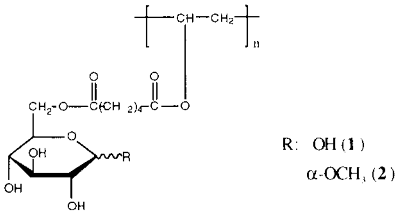
Generation of Superoxide by Synthetic Polymers Containing Reducing Sugar Branches

Masaru Kitagawa and Yutaka Tokiwa*†
Research Institute of Innovative Technology for the Earth (RITE) NIBH Branch
†*National Institute of Bioscience and Human-Technology (NIBH), 1-1 Higashi, Tsukuba, Ibaraki 305*

(Received November 11, 1997; CL-970860)

Generation of superoxide from a polymer containing reducing sugar branches, poly(6-*O*-vinyladipoyl-D-glucose), was observed by the reduction of nitroblue tetrazolium (NBT), and the activity tended to increase with increasing molecular weight of the polymer.

It has been found that oxygen radical species are generated by autoxidation of reducing sugars under physiological conditions.[1] The reactions are important in biological systems such as inactivation of viruses and cleavage of DNA.[2] Enolization of acyclic forms of reducing sugars may be a rate determining step in the reduction.[3,4] Extensive investigations have been conducted on superoxide generation from monosaccharides [1] and synthetic polysaccharides which were prepared by polycondensation of reducing aldoses or ketoses.[5] Although Kobayashi *et al.* reported the synthesis of poly(D-glucopyranose-3-*O*-oxymethylstyrene), little information is available regarding polymer containing reducing sugar branches.[6] We have examined enzymatic and chemical synthesis of polymers containing sugar branches.[7,8] It is important to investigate the function of the polymer on the development as an intelligent material. We here report superoxide generation activity of the polymer measured by NBT reduction.



Synthesis of reducing sugar branched polymer, poly(6-*O*-vinyladipoyl-D-glucose) **1** and non-reducing sugar branched polymer, poly(methyl 6-*O*-vinyladipoyl-α-D-glucoside) **2** were reported. Specially, polymerizable sugar esters, 6-*O*-vinyladipate of sugars, were synthesized by use of enzymatic catalyst,[7] and then the polymers were obtained by radical polymerization.[8] Generation of superoxide from polymers was studied by determining the rate of formazan formation (ΔA_{560}/min) by superoxide from NBT according to the method of Beauchamp and Fridovich.[9] Measurements were made using various concentrations of the compounds and 50 μM NBT immediately after dissolution of the compounds in a 0.015 M carbonate buffer (pH 10.4) at 25 °C. The reaction mixtures were equilibrated with air or were purged with nitrogen gas, and A_{560} after 20 min of reaction increased linearly. The change in optical density of the mixture was measured using a Shimadzu spectrophotometer UV-160A equipped with a constant temperature cell compartment.

We first compared the NBT reduction activity of reducing sugar branched polymer **1** with non-reducing sugar branched polymer **2**. As shown in Table 1, the reducing sugar branched polymer **1** was found to show much higher activity than the non-reducing sugar branched polymer **2**. Furthermore, the polymer greatly increased the NBT reduction rates compared with the corresponding monomers. The NBT reduction became higher in the order of D-glucose, 6-*O*-vinyladipoyl-D-glucose, and polymer **1**. Under nitrogen gas, the NBT reduction of the reducing sugar branched polymer **1** was reduced to less than half (1.00 ΔA_{560}/min x 10^{-3}) as compared with that in the presence of air. Non-reducing sugar, methyl α-D-glucoside, did not reduce NBT even at a high concentration (60 mM). Although the reason is not clear, methyl 6-*O*-vinyladipoyl-α-D-glucoside and its polymer **2** showed low reduction activity even without reducing sugar. The

Table 1. NBT reduction by the polymer containing sugar branches

Compounds	Concentration[a] (mM)	NBT reduction (ΔA_{560}/min × 10^{-3})
D-Glucose	30	0.03
6-*O*-Vinyladipoyl- D-glucose	30	0.13
Polymer **1**	10	2.50
Polymer **1**[b]	10	1.00
Methyl α-D-glucoside	60	0.00
Methyl 6-*O*-vinyladipoyl - α-D-glucoside	60	0.10
Polymer **2**	15	0.25

[a] The concentrations of the polymer solution represents monomer concentration obtained by dividing the amount of the polymer by the molecular weight of the monomer unit. The molecular weights of polymer **1** and **2** are 7100 and 7200.
[b] The reaction was carried out under nitrogen gas.

