Antimutagenic effect of heteroolans, arabinogalactans, pectins and mannans in the Euglena assay

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Summary

A series of plant cell wall polysaccharides – heteroolans, arabinogalactans, pectins and mannans exerted antimutagenic (antibleaching) activity against acridine orange- and ofloxacin-induced mutagenicity in the Euglena assay. All polysaccharides tested exhibited a significant dose-dependent antibleaching activity and the percentage inhibition of mutagenicity ranged from 52 to 96%. It can be assumed that the antimutagenicity of the polysaccharides depends on their structural and compositional properties as well as on the different mode of action of both the mutagens tested.

Introduction

Plant cell walls represent a highly ordered dynamic and developmentally regulated network of polysaccharides, structural proteins and phenolic substances (lignin, ferulic acid etc.) that is largely based on non-covalent interactions (Schindler 1998). In higher plants, cellulose is the major cell wall component, followed by polysaccharides often divided into hemicelluloses and pectin, and by lignin (O’Neill et al. 1990). Hemicelluloses include a large variety of xyloglucan, xyloglucan, glucomannan and galactoglucomannan. Arabinogalactans (AGs) are rather tenuously connected with the above mentioned structural polysaccharides. Pectins comprise a heterogeneous group of polysaccharides present mainly in parenchyma cell walls of fruits and vegetables. The polygalacturonic acid and rhamnogalacturonans are considered to represent different domains of a native pectin polymer chain, whereas neutral arabinogalactan, galactan and arabinan form side chains of the domains.

In western diets, more than 95% of the dietary fibres (DFs) are contributed by whole plant cell walls. DFs may either enhance or protect against the development of colorectal cancer, depending upon the extract amounts and chemical nature of the fibres (Heitman et al. 1992; Aoe et al. 1993; Alabaster et al. 1995; Harris & Ferguson 1999).

There are few reports dealing with the antimutagenic activity of DFs and isolated plant cell-wall components on microbial systems (Takeuchi et al. 1988; Yamaguchi 1992; Edenharter et al. 1995; Higashimoto et al. 1998). No solvent fraction prepared from a series of fruits or vegetables reduced mutagenic activity induced by 2-amino-3,4-dimethylimidazol[4,5-f]quinoxaline (IQ) in S. typhimurium TA98NR. In contrast, mutagenicity was strongly reduced by lignin, weakly by alginic acid and pectin, while cellulose, gum Arabic, gum guar and xylan were ineffective (Edenharter et al. 1995). Among many components of citrus fruits, DFs such as lignin and pectin showed strong antimutagenic effects against the mutagenicity of 1-methyl-1,2,3,4-tetrahydro-beta-carboxyline-3-carboxylic acid in a reaction mixture containing nitrite and ethanol (Higashimoto et al. 1998). Recently, Hensel & Meier (1999) reported dose-dependent antimutagenic effects of xyloglucans and different pectins and pectin-like rhamnogalacturonans against 1-nitropyrene-induced mutagenicity.

The unicellular photosynthetic flagellate Euglena gracilis possesses a multigenomic system with nuclear, mitochondrial and chloroplast DNAs. The detection ability of this model is based on the preferential and selective sensitivity of the chloroplast genome to xenobiotics resulting in elimination of the functional chloroplasts from the cells. This phenomenon is accompanied by a decrease or complete loss of chloroplast DNA. The antichloroplastic activity of mutagens is macroscopically manifested by the formation of heterotrophic colourless colonies (irreversible bleaching effect). Our laboratory’s results proved that the E. gracilis model is...
appropriate for detecting mutagens, as well as testing and evaluating antimutagens (Ebringer 1990; Ebringer et al. 1993, 1996).

In the present work we examine the antimutagenic activity of heteroxylans, arabinoxylans, pectins and mannans of well defined structural and molecular properties against acridine orange (AO)- and ofloxacin (OFL)-induced mutagenicity in _Euglena_ assay. These components are known to be responsible for many of the physiological effects of dietary fibres.

