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Direct Evidence for the Radioprotective Effect of Various Carbohydrates on Plasmid DNA and *Escherichia coli* Cells

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Abstract Damage to cells exposed to radiation is primarily attributed to direct effects on the structure of cellular DNA. Radiation-induced damage of pBluescript SK plasmid DNA and *Escherichia coli* DH5α were examined in the presence of various branched oligosaccharides, polysaccharides, and/or 8-MOP (8-methoxypsoralen). Branched oligosaccharides efficiently protected DNA and cells exposed to ultrasoft X-ray and UV irradiation. In the presence of 0.2% (w/v) branched oligosaccharides and polysaccharides, DNA can be protected from damage due to UV and ultrasoft X-ray by a factor of 1.3–2.1 fold and 3.2–8.3 fold, respectively. The protective effect of cells exposed to UV or ultrasoft X-ray was also observed by branched oligosaccharides. The combination of MOP, a photoreagent, with carbohydrates increased the protective effects for DNA and cells, compared with that of a single use of MOP or carbohydrate alone.

Key words: Radiation protection, oligosaccharides, UV, ultrasoft X-ray, *Escherichia coli*

DNA is the primary target for lethal radiation damage in organisms [11, 18, 20]. Radiation causes mutation or carcinogenesis by radiation-induced scission of DNA molecules. Cole [2] has found that mammalian cells and yeast cells were killed by electron beams of various energies. X-ray irradiation of mammalian cells produces single-strand scissions in DNA [14]. Therefore, there has been a search for substances that protect DNA from radiation-induced damage. One such substance is dimethyl sulfoxide (DMSO) which prevents single strand breaks of DNA [13]. Since DMSO is toxic and needed in large amounts, nontoxic protectors effective in small concentrations has been searched. Previously, tea catechin was reported to be a strong protectant [19]. Another candidate is trehalose, which is a naturally occurring disaccharide and known to be a multipotent protector for living things from the stress of freezing, heating, drying, and irradiation [20]. However, the availability of other carbohydrates in protecting DNA and microorganisms from radiation-induced damage is not known. Therefore, an attempt was made to measure the protective effect of monosaccharides (glucose, fructose), disaccharides [sucrose, maltose, isomaltose, lactose, trehalose, gentiobiose (β-D-glucopyranosyl-1→6-D-glucopyranose)], trisaccharides, oligosaccharides, and polysaccharides [acarbose (4,5,6-trihydroxy-3-hydromethyl-2-cyclohexen-1-yl 4-amino-4,6-dideoxy-D-glucopyranosyl maltose), maltotriose, maltopentaose, isomaltosyl maltooligosaccharides, isomaltosyl raffinooligosaccharides, dextran, levan, pullulan, xylan] against radiation damage.

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MATERIALS AND METHODS

Strains, Plasmids, and Chemicals

Either plasmid pBluescript SK or *E. coli* DH5α bearing pBluescript SK were used for radiation experiments. For the growth of *E. coli* bearing pBluescript SK, Luria-Bertani (LB) medium containing 50 μg/ml ampicillin was used [8]. The composition of the LB medium was 1% (w/v) trypton, 0.5% (w/v) yeast extract, and 0.5% (w/v) NaCl. For the preparation of plasmid DNA, a QIAprep Spin Miniprep kit (Qiagen, Germany) was used. The plasmid was normally prepared as >90% in the closed circular (CC) form. Then, it was dissolved in a buffer (SSC) of 150

mM NaCl and 15 mM sodium citrate containing various monosaccharides (glucose, fructose), disaccharides (sucrose, maltose, trehalose, lactose, gentiobiose), and degree of polymerization 3 or higher saccharides (acarbose, maltotriose, maltopentaose, α-cyclodextrin, β-cyclodextrin, isomaltosyl maltooligosaccharides, BOS-12, BOS-9, amylodextrin, isomaltosyl raffinooligosaccharides, dextran T-10, dextran T-40, levan, pullulan, xylan). All other chemicals were GR grade.

