served for certain effects in mammalian systems such as mice (*11*)—and the dose-effect curve departs from linearity when the frequency of radiation-induced mutations is only about five times the spontaneous frequency. This underlines the deficiencies of linear extrapolations from large effects. For example, the mutation frequency at 1 rad, estimated from the mutation frequency at 50 rads, would be more than twice as much as the observed rate.

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References and Notes

1. L. Ehrenberg and G. Eriksson, *Acta Radiol. Suppl.* **254**, 73 (1966); G. Eriksson, *Hereditas* **68**, 101 (1971); D. Lindgren, G. Eriksson, L. Ehrenberg, *Mutat. Res.* **10**, 335 (1970); G. G. Nayar, K. P. George, A. R. Gopal-Ayengar, *Radiat. Bot.* **10**, 287 (1970); A. H. Sparrow and W. R. Singleton, *Amer. Natur.* **87**, 29 (1953); A. H. Sparrow, R. L. Cuany, J. P. Miksche, L. A. Schairer, *Radiat. Bot.* **1**, 10 (1961).
2. L. W. Mericle and R. P. Mericle, *Radiat. Bot.* **5**, 475 (1965); *Health Phys.* **11**, 1607 (1965).
3. A. G. Underbrink, R. C. Sparrow, A. H. Sparrow, H. H. Rossi, *Radiat. Res.* **39**, 463 (1969).
4. ———, *ibid.* **44**, 187 (1970); *Int. J. Radiat. Biol.* **19**, 215 (1971).
5. L. W. Mericle and R. P. Mericle, *Radiat. Bot.* **7**, 449 (1967).
6. A. M. Kellerer and H. H. Rossi, *Radiat. Res.* **47**, 15 (1971).
7. L. J. Goodman and U. Koch, *Annual Report on Research Project NYO-2740-6* (Department of Radiology, Radiological Research Laboratories, Columbia University, New York, 1969), p. 96; H. H. Rossi, J. L. Bateman, V. P. Bond, L. J. Goodman, E. E. Stickley, *Radiat. Res.* **13**, 503 (1960).
8. H. H. Rossi, *Phys. Med. Biol.* **15**, 255 (1970).
9. The absorbed dose is defined as $D = E/m$, where E is the energy deposited by charged particles in mass m. However, microscopic regions of diameter d and mass μ exhibit a range of specific energy $z = \epsilon/\mu$, where ϵ is a (generally) highly variable local energy deposition. The probability distribution of increments of specific energy is $f_1(z)$; $\bar{z}_D$ is the ratio of the second and first moments of this distribution (*10*).
10. H. H. Rossi, *Advan. Biol. Med. Phys.* **11**, 27 (1967); A. M. Kellerer and H. H. Rossi, *Proc. Symp. Microdosim. 2nd*, 841 (1969).
11. K. G. Lüning and A. G. Searle, *Mutat. Res.* **12**, 291 (1971).
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Hydroxyproline Heterooligosaccharides in Chlamydomonas

Abstract. *Most of the hydroxyproline in* Chlamydomonas reinhardtii *is glycosidically linked to oligosaccharides and a monosaccharide that are different from the arabinosides found in hydroxyproline-containing plant cell walls previously examined. Of particular interest is the presence of hydroxyproline-*O*-galactose. These differences may be common to the volvocalean green algae and may be related to lower tensile strength of the cell walls of this group of plants.*

In the search for a cell wall component capable of regulating cell wall extensibility, the hydroxyproline-rich protein extensin features as a possible candidate because of its ability to cross-link polysaccharides through the hydroxyproline-*O*-arabinose linkage (*1*). This linkage is detected by the appearance of a series of hydroxyproline arabinosides (Hyp-Ara_n, where $n = 1$ to 4) released from the cell wall by alkaline hydrolysis. These arabinosides have been isolated from all plants examined, ranging from the spermatophytes to the green alga *Chlorella* (*1*, *2*). Especially remarkable in the survey (*2*) was the constancy of arabinose as the only hydroxyproline substituent, with a limit of four arabinose residues.

This survey has been extended to *Chlamydomonas*, an organism considered phylogenetically to be more primitive than *Chlorella*. We now report that alkaline hydrolysis of a crude cell wall fraction from *Chlamydomonas* releases a striking variety of hydroxyproline-*O*-glycosides. These include hydroxyproline-*O*-galactose observed in nature for the first time and a number of hydroxyproline heterooligosaccharides.

