Virus Inactivation in Superoxide Dismutase Preparations by Ultraviolet Light Irradiation

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Viral inactivation in superoxide dismutase (SOD) derived from human red cells was carried out by ultraviolet light C (UVC) irradiation. With 400 J/m² UVC irradiation, the titer of canine parvovirus (CPV, a nonenveloped virus), M13 bacteriophage (M13, a nonenveloped phage) and vesicular stomatitis virus (VSV, an enveloped virus), which were spiked into SOD solution, were reduced by $\geq 4.6 \log_{10}$ (detection limit), 7.0$\log_{10}$, and 6.2$\log_{10}$, respectively. The SOD activity was maintained and the band pattern of SOD on an electrophoresis gel was not changed even by 1000 J/m² UVC irradiation. These results indicate that UVC irradiation is a promising method for the inactivation of both enveloped and nonenveloped viruses in SOD preparations while maintaining its activity.

Key words: viral inactivation; superoxide dismutase; ultraviolet light C.

Copper, zinc superoxide dismutase (SOD) is known to catalyze the dismutation of the superoxide radical to O₂ and H₂O₂. SOD has been investigated as a medical materials because its therapeutic benefits have reported: SOD counteracts many deleterious physiological effects induced by superoxide radicals such as aging, tumorigenesis, dermatitis, diabetes, rheumatoid arthritis, hepatitis and Alzheimer syndrome.

Human red cells are believed to be one of several feasible sources of a significant amount of SOD as well as recombinant SOD. The present study was designed to make good use of out-dated human red cells in donated blood (the preservation limit in Japan: 21 d).

The use of biological raw materials involves a slight risk of viral transmission. The viral safety of blood products has been significantly enhanced as a consequence of improvements in donor screening and virucidal procedures. However, viruses cannot be detected by serological tests during a window period (the time before current tests detect the presence of antibodies in virus-infected individuals). Screening tests for the detection of parvovirus B19 and some other pathogenic viruses have not yet been carried out. A solvent detergent (SD) method and heat treatment have been introduced for viral inactivation in the preparation process of coagulation factors and other protein materials derived from donated blood. Both the SD method and heat treatment provide markedly enhanced viral safety with respect to human immunodeficiency virus (HIV), hepatitis B virus (HBV) and hepatitis C virus (HCV). However, there are still risks of transmission of nonenveloped and heat-stable viruses such as parvovirus B19.

Ultraviolet light C (UVC) irradiation is known to be effective for inactivation of not only enveloped viruses, but also nonenveloped viruses. Some nonenveloped viruses reportedly spiked into plasma, Factor VIII or fibrinogen preparations were sufficiently inactivated by UVC irradiation. UVC irradiation targets nucleic acids rather than proteins due to the much higher extinction coefficient of the former at 260 nm and their larger target size. The photon absorbed by nucleic acids primarily causes the dimerization of adjacent pyrimidines. The resultant intranucleotide cross-link renders the viral genome incapable of replicating. Thus, UVC can inactivate a wide variety of viruses, especially those having single-strand nucleic acid. Therefore, we selected UVC irradiation for virus inactivation in SOD solution.

We report here viral inactivation in SOD solution by UVC irradiation, in which canine parvovirus (CPV, a nonenveloped virus), M13 phage (M13, a nonenveloped virus) and vesicular stomatitis virus (VSV, an enveloped virus) were selected as model viruses. The damage to SOD by UVC irradiation was also investigated.

MATERIALS AND METHODS

Chemical reagents Superoxide dismutase (Cu, Zn SOD) from human erythrocytes was purchased from Sigma Chemicals (St. Louis, MO). The superoxide dismutase assay kit (SOD-525) was purchased from Calbiochem–Navabiochem Corporation (San Diego, CA). The Vectastain ABC kit was from VECTOR Laboratories (Burlingame, CA). Isopropyl-β-thiogalactopyranoside (IPTG) and 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal) were obtained from Gibco Laboratories (New York, NY). Methylen blue (MB) was from J. T. Baker (Phillipsburg, NJ).

Virus titration The VSV assay was performed according to the method of Armstrong. Briefly, we added 1-in-10 serial dilution of the virus samples to 96-well microtiter cell culture plates containing Vero cells (American Type Culture Collection, Rockville, MD). Vero cells were grown in Dulbecco’s modified Eagle medium (DMEM) containing 10% fetal calf serum (FCS). After incubation for 72 h at 37 °C in 5% CO₂, the plates were scored for cytopathic effects induced by the presence of viruses. Infectivity was calculated according to the Reed and Muench method.

Canine parvovirus (CPV) titration was performed by the plaque-forming assay. Briefly, CPV solution was adsorbed

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to Crandell feline kidney (CRFK) cells (American Type Culture Collection) in a 6-well plate for 1 h at 37°C, and the cells were overlayed with DMEM containing 0.1% methylcellulose and 10% FCS and incubated at 37°C in 5% CO₂.

Fig. 1. The Inactivation of CPV, M13, and VSV by UVC Irradiation

Each virus suspension diluted 10-fold with PBS was irradiated by UVC at the indicated doses. After irradiation, virus infectivity was measured as described in Materials and Methods. (mean ± S.D., n = 3).