Preparation of Branched Oligosaccharides (BOS-9 and BOS-12)

BOS-9 and BOS-12 were prepared from mixed culture fermentation of *Lipomyces starkeyi* and *Leuconostoc mesenteroides* as previously described [5, 7]. *L. starkeyi* was grown with YS medium for 40 h at 30°C. The composition of YS medium was 0.5% yeast extract, 0.5% KH_2PO_4, 1% soluble starch, and 2% sucrose. After fermentation of *L. starkeyi* for 40 h, *L. mesenteroides* B-512FMCM was inoculated. Then, 9% sucrose (based on total volume) was added to the fermentor continuously and produced BOS-9. By a similar fermentation procedure, except that 12% sucrose was continuously added, BOS-12 was prepared. Following fermentation, the supernatant was separated from cells by centrifugation (6,000 ×g). Fructose in the supernatant was removed by alginate immobilized *Saccharomyces cerevisiae*, and dextran was precipitated by the addition of ethanol up to 43%. The remaining supernatant was concentrated, and the branched oligosaccharides were prepared using a rotary vacuum evaporator (N-N series, Eyela, Japan).

Preparation of Malto- and Raffinooligosaccharides

Glucansucrases from *L. mesenteroides* B-512FMCM and NRRL B-1355 were prepared from culture supernatants of LW liquid cultures [5, 7]. The composition of LW medium was 0.5% yeast extract, 0.5% peptone, 2% K_2HPO_4, and 2% sucrose. Each 200 mM sucrose or maltose was prepared with 20 mM Na-acetate buffer (pH 5.2). Total carbohydrate concentration in an acceptor reaction digest was fixed at 100 mM. For the preparation of isomaltosyl maltooligosaccharides, maltose and sucrose were mixed 1:1 (200 mM:200 mM), and B-512FMCM dextransucrase (20 U/ml) was added to this mixture with the ratio of 1:1 (v/v). For the preparation of isomaltosyl raffinooligosaccharides, the same conditions were applied, except that B-1355 glucansucrase (10 U/ml) was used instead of B-512FMCM dextransucrase. Both enzyme reactions were carried out at 30°C until all the sucrose was consumed.

Source of Radiation (Light Source)

Ultrasoft X-ray was obtained from the LIGA beamline of the Pohang Accelerator Laboratory (South Korea) using a specially constructed chamber. An aluminum target was placed inside of the exposure chamber, and the ultrasoft X-ray was generated by irradiating this target with a white beam. The chamber was filled with helium (He) gas which is effectively transparent to the ultrasoft X-rays. The binding energies of Al orbitals K(1), L(1), L(2), and L(3) were 1,559.6 eV, 117.8 eV, 73.1 eV, and 72.7 eV, respectively. If an electron of K-shell is taken out by the incident photon, an electron from a neighboring orbital moves to that empty orbital. The difference in the orbital energies is emitted as a photon of the energy range of the ultrasoft X-ray. In this experiments, the Al K-ray corresponded to an energy of 1.487 KeV.

Radiation-Induced Damage on Plasmid DNA and Cells of *E. coli*

The DNA (20 µl of a 50 µg/ml concentration) or *E. coli* (100 µl of 10^{10} cells/ml), with or without carbohydrates, were allocated in the wells of 96-well microplates (Nalge NUNC International, U.S.A.), and UV or ultrasoft X-ray were evenly exposed. In the UV-irradiation experiment, the plate was exposed to UV at a distance of 10 cm away from the UV lamp (254 nm, 10 W, G10T8-AN, Germicidal, Sankyo Denki, Japan) for 10 min. In the ultrasoft X-ray-irradiation, 6.4×10^6 photons/s were irradiated to each sample in a well. After irradiation by ultrasoft X-ray or UV (on DNA), the conformational change of the DNA was analyzed by agarose gel electrophoresis, comparing with the amount of plasmid DNA of covalently closed circular and open circular (single-strand break) forms. Following irradiation, 10 µl plasmid DNA samples were separated into CC and open circular (OC, single-strand break) by electrophoresis on 0.8% agarose gel. The band stained with 1 µg/ml of ethidium bromide represented the DNA bands, and the conformational change was quantitatively analyzed with the Kodak digital science 1D Image Analysis program (Eastman Kodak Co., U.S.A.). The small amount of OC form containing un-irradiated DNA was substracted as survival (%)=CC/(CC+OC)×100. No linear form of DNA caused by double-strand breaks was found during this study. The survival number of *E. coli* colonies after irradiation was counted on LB-ampicillin agar plates [10].