**Materials and Methods**

**Polysaccharide samples**

The xylan GX4 from beechwood sulphite pulp was a gift from Lenzing AG (Lenzing, Austria). All the other polysaccharide samples used were prepared in the Institute of Chemistry, Slovak Academy of Sciences, Slovak Republic. Xylans GX1-3 were isolated from beechwood (Ebringerová et al. 1989), AGX1, 2 from corn cobs (Ebringerová et al. 1995), AX1, 2 from rye bran (Hromádková & Ebringerová 1987), and HX from corn bran (Hromádková & Ebringerová 1995). Arabinoxylans, AG1 and AG2 were prepared from the wood of _Larix sibirica_ (Karácsonyi et al. 1984) and _L. dahurica_ (Odonnažig et al. 1994), respectively. AG3 and the mannans M1, 2 were isolated from delignified green coffee beans according to Bradbury & Halliday (1990). The pectins P1 and P2 were isolated from rhubarb stalks (Ebringerová et al. 1993) and the roots of the holoparasite _Cistanche deserticola_, a traditional Mongolian medicine (Ebringerová et al. 1997). The composition and basic structural features of all polysaccharide samples are summarized in Tables 1 and 2.

**Chemicals**

OFL was purchased from Hoechst-Biokita, Slovak Republic and AO from Loba Chemie-Wien, Fischamend, Austria. Dimethyl sulfoxide (DMSO) was obtained from Merck, Darmstadt, Germany. Stock solutions of mutagens were prepared freshly by dissolving in 0.1 M NaOH (OFL) or in distilled water (AO). Polysaccharides were dissolwed in DMSO.

**E. gracilis assay**

_Euglena gracilis_ strain Z was obtained from Dr. S.H. Hutner, Haskins Laboratory, Pace University, New York, USA. The media formulation and assay procedure used were the same as those described previously (Križková et al. 1998).

Briefly, the mutagen and polysaccharide were simultaneously added in appropriate concentrations to an exponentially growing culture of _E. gracilis_. The cells (about 0.5–1 × 10⁵ cells per ml) were incubated for 1 day at 27 °C under permanent illumination of 1 klx. After 24 h, cells were diluted and spread on agar plates which were incubated for 10–14 days in the light at 27 °C; then green and white (bleached) colonies were counted. The percentage inhibition of mutagenicity (MIP) was calculated for all samples according to equation:

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MIP = (1 - N_W/N_C)100\%,
\]

where _N_W_ is the number of white mutants on the test plates observed in the presence of the polysaccharide with mutagen and _N_C_ the average number of white mutants on control plates in the presence of mutagen without polysaccharide.

Only green colonies were found in all sets of controls (no spontaneous white mutants). Polysaccharides alone in all concentrations tested induced no white _Euglena_ colonies.

**Statistical analysis**

The Student’s t-test and ANOVA (F-test) were used in statistical analyses. The value of _P_ < 0.05 was considered to be statistically significant. The results represented are means ± standard deviation (SD).

**Results**

**The activity of polysaccharides against acridine orange**

AO at a concentration of 5 µg ml⁻¹ induced 68% of white _Euglena_ mutants. The effect of several groups of plant polysaccharides (pectins, xylans, arabinoxylans and mannans) on AO-induced mutagenicity is demonstrated in Figure 1. All polysaccharides tested exhibited a dose-dependent antibleaching activity. Xylans, represented by various structurally different types, at the lower concentration (500 µg ml⁻¹) decreased the frequency of bleached mutants to 19–45%, whereas at the higher concentration of 2000 µg ml⁻¹, the effect was more pronounced and the frequency was reduced to 8–30%. Generally, soluble xylans showed a better antimutagenic effect against AO than the insoluble ones. The coffee bean AG3, similar to the pectins are among the most potent polysaccharides, decreased at a concentration of 2000 µg ml⁻¹ the amount of chloroplast-free mutants to 6–11%. The two larchwood arabinoxylans, AG1 and AG2 exhibited a moderate effect (23–29%). The test results exhibited a high level of statistical significance (_t_-test _P_ < 0.001, ANOVA test _P_A_ < 0.001).

**The activity of polysaccharides against ofloxacin**

OFL at a concentration of 30 µg ml⁻¹ induced 92% of irreversible bleached cells of _E. gracilis_. Statistically significant dose dependent anti-OFL effect for all polysaccharides was documented, except of the insoluble corn cob xylan (AGX2) (Figure 2). The lowest