Cells of *Chlamydomonas reinhardtii* (IUCC 89) were grown in 12-liter flasks containing 6 liters of Sager and Granick's acetate medium (*3*). Cultures were bubbled continuously with air and maintained under a bank of six fluorescent tubes yielding an intensity of 33,000 lu/m^2. Cells were harvested by centrifugation at the end of the logarithmic growth phase, washed with sterile water, resuspended in water, and sonicated at the maximum setting for 20 minutes (Branson Sonic Power Sonifier). Microscopic examination showed that this treatment completely shattered all cells. The homogenate was made to 10 percent with trichloroacetic acid and centrifuged at 5000*g* for 20 minutes. The pellet was resuspended in water, neutralized with KOH, and sedimented again, yielding a crude fraction containing the cell walls. Alkaline hydrolysis [0.2*M* $Ba(OH)_2$ for 6 hours at 100°C] of this fraction yielded a mixture of hydroxyproline glycosides, which were separated chromatographically on a column (0.6 by 75 cm) of Chromobeads B (Technicon Corp.) and monitored for hydroxyproline by automated analysis as described (*2*), except that a 0 to 0.2*N* HCl gradient was used to improve the resolution of the hydroxyproline glycosides (Fig. 1). The glycosides were hydrolyzed in 2*N* trifluoroacetic acid (*4*). Sugars from the hydrolyzates were identified by paper chromatography in ethyl acetate, pyridine, water (8 : 2 : 1) solvent (*5*) and development with alkaline silver nitrate (*6*). These identifications were confirmed by gas chromatography of the sugar alditol acetates (*4*). Amino acids other than hydroxyproline were assayed for by paper electrophoresis (*7*). The hydroxyproline, arabinose, and galactose were estimated (*7*), and glucose was estimated with Glucostat (an enzymic assay obtained from Worthington Biochemical).

For determination of the sequence of sugars, each glycoside was partially hydrolyzed in 0.1*N* trifluoroacetic acid at 90°C for 0.5 hour. This mild acid hydrolysis released a mixture of hydroxyproline glycosides as breakdown products of the original hydroxyproline glycosides. Separation and analysis of these glycosides enables us to determine a tentative sugar sequence.

Figure 1 shows the profile of hydroxyproline glycosides eluted from the

Table 1. Composition of hydroxyproline (Hyp) glycosides from *Chlamydomonas reinhardtii*.

Glycoside	Hyp (% of total)	Theoretical* molar ratios of glycosides
Hyp A_1	12.7	Ara_5-Gal_5-Glc_2-Hyp
Hyp A_2	4	
Hyp B	12.3	Ara_3-Gal-Glc-Hyp
Hyp C	17.6	Ara_3-Gal-Glc-Hyp
Hyp D	12.6	Ara_3-Gal-Hyp
Hyp E	13	Ara_3-Gal-Hyp
Hyp F	6.2	Ara_2-Gal-Hyp
Hyp G	10.8	Ara_2-Gal-Hyp
Hyp H	0.9	Ara_2-Hyp
Hyp I	4.1	Gal-Hyp
Hyp J	2.4	Gal-Hyp
Hyp K	1	Ara-Hyp
Free Hyp	2.4	*trans* and *cis* Hyp

* For estimated molar ratios, see Table 2.

chromatography column. Under conditions of alkaline hydrolysis, racemization of hydroxyproline occurs, giving rise to a mixture of *cis* and *trans* hydroxyproline glycosides, which separate on the column and elute as pairs of glycosides. Hence, hydroxyproline glycoside pairs B and C, D and E, F and G, and I and J are identical both in composition and sequence of the sugars in the attached oligosaccharide except that the first of each pair contains hydroxyproline as the *trans* epimer and the second as the *cis* epimer.

Table 1 lists the composition of the hydroxyproline glycosides (*8*). Free hydroxyproline, Hyp-Ara_1, and Hyp-Ara_2 are all present, though in small amounts, compared with figures for plant cell walls similarly analyzed (*2*). Hyp-Ara_3 and Hyp-Ara_4 are absent or present in quantities so small as to be obscured by peaks Hyp D to Hyp G (*9*).