UV Absorbance of Carbohydrate Solution

The properties of each saccharide solution (0.2%) were measured by UV spectrophotometery from 220 to 300 nm (SmartSpec 3000, Bio-Rad, U.S.A.).

RESULTS AND DISCUSSION

Protective Effect from UV-Induced Damage

DNA single-strand breaks increased with increasing dose of UV-inadiation (Fig. 1). The protective effects of DNA by BOS-12, BOS-9, isomaltosyl raffinooligosaccharides,

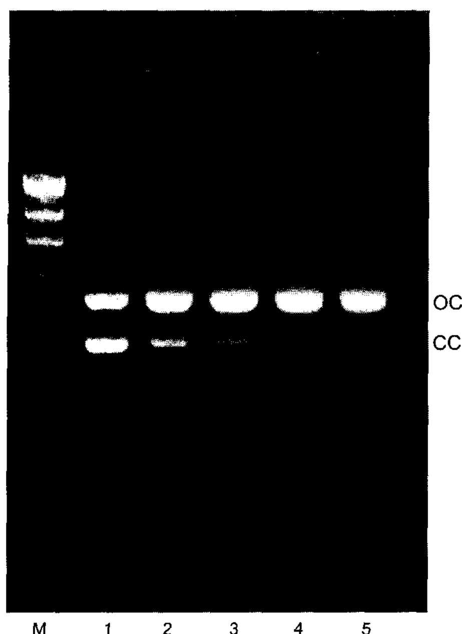

Fig. 1. Electrophoretic pattern of ultrasoft X-ray irradiated pBluescript plasmid DNA.
Lane M, λ/*Hin*dII; lane 1, control (before the irradiation); lanes 2–5, DNA exposured for 5, 10, 20, 30 min, respectively.

isomaltosyl maltooligosaccharides, and levan were approximately 2.0, 1.7, 1.3, 1.4, and 2.1 times, respectively, compared with the control DNA without carbohydrates (Table 1). Similarly, these carbohydrates also reduced UV damage to *E. coli* cells (Fig. 2). Among those tested, BOS-12 and levan showed a greater protective effect for both DNA and cells.

Protective Effect from Ultrasoft X-ray-Induced Damage

Similar to the protective effect of carbohydrates on DNA and *E. coli* damage by UV rays, BOS-12, BOS-9, and isomaltosyl raffinooligosaccharides also showed protective effects on *E. coli* damaged by ultrasoft X-ray exposure (Fig. 3 and Table 2). BOS-12 showed the highest protective effect among carbohydrates tested for both DNA and cells.

Table 1. Relative protection effects from ultraviolet damage of various saccharides on DNA.

Carbohydrate	Relative ratio of protection effects
No sugar[a]	1
BOS-12[b]	2.03
BOS-9[c]	1.74
Isomaltosyl maltooligosaccharides	1.40
Isomaltosyl raffinooligosaccharides	1.31
Levan	2.11

[a]No addition of any carbohydrate.
[b,c]See Ref. [5].
*Each sample (2.5%) was exposed to a 254 nm UV lamp for 10 min.

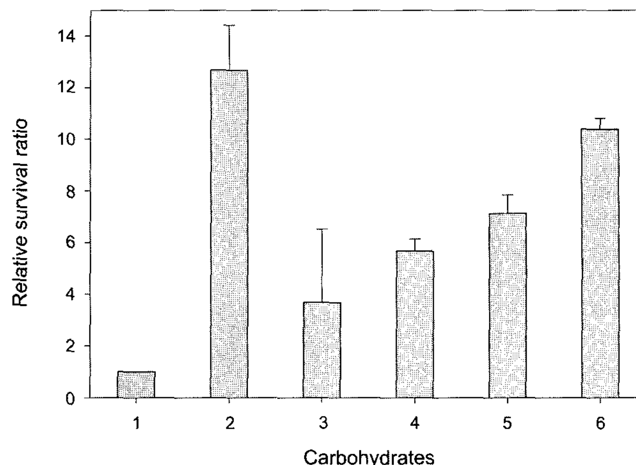

Fig. 2. Relative protection of *E. coli* from UV damage by various carbohydrates.
1, Control (without carbohydrate addition); 2, BOS-12; 3, BOS-9; 4, isomaltosyl raffinooligosaccharides; 5, isomaltosyl maltooligosaccharides; 6, levan. *Sample (2.5%) was exposed to a 254 nm UV lamp for 10 min.

