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Effect of the Radioprotector 2-Mercaptopropionylglycine (MPG) on the Radiation Inactivation of Catalase *In Vitro*

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Studies were performed to provide mechanistic insights into the action of the radioprotector drug, 2-mercaptopropionylglycine (MPG) following the radiolysis of catalase, a detoxifying enzyme. The enzyme solution was γ-irradiated in the presence and absence of MPG. The enzyme activity was monitored *in vitro* using H_2O_2 as a substrate. MPG behaved primarily as a radioprotective drug. However, due to the presence of Fe^{++}/Fe^{+++}, and the in homogeneity of catalase, under certain conditions there was circumstancial interaction of Fe^{++}/Fe^{+++} with MPG, resulting in the formation of an unstable catalase Fe^{++}/Fe^{+++}-MPG chelate/complex. This resulted in the radiosensitizing effect of MPG on enzyme catalase.

INTRODUCTION

2-mercaptopropionylglycine (MPG) has been widely used as a potent radioprotector *in vivo* and *in vitro*[1-3]. The proposed radioprotective mechanism varies from a release of endogenous protectors[4], to a high redox potential[5], to free radical scavenging[6]. However, MPG did not exhibit the protection characteristic of spontaneous or chemically induced lipid peroxidation[7], or certain conditions of radiation-induced microsomal lipid peroxidation[2]. From these view points, we have strived to reason the protection/non-protection offered by MPG. We have chosen an enzyme catalase because: (i) it acts as an oxidizing agent to ethanol, with the consumption of 1 mole of peroxide; that is, it decomposes hydrogen peroxide (detoxification), providing a convenient assay procedure, and (ii) it has been used extensively to decipher the mechanism of radiolysis of protein[8]. Our experimental goal was to procure insight into the mechanism of radioprotection or lack of protection by MPG in the radiation-induced inactivation of catalase.

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EXPERIMENTAL

Chemicals

All chemicals used were of analytical grade and were used without further purification. Water was deionized before glass-double distillation.

Catalase (EC.1.11.1.6)

Catalase was obtained from Sigma Chemical Co., USA. Freshly prepared catalase solution in 50 mM phosphate buffer, pH 7.0, was used. The following concentrations of catalase were used in this work (in μg/ml): 0.44, 1.32, 2.20, 3.10, 3.70, and 4.80. One unit of enzyme decomposes 1.0 μmole of H_2O_2 per minute, at pH 7.0 and 25°C.

Catalase Assay System

Immediately after irradiation at room temperature (23°C), assay was performed according to the method of Aebi (1984)[9], with minor modifications. The duration of assay was 30 sec. The enzyme activity is expressed as a difference in absorbance (ΔA_{240}) per unit time. Each data point represents a mean ± S.E. for 10 to 15 assays in a Hitachi spectrophotometer.

2-mercaptopropionylglycine (MPG)

MPG was obtained from Prof. Tsutomu Sugahara, Kyoto (Japan), under the trade name, Tiopronin, marketed by Santen Pharmaceutical Co. Ltd., Japan. MPG, 0.002 M, was freshly prepared in 50 mM phosphate buffer, pH 7.0. The concentration of MPG in the experiments was 0.001 M.

Gamma-irradiation

A ^{60}Co-source (Model-Gamma Chamber-900, BARC, India) delivering γ-radiation at a dose rate of 1 Gy/sec, was used. 1 ml enzyme solutions of appropriate concentrations were mixed with 1 ml phosphate buffer or 1 ml MPG, as required, were allowed to stand for 15 minutes at room temperature (23°C), then irradiated under normal atmospheric conditions with doses of 10, 20, 40 and 80 Gy.