The rest of the hydroxyproline glycosides are different from any yet discovered. Of special interest is the presence of some of the hydroxyproline glycosidically linked to galactose. This glycoside when hydrolyzed yielded no sugar other than galactose, and no amino acid other than hydroxyproline. Table 2 lists the data used to determine the sugar sequence of the other oligosaccharides (*10*). These include Hyp-Ara-Ara-Gal, Hyp-Ara-Ara-Gal-Ara, Hyp-Ara-Glc-Ara-Gal-Ara, and the very large glycoside tentatively estimated to contain Hyp-Ara_5-Gal_5-Glc_2 (*11*).

These data show that *C. reinhardtii* differs in its hydroxyproline glycoside composition from all other plants so far analyzed. Five new hydroxyproline glycosides have been demonstrated including hydroxyproline-*O*-galactose.

Because of the crude preparations used, the data presented so far only provide indirect evidence for the localization of these new hydroxyproline heterooligosaccharides in the cell wall. At least 40 percent of the cellular hydroxyproline in *C. reinhardtii* is wall-bound (*12*), whereas our data show that 95 percent of the total cellular hydroxyproline has attached heterooligosaccharide. Therefore most of the cell wall hydroxyproline must have similarly attached heterooligosaccharide. We confirmed this by taking advantage of the fact that *Chlamydomonas gymnogama* sheds its wall intact during sexual reproduction (*13*). We obtained enough of the wall that was shed to show that alkaline hydrolysis yielded a hydroxyproline oligosaccharide

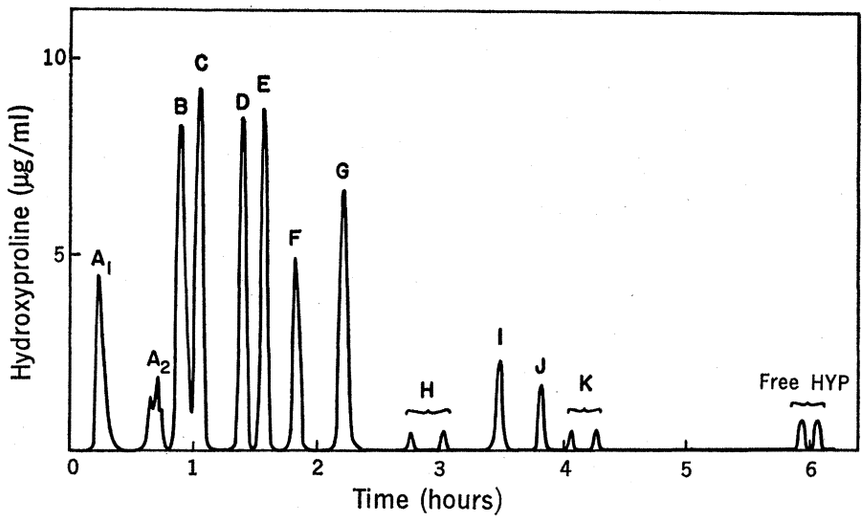

Fig. 1. Profile of hydroxyproline glycosides released by alkaline hydrolysis and eluted from Chromobeads B column.

profile similar to that of *C. reinhardtii* (Fig. 1). In addition, alkaline hydrolysis of the extracellular matrix of *Volvox carteri* also gave a similar glycoside profile. These results indicate that the hydroxyproline heterooligosaccharides are in fact an integral (or structural) part of the cell wall (or are at least extracellularly located in *Volvox*), and, further, they may be characteristic of the whole volvocalean order of green algae.

It is not at all clear what this variety of hydroxyproline heterooligosaccharides in *Chlamydomonas* signifies compared with the relatively unvarying hydroxyproline homooligosaccharides encountered so far in all higher plant groups. However, during cell breakage we noted the extreme fragility of the cell wall of *Chlamydomonas* as compared with those of *Chlorella vulgaris*: short sonications (less than 1 minute) rupture about 80 percent of *Chlamydomonas* cells, and the released cell walls are rapidly disintegrated into tiny fragments by further sonication. A similar sensitivity to a Virtis homogenizer

Table 2. Sugar sequence of hydroxyproline glycosides from *Chlamydomonas reinhardtii* as determined by partial hydrolyses. Since both members of each pair of glycosides yielded the same mixture of breakdown products, only those released from the first of each pair of glycosides are listed.

