## Notes

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# Stability of Enzyme Preparations on Radiosterilization

Kazuhiro Morimoto, Shojiro Kimura, Yoshihiko Inamori and Katsuaki Morisaka

*Osaka College of Pharmacy*[1]

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The sterilizing effects of gamma rays and the stabilities of enzymes to gamma radiation were studied. The sterilizing effects of gamma rays were similar in powder and liquid states of four enzymes with *E. coli* and *Staph. aureus* as contaminants. $D_{10}$ values for *E. coli* solution and *E. coli* lactose powder were both 37 krad, and those for *Staph. aureus* solution and *Staph. aureus* lactose powder were 30 krad and 25 krad, respectively. The stability of enzymatic activity to gamma radiation depended on the form of the enzyme material. In solution the activity of each enzyme was reduced by a dose of 10 krad. Trypsin and kallikrein were inactivated completely by 50 krad and chymotrypsin and bromelain by 100 krad. However, in the powder state, no enzyme was found to show loss of activity at less than 500 krad. All the enzymes retained more than 90% activity after 1 Mrad irradiation, and substantial activities were retained after 5 Mrad.

No difference in the stability of enzymatic activity was found between the powder state and lactose mixture. When water or ethanol was present in the lactose mixture, the stability to gamma radiation was greatly reduced.

**Keywords**——sterilization; stability of enzyme preparation; gamma irradiation; *Escherichia coli*; *Staphylococcus aureus*; trypsin; chymotrypsin; kallikrein; bromelain

The sterilization of enzyme preparations is a difficult problem, because of the instability of enzymes on heating and the likelihood of bacterial contamination. Among various sterilization procedures, the irradiation method, which can be carried out in the cold with packaged materials, has been used for the sterilization of disposable medical supplies and for the preservation of foods, as well as for the sterilization of amylase preparations,[2] protease preparations[3] and other enzyme preparations.[4,5] The efficacy of radiation sterilization against various bacteria was described by Sato.[6]

In this work, the effects of gamma radiation were investigated on three different preparations of four enzymes. The sterilizing effects of gamma radiation were studied on *Escherichia coli* and *Staphylococcus aureus*.

### Materials and Methods

**Enzyme Preparations**——Enzymes studied were trypsin (Miles No. E7: activity 4500 NFU/mg), chymotrypsin (Miles No. 1a: activity 1150 NFU/mg), kallikrein (Canada Packer No. 27057: activity 70 ku/mg), and bromelain (Amano Pharm. Mfg Co. No BRB– 10527: activity 853 U/mg). The effects of gamma radiation were studied on three different forms of the materials.

Powder state: 30 mg of the material was enclosed in an ampoule.

1) Location: *Kawai, Matsubara-shi, Osaka 580 Japan.*
2) K. Kawashima, Y. Nango, T. Doi and K. Umeda, *J. of Food Industry of Japan*, **20**, 9 (1973).
3) K. Kawashima, Y. Nango, T. Doi and K. Umeda, *J. of Food Industry of Japan.*, **21**, 15 (1974).
4) T. Ueno, F. Yoshida, A. Nishimura and T. Kotaka, *ANN. Rep. Rad. Center, Osaka*, **16**, 62 (1975).
5) T. Sakai, Y. Yoshida, H. Demura, T. Yanagita, M. Meiwa and K. Ohyama, *Chem. Pharm. Bull.*, **26**, 1130 (1978).
6) K. Sato, *J. Antibact. Antifung. Agents*, **6**, T399 (1978).

Lactose mixture: 1 g of lactose was added to 100000 NFU of trypsin, 200000 NFU of chymotrypsin, 10 kU of kallikrein or 85 kU of bromelain. After mixing, 100 mg was enclosed in an ampoule.

Water- or ethanol-containing lactose mixture: Water or ethanol was added to a level of 10% or 20% of the lactose mixture.