M13 bacteriophage and Escherichia coli XL-1 Blue were purchased from Stratagene (La Jolla, CA). Two milliliters of phage suspension and 2 ml of XL-1 Blue overnight culture were inoculated to 200 ml of LB medium and incubated at 37°C overnight with shaking. After centrifugation at 8000 g for 15 min, the supernatant containing the phage was made 2.6% (w/v) in polyethylene glycol 6000 and 0.33 M in NaCl, and then centrifuged at 18000 g for 15 min at 4°C. The pellet was suspended in phosphate-buffered saline (PBS), and the phage was purified by CsCl density gradient (1.3 g/ml) centrifugation at 100000 g for 24 h at 22°C (Beckman SW25Ti, Beckman Instruments, Fullerton, CA). After dialysis against PBS, the phage suspension was stored at −80°C until use. The M13 phage titer was determined by a standard top agar method using XL-1 Blue as a host strain. This phage forms blue plaques as a result of lac Z gene complementation. XL-1 Blue was supplemented with IPTG for lac Z induction, and with X-gal for lac Z detection.

Phototreatment One milliliter of virus suspension diluted 10-fold with PBS, which contained 0.5 mg SOD, was added to each well (diameter 35 mm) of a 6-well plate. The plate containing the virus suspension was placed on a shaker inside a clean bench with the cover of the plate removed. The sample was irradiated by a UVC lamp (2000 erg/cm²/s, GL-6, Toshiba, Japan) with shaking (80/min). The irradiation dose was controlled by the irradiation time. The fluence rate was measured with a radiometer (model 4090, Springfield Jarco Instruments, Yellow Springs, OH). The white light was from a 1-kW tungsten-halogen lamp.

SOD Assay The SOD activity was measured by the SOD assay kit according to the supplier's instruction. The assay is based on the SOD mediated increase in the rate of autoxidation of 5,6,6a,11b-tetrahydro-3,9,10-trihydroxybenzo(fluorene in aqueous alkaline solution to yield a chromophore with maximum absorbance at 525 nm. The treated sample containing 0.5 mg/ml SOD was diluted 5-fold with PBS prior to the assay, because 0.5 mg/ml SOD was beyond the measurement limit of the assay kit. For a KCN-treated sample (which sufficiently inactivated the SOD activity), a sample was incubated with 0.13 M KCN prior to the assay.

SDS-PAGE Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) was carried out by the method of Laemml[14] using 5–20% gradient polyacrylamide gels. Phototreated or control virus suspension denatured with 1% SDS was applied to each lane of the gel. The bands were stained with Coomassie Brilliant Blue R-250.

RESULTS AND DISCUSSION

This study was designed to investigate the possible use of UVC irradiation for virus inactivation in SOD preparations after the final column chromatography of its purification process.15 The SOD concentration after final column chromatography was previously found to be about 0.5 mg/ml; therefore, the concentration of SOD suspension used for the present experiments was adjusted to 0.5 mg/ml.

SOD solution containing virus was irradiated by UVC (Fig. 1). With 400 J/m² UVC irradiation, the log reduction values of CPV (nonenveloped virus), M13 (nonenveloped phage) and VSV (enveloped virus) were >4.6 log₁₀ (detection limit), 7.0 log₁₀ and 6.2 log₁₀ respectively. These values indicate that 400 J/m² irradiation sufficiently inactivates three viruses.
Measurement of the activity of SOD irradiated by UVC (Fig. 2) showed that the SOD activity was not reduced, even by 1000 J/m² UVC irradiation.

SOD solution irradiated by UVC was subjected to SDS-PAGE (Fig. 3). Similar to the case of SOD activity, the sharpness of the SOD band on the electrophoresis gel was not changed even by 1000 J/m² UVC irradiation. These results indicate that UVC irradiation can sufficiently inactivate three viruses while retaining the activity of SOD. This SOD property is in sharp contrast to the fact that the activity of many coaguration factors in plasma were inactivated by 1000 J/m² UVC irradiation.  

UVC-induced photochemical damage of proteins can principally involve photodynamic reaction, which are mediated both by type I reaction (superoxide or hydroxy radical) and type II reaction (singlet oxygen). As described above, SOD activity is resistant to 1000 J/m² UVC irradiation. Superoxide is thought to be easily scavenged by SOD itself. It is well known that MB is a singlet oxygen generator when irradiated. As shown in Fig. 3, the band of SOD treated with MB plus irradiation shifted upward, indicating that SOD is damaged by singlet oxygen. (The activity was decreased by ca. 25% (data not shown)) The reason for the apparent increase in molecular weight of 30 J/cm² irradiated SOD is not clear at present. Therefore, the amount of active oxygens, such as singlet oxygen or hydroxy radical, produced by UVC irradiation may be too little to inactivate the activity of SOD.

It was reported that porcine parvovirus, hepatitis A virus and encephalomyocarditis virus (each of which is a non-enveloped viruses) were sufficiently inactivated with 1000 J/m² UVC irradiation. UVC irradiation is thus expected to be able to inactivate other kinds of nonenveloped viruses in SOD preparations as well as enveloped viruses. UVC irradiation is concluded to be a promising method for inactivating viruses in SOD preparations, and may also be applicable to other biological or medical materials.

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