Glycosides released	Hyp (% of total)	Molar ratios				Tentative sequence of Hyp oligosaccharides
		Ara	Gal	Glc	Hyp	
			Hyp B glycoside			
B_1	34	2.5	0.8	0.6	1	Hyp-Ara-Glc-Ara-Gal-Ara
B_2	12	1.9	1	0.8	1	Hyp-Ara-Glc-Ara-Gal
B_3	9	2.1	0.6	0	1	Hyp-Ara-Ara-Gal-Ara*
B_4	7	1.3	0	0.73	1	Hyp-Ara-Glc-Ara
B_5	22	0.84	0	0.84	1	Hyp-Ara-Glc
B_6	16	0.66	0	0	1	Hyp-Ara
			Hyp C glycoside			
C		3	0.76	0.43	1	Hyp-Ara-Glc-Ara-Gal-Ara
			Hyp D glycoside			
D_1	49	3.6	0.87	0	1	Hyp-Ara-Ara-Gal-Ara
D_2	10	2.6	0.8	0	1	Hyp-Ara-Ara-Gal
D_3	14	2	0	0	1	Hyp-Ara-Ara
D_4	27	1.2	0	0	1	Hyp-Ara
			Hyp E glycoside			
E		3.4	1.1	0	1	Hyp-Ara-Ara-Gal-Ara
			Hyp F glycoside			
F_1	48	2.4	0.96	0	1	Hyp-Ara-Ara-Gal
F_2	13	2.2	0	0	1	Hyp-Ara-Ara
F_3	39	1	0	0	1	Hyp-Ara
			Hyp G glycoside			
G		2.3	1.02	0	1	Hyp-Ara-Ara-Gal

* Peaks Hyp B and Hyp C both gave rise to small amounts of this glycoside. The molar ratios indicate an empirical formula of Hyp-Ara_3-Gal_1. The sequence shown is tentative and is suggested by the similarity of B_3 and C_3 to Hyp D and Hyp E. Complete sequencing of B_3 and C_3 was not possible because they were present in such small quantities.

has been observed (*14*). Cells of *Chlorella vulgaris*, however, require much longer periods of sonication to be ruptured, and the released cell walls are quite resistant to further disintegration. Thus *Chlamydomonas* walls are more susceptible to mechanical rupture than *Chlorella* walls are, though the thickness of their walls is nearly the same [21 nm for *Chlorella* (*15*) compared to 19 nm for *C. reinhardtii* (*16*)]. This weakness of the wall may be correlated with the fact that *Chlamydomonas* maintains its osmotic balance via energy-expending contractile vacuoles, rather than via wall pressure as in more advanced plant types. Although the weak walls of *Chlamydomonas* appear to lack cellulose (*16*), this does not mean that the absence of cellulose is the cause of the weak wall. Coenocytic algae such as *Codium* (*17*) also lack both cellulose and contractile vacuoles but possess relatively rigid walls that contain hydroxyproline (*12*). Therefore it seems possible that the changeover from hydroxyproline heterooligosaccharides to homooligosaccharides marks a fundamental evolutionary transition from wall organization which was mechanically weak to a wall organized in such a way (a highly cross-linked covalent network) as to provide sufficient tensile strength to balance internal and external osmotic pressures. Experimental proof of this hypothesis awaits further study.