Solution: The enzymes were dissolved in saline solution to give the following concentrations; trypsin (22.5 NFU/ml), chymotrypsin (200 NFU/ml), kallikrein (19.6 kU/ml), and bromelain (500 U/ml); 2 ml of the resulting solution was enclosed in an ampoule.

**Bacteria Preparations**——The effects of gamma radiation on contaminating bacteria were studied with *Escherichia coli* strain NIHJ JC-2 and *Staphylococcus aureus* strain 209-P grown in nutrient broth (Eiken Chemical Co.). Bacteria solutions were prepared by adding diluted ($1\times10$) bacteria suspensions to 2 ml of sterilized water and enclosing the whole in an ampoule. Bacterial powders were prepared by adding 0.05 ml of the diluted suspensions to 1 g of lactose, mixing them, and enclosing the whole in an ampoule.

**Gamma Irradiation**——For gamma irradiation, a $^{60}$Co source (6000 Ci) at the Research Institute of Kyoto University was employed. The intensity of the $^{60}$Co source was determined by Firck's ferrous chemical densimetry.[7] Radiation doses were controlled by changing the duration of irradiation. The doses ranged from 10 krad to 1 Mrad. Irradiation was carried at room temperature.

**Determination of Enzyme Activities**——Activities of trypsin and kallikrein were determined with BAEE as a substrate according to Schwert and Takenaka,[8] that of chymotrypsin with ATEE as a substrate according to Schwere and Takenaka[8] and that of bromelain by the JP-IX method.

**Determination of Survival Ratio of Bacteria**——The irradiated samples were incubated in nutrient agar at 37° for 48 hr. The number of colonies was compared with that of a non-irradiated sample, and the survival ratio was determined.

## Results and Discussion

The enzymes in a powder state were fairly stable to gamma radiation. A significant dose of gamma radiation was required (500 krad—1 Mrad) to reduce the enzymatic activities, as shown in Fig. 1. Lactose mixtures of the enzymes were also stable to gamma radiation, as shown in Fig. 2. Enzymatic activities were reduced only when more than 500 krad was given. However, when water or ethanol was included in the lactose mixture, the reduction of the enzymatic activities by radiation was enhanced (Fig. 2). The reductions of activity were not as marked as in the case of solutions (Fig. 1). It can thus be considered that enzymatic activities are reduced not only by the direct action of gamma rays, but also by indirect action of water radicals.

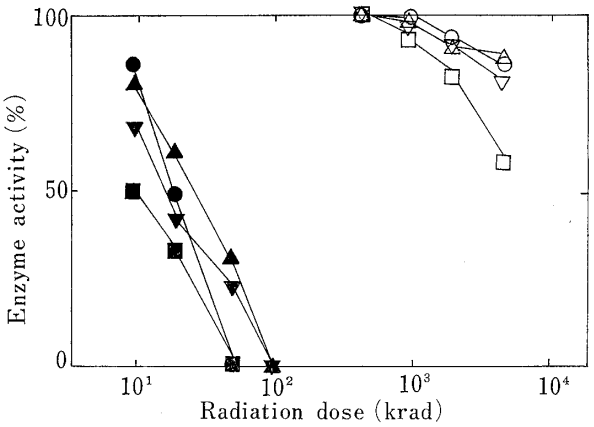


Fig. 1. Effect of Irradiation Dose on the Retained Activities of Various Enzyme Solutions and Powders

Solution: ● trypsin, ▲ chymotrypsin, ■ kallikrein, ▼ bromelain; Powder: ○ trypsin, △ chymotrypsin, □ kallikrein, ▽ bromelain.

While in solution, the enzymatic activities were greatly reduced by a small dose of gamma rays (10 krad); 50 krad destroyed the activities of trypsin and kallikrein, and 100 krad destroyed those of chymotrypsin and bromelain (Fig. 1).