RESULTS AND DISCUSSIONS

Changes in enzyme activity in terms of change in absorbance at 240 nm following various doses ^{60}Co irradiation, with and without MPG, are shown in Figures 1 and 2. A relatively sharp reduction in catalase activity (50 to 68%) was observed in all cases, when it was supplemented with MPG. At low enzyme concentrations (0.44 and 1.32 μg/ml), the initial depression in activity at 10 Gy γ-ray shows increased activity up to 40 Gy (Figures 1A and 1B). At higher concentrations of enzyme (2.20, 3.10, 3.90 and 4.80 μg/ml), however, the observed activity at 10 Gy is followed by a gradual decline in activity with increasing γ-ray doses (Fig. 2A, B, C and D). While the radiation dose remains the same for the low enzyme concentration group (Fig. 1A and B) and the high enzyme concentration group (Fig. 2A, B, C and D), only the former shows a trend of reversibility of effect after a dose of 10 Gy. The damage seems to be severe,

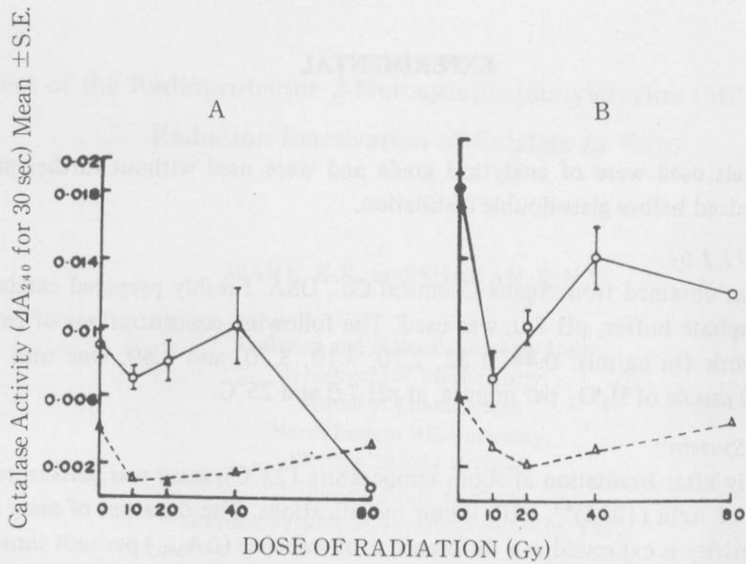

Fig. 1. Catalase activity following various doses of gamma-irradiation with (Δ- - - - - - - - - - -Δ) and without (o————————o) MPG (0.002 M). Concentration of Catalase is 0.44 μg/ml in A and 1.32 μg/ml in B.

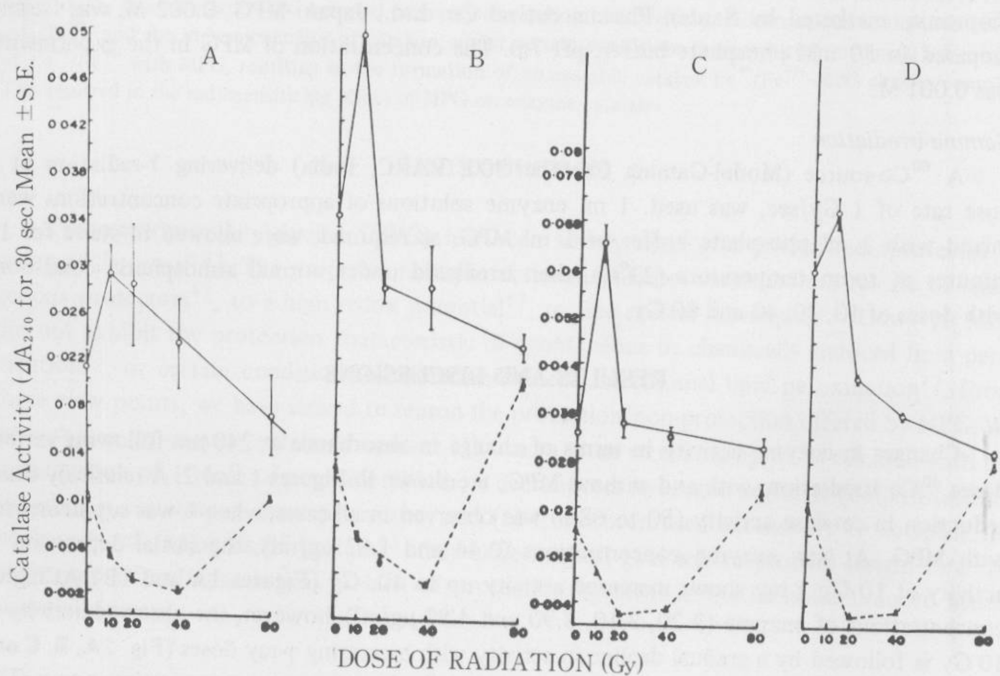

Fig. 2. Catalase activity following various doses of gamma-irradiation with (Δ- - - - - - - - - -Δ) and without (o————————o) MPG (0.002 M). Concentration of Catalase in 2.20 μg/ml in A, 3.10 μg/ml in B, 3.90 μg/ml in C and 4.80 μg/ml in D.

and to some extent dose-dependent, beyond a radiation dose of 10 Gy for higher concentrations of enzyme (Fig. 2A, B, C and D). These results indicate that the concentration of the enzyme may influence radiation damage after a dose of 10 Gy.