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References and Notes

1. D. T. A. Lamport, *Nature* **216**, 1322 (1967).
2. ——— and D. H. Miller, *Plant Physiol.* **48**, 454 (1971).
3. R. Sager and S. Granick, *Ann. N.Y. Acad. Sci.* **56**, 831 (1953).
4. P. Albersheim, D. J. Nevins, P. D. English, A. Karr, *Carbohyd. Res.* **5**, 340 (1967).
5. K. T. Williams and A. Bevenue, *J. Ass. Office Agr. Chem.* **36**, 969 (1953).
6. W. E. Trevelyan, D. P. Procter, J. S. Harrison, *Nature* **166**, 444 (1950).
7. D. T. A. Lamport, *Biochemistry* **8**, 1155 (1969).
8. It is possible that hydroxyproline glycosides other than those listed are present in small amounts, as trace amounts of xylose and mannose were present when glycosides A, B, and C were hydrolyzed. However, these trace amounts cannot be considered to be constituents of the listed glycosides for they are always present in less than 1/5 molar equivalent to 1 molar equivalent of hydroxyproline.
9. The elution positions of Hyp-Ara_3 and Hyp-Ara_4 are within the same range that peaks Hyp D to Hyp G occupy. The presence of trace quantities of Hyp-Ara_3 and Hyp-Ara_4 might account for the somewhat higher than expected yields of arabinose in peaks Hyp D to Hyp G. There can be only trace amounts of Hyp-Ara_{3-4}, however, because substantial quantities of them would lower the Gal : Hyp molar ratios and any large amount of Hyp-Ara_4 would appear as Hyp-Ara_3 in the mixture of Hyp glycosides released from Hyp D and E. Such a glycoside was not released in any detectable amount.
10. Mild acid hydrolysis of glycosides B to G released these Hyp glycosides. Quantitative analysis of each glycoside indicates a tentative sequence for each oligosaccharide; the present data do not exclude a branched structure. In a few cases, where no sugar is indicated, trace amounts (less than 5 percent of the total sugar present) appeared on paper chromatograms. These traces probably arose from sugars actually present and undergoing epimerization as a result of the acidic conditions. Glycosides are listed in the order they eluted from the column, from largest to smallest. The percentages of total hydroxyproline give an indication of the relative lability of the terminal glycoside bond to acid hydrolysis.
11. The theoretical molar ratios of this glycoside can only be tentative because the glycoside eluted with the void volume of the column, and this fraction may contain free sugars released during alkaline hydrolysis. Hence the exact sugar content is not yet known, but the elution position of this glycoside on a Sephadex G-25 column indicates a molecular weight consistent with this empirical formula.
12. Inta B. Gotelli and R. Cleland, *Amer. J. Bot.* **55**, 907 (1968).
13. T. Deason, *J. Phycol.* **3**, 109 (1967).
14. T. Punnett and E. C. Derrenbacker, *J. Gen. Microbiol.* **44**, 105 (1966).
15. D. H. Northcote, K. J. Goulding, R. W. Horne, *Biochem. J.* **70**, 391 (1958).
16. R. W. Horne, D. R. Davies, K. Norton, M. Gurney-Smith, *Nature* **232**, 493 (1971).
17. R. D. Preston, *Sci. Amer.* **218** (6), 102 (1968).
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An Eagle's Eye: Quality of the Retinal Image

Abstract. *The optical quality of a living eagle's eye was determined by an ophthalmoscopic method. The performance of the eye was substantially better than that reported for humans, but did not confirm some of the wilder claims made for such birds.*

Although man has assigned to himself the highest niche in the order of evolution, many lower forms may surpass human abilities in certain aspects of sensory performance. For instance, almost any lower vertebrate is thought to have a keener sense of smell, bats and dogs to be sensitive to a wider range of auditory frequencies, cats and owls to have better visual performance under low levels of illumination, and hawks and eagles to possess keener vision. The last of these assertions is supported largely by anecdotal evidence, and by microscopic examination of bird retinas. Rochon-Duvigneaud estimated that the density of cones in the central fovea of the hawk reaches 1 million per square millimeter. (*1*), which may be compared with perhaps 147,000 per square millimeter in the center of the human fovea (*2*). Both Rochon-Duvigneaud and Polyak surmised that the visual acuity of the birds of prey surpassed that of man, but they did not offer quantitative estimates (*1*, *3–6*). However, Walls (*7*) states that in the central fovea an eagle could reach acuities eight times that of man!

Walls's claim is undoubtedly exaggerated—the human visual system becomes diffraction limited and reaches its maximum performance at a pupil diameter of 2.3 to 2.4 mm (*8*, *9*). The cutoff frequency of a perfect optical system is directly proportional to the diameter of its entrance pupil. A bird would therefore require a pupil at least 18.4 mm in diameter to be theoretically capable of fulfilling Walls's expectations, a dimension which is beyond the capability of even the largest birds of prey (*3–5*, *10*).

The size and organization of the eagle eye does suggest that its resolving power is extremely high, but the retinal mo-

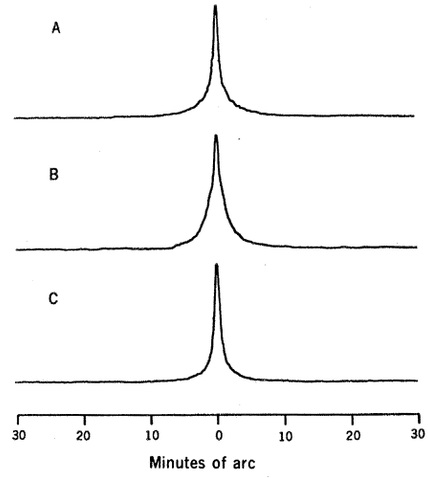

Fig. 1. Three selected linespreads measured external to the eagle's eye. Except for small errors introduced by source and slit width differences, these waveforms may be compared directly to the external linespreads obtained for the human eye by Campbell and Gubisch (*8*).