Protective effects for DNA and cells following ultrasoft X-ray exposure were 1.9 and 8.3 times higher than the control.

Almost the same constant rate of 4×10^{-8} /M·s for the OH radical scavenging reaction by DNA and glucose was reported, suggesting that equimolar DNA and disaccharides or oligosaccharides can consume the radical at equivalent kinetic rates [20]. The DNA concentration in the experiment was 1×10^{-3} g/l, equivalent to 3.08×10^{-6} M of nucleotide (assuming an average molecular weight of 325).

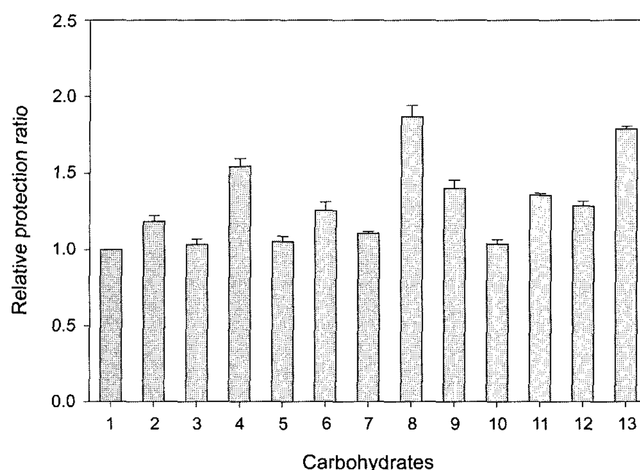

Fig. 3. Relative protection effects of *E. coli* from ultrasoft X-ray exposure by various carbohydrates.
1, Control (without carbohydrate addition); 2, trehalose; 3, maltose; 4, BOS-9; 5, pullulan; 6, xylan; 7, gentiobiose; 8, BOS-12; 9, acarbose; 10, amylodextrin; 11, maltopentaose; 12, isomaltosyl maltooligosaccharides; 13, isomaltosyl raffinooligosaccharides.

Table 2. Time required for the conversion of 50% CC form DNA to OC form DNA after ultrasoft X-ray exposure.

Carbohydrates	Time for 50% conversion (min)
No sugar (control)[a]	7.7
Glucose[†]	6.6
Fructose[†]	13.0
Sucrose[†]	8.6
Trehalose[†]	17.0
Gentiobiose[†]	13.0
Lactose[†]	13.0
Maltose[†]	10.5
Maltotriose[†]	12.6
Acarbose[†]	23.7
Maltopentaose[†]	13.6
α-Cyclodextrin[†]	10.9
β-Cyclodextrin[†]	7.4
Isomaltosyl maltooligosaccharides[‡]	14.7
Isomaltosyl raffinooligosaccharides[‡]	24.8
Amylodextrin[‡]	9.9
BOS-9[b‡]	57.3
BOS-12[c‡]	63.8
Pullulan[‡]	15.2
Levan[‡]	7.3
Xylan[‡]	7.6
Dextran T-10[‡]	13.2
Dextran T-40[‡]	6.3

[a]No addition of any saccharide.
[b,c]See Ref. [5].
[†]The final concentration was 100 mM.
[‡]The final concentration was 2.5% (w/v).