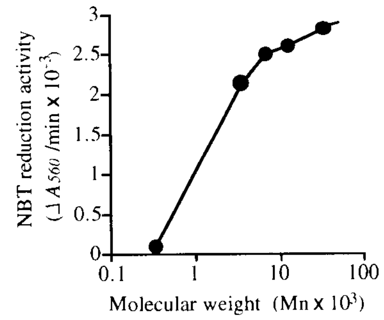


Figure 1. Effect of molecular weight of reducing sugar branched polymer **1**. Measurements were made using 10 mM of the polymer. The concentrations of the polymer solution represents monomer concentration obtained by dividing the amount of the polymer by the molecular weight of the monomer unit. The molecular weights (Mn) are 344, 3600, 7100, 12900 and 34400 from the left.

above results demonstrate that the reducing sugar branched polymer **1** generates superoxide by reaction with oxygen dissolved in the aqueous solution and that the increased activity of the polymer can be attributed to the polymer effect.

Prompted by the above results, we examined the effect of molecular weight of the polymer as shown in Figure 1. NBT reduction activities were observed to be much higher in most polymers than in monomer, and tended to increase with increasing molecular weights of the polymer. In this experiment, the superoxide generation was enhanced more by the polymers possessing high molecular weight.

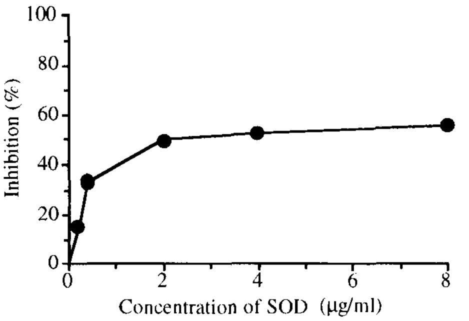


Figure 2. Inhibition of NBT reduction with reducing sugar branched polymer **1** by SOD. Measurements were made using 10 mM of the polymer (Mn=7,100) in the presence of various concentrations of SOD. The concentrations of the polymer solution represents monomer concentration obtained by dividing the amount of the polymer by the molecular weight of the monomer unit.

To confirm the generation of superoxide in NBT reductions, the effect of superoxide dismutase (SOD, EC1.15.1.1, bovine erythrocytes) on the reaction was examined as shown in Figure 2. SOD was found to inhibit the NBT reduction, by about 50%. NBT reduction was decreased by more than 50 % under nitrogen gas as mentioned above. Although it was suggested that chemical species such as intermediate anions (enediol anions) other than superoxide may reduce NBT, these results give evidence that the polymers generate superoxide, and are responsible for half of the reduction of NBT.

The present work shows that the amounts of superoxide from the reducing sugar branched polymer **1** containing glucose was much higher than that of monomer. NBT reduction of D-glucose is known to be lower than that of mono-saccharides such as ribose, fructose and so on.[1] We are now conducting experiments regarding the reduction activity of other reducing sugar branched polymers. Further, the reason for the polymer effect on the rate of the NBT reduction remains to be studied.

This study was performed as a part of the development of Biodegradable Plastics supported by the New Energy and Industrial Technology Development Organization (NEDO).

References and Notes

1 N. Kashimura, J. Morita, and T. Komano, *Carbohydr. Res.*, **70**, C3 (1979).
2 N. Kashimura, J. Morita, I. Sato, Z. Kumazawa, S. Nishikawa, S. Ito, Y. Koma, and M. Komada, in "Amino-Carbonyl Reaction in Food and Biological System," ed by M. Fujimaki, M. Namiki, and H. Kato, Elsevier-Kodansha, Holland (1986), pp401.
3 H. S. Isbell, *Carbohydr. Res.*, **49**, C1 (1976).
4 P. Thornalley, S. Wolff, J. Crabbe, and A. Stern, *Biochim. Biophys. Acta*, **797**, 276 (1984).
5 J. Morita, N. Kashimura, and T. Komano, *Agric. Biol. Chem.*, **44**, 2971 (1980).
6 K. Kobayashi and H. Sumitomo, *Macromolecules*, **13**, 234 (1980).
7 S. Shibatani, M. Kitagawa, and Y. Tokiwa, *Biotechnol. Lett.*, **19**, 511 (1997).
8 M. Kitagawa and Y. Tokiwa, *Carbohydr. Lett.*, **2**, 343 (1997).
9 C. Beauchamp and I. Fridovich, *Anal. Biochem.*, **44**, 276 (1971).