Antigenotoxic potential of glucomannan on four model test systems

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Abstract

Antimutagenic, anticlastogenic, and bioprotective effect of polysaccharide glucomannan (GM) isolated from Candida utilis was evaluated in four model test systems. The antimutagenic effect of GM against 9-aminoacridine (9-AA) and sodium azide (Na3N) induced mutagenicity was revealed in the Salmonella typhimurium strains TA97 and TA100, respectively. GM showed anticlastogenic effect against N-nitroso-N’-methylurea (NMU) induced chromosome aberrations in the Vicia sativa assay. The bioprotective effect of GM co-treated with methyl-methane-sulphonate (MMS) was also established in Chlamydomonas reinhardtii repair deficient strains urs10 and urs14. The statistically significant antimutagenic potential of GM was not proved against 4-nitroquinoline-1-oxide (4-NQO) induced mutagenicity in Saccharomyces cerevisiae D7 assay. It may be due to bioprotective activity of α-mannan and β-glucan, which are integral part of S. cerevisiae cell walls. Due to the good water solubility, low molecular weight (30 kDa), antimutagenic/anticlastogenic, and bioprotective activity against chemical compounds differing in mode of action, GM appears to be a promising natural protective (antimitogenic) agent.

Introduction

The negative effects (e.g. toxic/genotoxic) of many environmental pollutants (Miadoková et al., 1996; Gold et al., 1999; Marzin 1999; Snyder and Green, 2001; Bolognesi, 2003) generate efforts to find different approaches to solve this problem. From this point of view the study of natural compounds with bioprotective effects is of great importance (Weisburger, 2001; Steinkellner et al., 2001; Miadoková et al., 2002; Park et al., 2003; Ram, 2004). Various polysaccharides isolated from yeast cell walls possess marked immunological properties ranging from non-specific stimulation of host immune system, resulting in antitumor, antiviral and anti-infective effects to antioxidant, antimutagenic and haematopoetic activity (Real et al., 1992; Liu et al., 1997; Babincová et al., 1999; Chorvatovičová et al., 1999; Bao et al., 2002; Tokunaka et al., 2002). One of the naturally occurring polysaccharides is also glucomannan (GM) isolated from Candida utilis (Kogan et al., 1993). Although C. utilis glucomannan is functionally and structurally very similar to the mannans of
other *Candida* and *Saccharomyces* species, as to their highly branched structure, glycosidic linkages, high content of mannose, immunochemical studies showed low cross-reactivity (Kogan et al., 1993). It seems that the glucose units at the terminal position of the side chains, play an important role in its biological activity. Biological effects of glucomannan are probably more similar to those of the cell wall skeletal β-glucans than to those of the surface mannans of yeast cells. Recently it has been proved that glucomannan isolated from *C. utilis* has a protective effect against mutagenicity induced by cyclophosphamide (Chorvátovcová et al., 1999), ofloxacin and acridine orange (Krížková et al., 2001).

The aim of this work was to further explore the possible antimutagenic and bioprotective activities of GM towards several chemicals differing in the mode of action in the four test systems: the *Salmonella typhimurium* assay as a prokaryotic model organism (Maron and Ames, 1983); the *Saccharomyces cerevisiae* toxicity and mutagenicity assay as a lower heterothrophic eukaryotic model organism (Zimmermann et al., 1984); the *Chlamydomonas reinhardtii* bioprotective assay as a lower photoautotrophic model organism (Miadoková et al., 1995), and the *Vicia sativa* chromosome aberration assay as a higher photoautotrophic model organism (Murín, 1984).

In addition to bacterial, yeast and plants assays, which are widely used for genotoxicity assessment, the unicellular green alga *C. reinhardtii* was included in experiments as another model system. This alga is an excellent biomarker for detection of environmental chemicals having also an ability to store and metabolize promutagenic aquatic pollutants to mutagenic products (Vlček et al., 1997a; Miadoková et al., 1998; Vlčková et al., 1999, 2000). Similarly as with the bacteria and yeasts, the collection of repair deficient strains of *C. reinhardtii* is available (Small, 1987; Podstavkovcová et al., 1992; Vlček et al., 1997b). Some of these strains can be used for genotoxicity assessment of environmental chemicals (Miadoková et al., 1995; Vlček et al., 1997a; Vlčková et al., 1999, 2000).

**Material and methods**

**Chemicals**

GM was isolated at the Institute of Chemistry, Slovak Academy of Science, Bratislava, Slovak Republic as described by Kogan et al. (1993); GM contains mannose and glucose in the ratio of 8.4:1, 1.5% protein content and has a molar mass 30 kDa as determined by gel filtration. Cell-wall GM was isolated from the lyophilized glycoprotein using extraction with 2% KOH and purification with Fehling’s reagent. Physico-chemical characterization of GM by means of gel-permeation chromatography, methylation analysis, NMR spectroscopy, as well as its immunochemical investigation have been described previously (Kogan et al., 1993). GM was dissolved in sterile distilled water. Other chemicals used: 9-aminoacridine (9-AA), Mr = 248.7 (Aldrich); methyl-methanesulphonate (MMS), Mr = 110.1 (Aldrich); N-methyl-N'-nitro-N-nitrosoguanidine (MNNNG), Mr = 147.1 (Sigma); 4-nitroquinoline-N-oxide (4-NQO), Mr = 190.2 (Sigma); N-nitroso-N'-methylurea (NMU), Mr = 103.1 (Sigma); sodium azide (NaN₃), Mr = 65.0 (Sigma). These chemicals recommended as reference (diagnostic) mutagens in the used test systems were dissolved in dimethyl sulfoxide (DMSO), Mr = 84.2 (Sigma).

**Salmonella typhimurium mutagenicity/ antimutagenicity assay**

In the *Salmonella typhimurium* assay, the strains TA97, TA98, TA100, and TA102 were used. For mutagenicity and antimutagenicity of GM (750, 500, 250 μg/plate), the Ames