The survival ratio of bacteria is plotted against the dose of gamma radiation in Fig .3. Bacteria are clearly vulnerable to small doses of gamma radiation. $D_{10}$ was 37 krad for *E. coli* both in suspension and lactose mixture; the values were 30 krad and 25 krad for *Staph. aureus* in suspension and lactose mixture, respectively. However, the difference between the two states is not significant. The data are in agreement with those of Sato.[6]

7) N. Miller and J. Wilkinson, *Trans. Faraday Soc.*, **50**, 690 (1954).
8) G.M. Schwert and Y. Takenaka, *Biochim. Biophys. Acta*, **16**, 570 (19555).

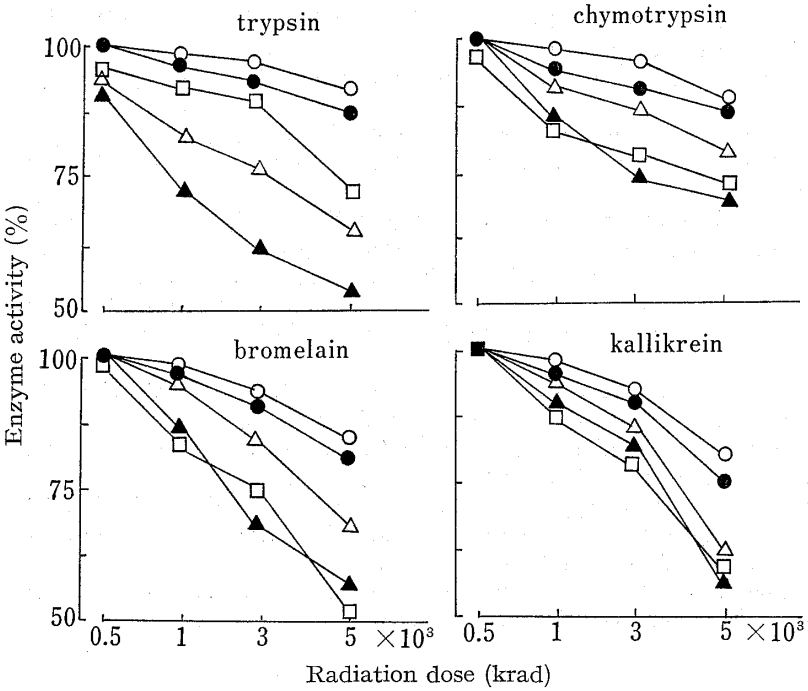


Fig. 2. Effect of Irradiation on Four Enzymes Preparation Containing Water or Ethanol

○, enzyme, △, enzyme+lactose+10% $H_2O$, □, enzyme+lactose+10% ethanol, ●, enzyme+lactose, ▲, enzyme+lactose+20% $H_2O$.

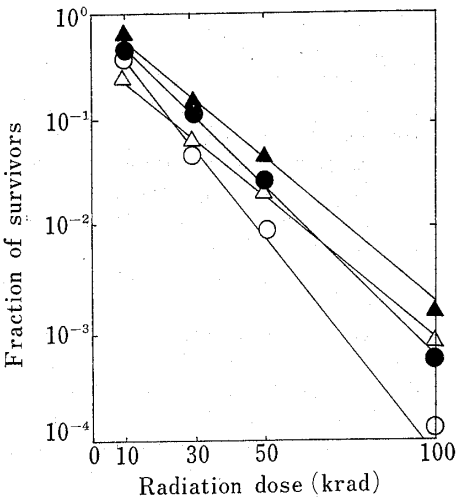


Fig. 3. Effect of Gamma Irradiation of Solutions and Lactose Powders Containing *E. coli* NIHJ JC-2 and *Staph. aureus* 209-P

| | $D_{10}$ value |
|---|---|
| *E. coli* solution ▲ | 37 krad |
| *E. coli* lactose powder △ | 37 krad |
| *Staph. aureus* solution ● | 30 krad |
| *Staph. aureus* lactose powder ○ | 25 krad |

In view of the instability of the enzymatic activities in the liquid state to gamma radiation, it is not practical to employ gamma radiation for the sterilization of enzyme solutions. In contrast, gamma radiation can be used for the sterilization of enzymes in powder form or lactose mixtures if the water content is maintained at a low level.

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