UV Absorption of Carbohydrates

The UV absorption intensity in the presence of different carbohydrate solutions was compared (Table 3). When the solution contained carbohydrates, the UV absorption increased. BOS-12 showed the highest absorption among other solutions tested. Therefore, it is assumed that this protective effect might be correlated with the intensity of UV absorption or ultrasoft X-ray in the presence of carbohydrates. This protective effect was expected, because the main factor for radiation-induced scission of DNA molecule is attributable to the OH radicals formed as a result of decomposition of the surrounding water molecules, and the protection of carbohydrates might be effected by scavenging the OH radicals [15]. With the addition of carbohydrates to CC form DNA, ultrasoft X-ray converted 50% of CC form DNA to OC form after 7.7 min of exposure under the experimental conditions (Table 2). However, by the addition of BOS-12, BOS-9, isomaltosyl raffinooligosaccharides, and acarbose, the time required for 50% conversion was extended to 63.8, 57.3, 24.8, and 23.7 min, respectively.

Table 3. UV absorption by various carbohydrates.

Carbohydrates	UV absorbance (220–300 nm)**
No sugar[a]	0.62
BOS-12[b]	1.524
BOS-9[c]	0.873
Isomaltosyl maltooligosaccharides	1.432
Isomaltosyl raffinooligosaccharides	1.035

[a]No addition of any saccharide.
[b,c]See Ref. [5].
**The highest absorption value of each carbohydrate (2.5%) between 220 nm and 300 nm.

Influence of Psoralen on Protective Effect

Psoralen is used as an ingredient of cosmetics and suntan products. 4'-Hydroxymethyl-4,5',8-trimethylpsoralen (HMT), 5-methoxypsoralen, and 8-methoxypsoralen are the psoralen derivatives. A combination of psoralen and ultraviolet A radiation, commonly referred to as "PUVA," is widely used in the treatment of psoriasis and for tumor photochemotherapy [3, 6]. This therapy consists of oral or tropical administration of 8-methoxypsoralen (8-MOP) followed by exposure to longwave UVA radiation (320–400 nm) [6]. However, the wide-spread use of PUVA therapy has made its potential long-term side-effects an issue of concern and debate [12, 17]. PUVA follow-up studies revealed increased risks of the development of squamous cell carcinoma in patients receiving PUVA therapy [18], although European studies appeared to be at odds with these reports [1]. The addition of MOP was shown to protect DNA against UV exposure, which yields its conversion to the open form (Table 4). The best protective effects were associated with the use of branched oligosaccharides compared with commercially available

Table 4. Protection of DNA from UV damage by various oligosaccharides and MOP.

Treatment	Conversion (%)
DNA (No UV exposure)	0
DNA+FOS (2.5%)	81
DNA+MOS (2.5%)	73
DNA+BOS-12 (2.5%)	46
DNA+BOS-9 (2.5%)	29
DNA+MOP	32
DNA+FOS (1%)+MOP	23
DNA+MOS (1%)+MOP	22
DNA+BOS-12 (1%)+MOP	4
DNA+BOS-9 (1%)+MOP	3
DNA+FOS (2.5%)+MOP	19
DNA+MOS (2.5%)+MOP	24
DNA+BOS-12 (2.5%)+MOP	0
DNA+BOS-9 (2.5%)+MOP	0

FOS, fructooligosaccharide; MOS, maltooligosaccharide; BOS-9 and BOS-12, See Ref. [5]; MOP, 8-methoxypsoralen (5 mM).
Values in parenthesis represent the final concentration of each sample.

fructooligosaccharides (FOS) and maltooligosaccharides (MOS). In the case of FOS, the percentage of conversion of DNA CC form to OC form was 81%, and that of MOS was 73%. BOS-12 and BOS-9 showed the conversion rates of 46 and 29%, respectively. The addition of MOP to oligosaccharides further increased the protective effect. FOS or MOS, in combination with MOP, decreased the conversion rate to 23% and 22%, respectively. This protection was even higher when MOP was added to branched oligosaccharides, decreasing the % conversion (CC form to OC form DNA) to 0–4%. Thus, the potential side-effects by ingredients of cosmetics and suntan products can be reduced by the addition of carbohydrates, especially by branched oligosaccharides.

The protective effect of DNA and cells by carbohydrates from radiation damages will be useful in developing methods to protect living cells from harmful environmental radiation from outer space, or leakage of radioactive materials from nuclear power plants. Carbohydrates can be readily added as ingredients in cosmetics and suntan products for protection from UV damage, while reducing potential MOP side-effects.

Acknowledgments

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