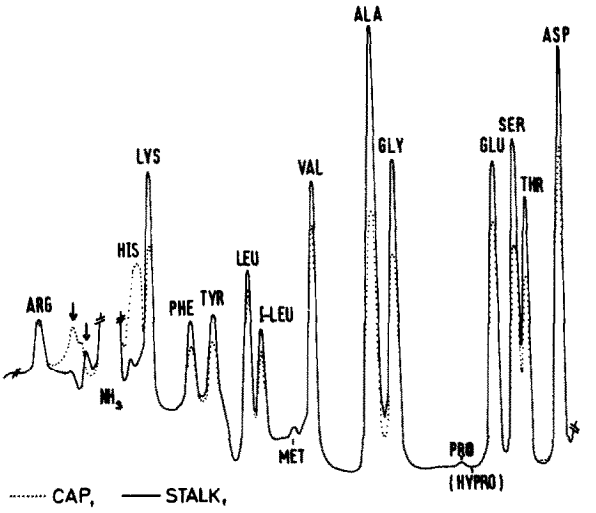

FIG. 1: The amino acid composition of stalk (———) and cap (·····) wall polypeptides of Polyphysa (A.) cliftonii. The total amount of amino acids of 100 ul hydrolysate applied to the amino acid analyzer was 11.7 µg (stalk) and 10.2. µg (cap), respectively. 100 µl correspond to 0.1 mg cell wall.
In both 'fractions' the 17 amino acids, indicated by their symbols, have been detected: ALA = alanine, ARG - arginine, ASP = aspartic acid, GLU = glutamic acid, GLY = glycine, HIS = histidine, HYPRO = hydroxyproline, I-LEU = isoleucine, LEU = leucine, LYS = lysine, MET = methionine, PHE = phenylalanine, PRO = proline, SER = serine, THR - threonine, TYR - tyrosine, VAL = valine. (↓) unknown ninhydrin-positive substances.

and cell wall polypeptides were found for glycine, histidine, lysine, serine, and alanine (Table 1). The stalk cell wall polypeptides contained only small amounts of histidine and were rich in glycine, lysine, serine, and especially in alanine. The cap cell wall polypeptides, on the other hand, showed a drastic increase in histidine. The unidentified ninhydrin-positive substances (Fig. 1) appeared more strongly in the cap cell wall 'fraction'. Hydroxyproline, which is the main component of 'extensine', a special type of cell wall polypeptide occuring in higher plants and most of the green algae (1), was present, too. But there are still some difficulties of separating hydroxyproline clearly from proline, presumably because it was present in too small amounts.

It is known that Acetabularia elongates by tip growth (15). One can assume that this type of elongation mainly depends on the continuous formation and deposition of new polysaccharide

TABLE 1:

The relative amounts of amino acids (%) of stalk and cap cell wall polypeptides of Polyphysa (A.) cliftonii.

		STALK	CAP			STALK	CAP
1	GLY	10.58	7.90	10	ARG	2.26	1.62
2	ALA	15.80	10.54	11	LYS	5.84	1.90
3	LEU	5.56	4.74	12	PHE	3.22	2.26
4	I-LEU	2.40	2.04	13	TYR	4.06	2.36
5	VAL	7.38	7.72	14	MET	0.16	0.10
6	SER	6.60	3.82	15	HIS	0.44	3.12
7	THR	4.12	2.96	16	PRO	0.08	0.08
8	ASP	11.60	9.26	17	HYPRO	-- traces ---	
9	GLU	11.02	9.14	18	u.n.s.[+)]	0.90	2.90

[+)] unknown ninhydrin-positive substances; see fig. 1 (↓)

material rather than as of a loosening of bonds, and subsequently, a gliding of the polysaccharide fibrils. However, if polypeptides are really involved in tip growth their functioning will be something different from that proposed for multinet-growth.

There is some evidence that polypeptides also may act as templates for the synthesis of polysaccharide fibrils (1,6). Our results support such a view: The corresponding amino acids which contribute to the differences of stalk and cap cell wall polypeptides may provide the molecular basis which might be necessary for the architecture of the different polysaccharides to be built up. - The fact that nearly half of the corresponding amino acids of stalk and cap cell walls vary in the same manner may be due to the existence of equivalent polypeptides for both cell structures. This would mean either that each polypeptide contains subunits which give rise to differences observed in stalk and cap cell wall polypeptides or that each cell structure possesses more than one single polypeptide.

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