In the presence of MPG (0.002 M added to the enzyme preparation 15 min prior to irradiation), from 20 to 40 Gy radiation for various concentrations of catalase induced further reduction in enzyme activity (meaning sensitization of catalase) instead of the expected increase in enzyme activity towards the normal value (which would have meant the protection of catalase). This was followed by an insignificant increase in enzyme activity (protection) in the low protein concentration group (Fig. 1) and a noticeable increase in enzyme activity (protection) in the high protein concentration group (Fig. 2).

The initial decrease in enzyme activity in the controls with the mere addition of MPG can be explained by the formation of MPG-Fe^{++} chelate compound, as suggested by Ayene and Srivastava (1985)[2]; Fe^{++} being provided by the four haematinic groups of the enzyme. We believe that Fe^{++} may not be fully released from the enzyme, but rather, that it remains partially exposed for this chelate formation (see below). This has also been suggested by Ayene and Srivastava (1985)[2]. This may possibly result in the formation of a complex like MPG-Fe^{++}-catalase. This presumption is advanced in an attempt to explain some of the observations in this experiment, cited. To determine whether of this complex involving Fe^{++} was formed, we attempted to supply Fe^{++} and Fe^{+++} ions exogenously to the catalase-MPG solution in order to check the competition of the exogenously supplied Fe^{++}/Fe^{+++} and the haematinic groups of the catalase for MPG. Our efforts were unsuccessful due to the drastic lowering of pH, and problems in solubility and denaturation. Further work to ascertain this is in progress in this laboratory. The possibility of an interaction of MPG with the substrate of catalase (i.e., H_2O_2) has been experimentally ruled out in our laboratory, but the results are not included here.

Hence, three factors may be operating on catalase in the presence only of MPG, or MPG and radiation:

i) Formation of MPG-Fe^{++} chelate/complex, partially disturbing the structural entity of catalase in the haematinic unit, may be the reason for the decrease in enzyme activity in controls;

ii) Radiation-mediated formation of the chelate/complex leading to the same factor as cited above;

iii) Interaction of free radicals produced as a result of radiation, with the amino acid moieties of the enzyme causing deactivation.

The results indicate that radiation probably enhances the MPG-Fe^{++}/Fe^{+++} chelate/complex formation which apparently induces the sensitization effect of MPG for up to 20 Gy (Fig. 1) or 40 Gy (Fig. 2) radiation. The free radical population may not be scavenged by MPG in the form of MPG-Fe^{++}/Fe^{+++} chelate/complex. With doses higher than 20–40 Gy, probably the MPG-Fe^{++}/Fe^{+++} chelate/complex is broken down to MPG and Fe^{++}/Fe^{+++} components. In this condition, while MPG can scavenge free radicals, Fe^{++}/Fe^{+++} can freely restore the structural entity of the haematinic groups by interaction with apocatalase for the restoration of enzyme activity, thereby showing protective effect.

The differences in the pattern of MPG-mediated protective action in Fig. 1 and Fig. 2 needs further clarification. In the case of the high enzyme concentration group (Fig. 2), the

free Fe^{++}/Fe^{+++} could probably restore catalase activity to the MPG treated control level (i.e. without radiation) indicating that the apocatalase portion was not irreversibly damaged by radiation. In the low enzyme concentration group (Fig. 1), since there was only partial MPG-mediated restoration of activity (below MPG treated control level), apparently, the apocatalase portion was damaged more significantly. This may be due to the concentration of free radicals available to react with unit amino acid residues of the apocatalase is higher in the case of low enzyme concentration group, and vice versa.

Thus, our results seem to indicate that the radioprotective effect of MPG is primarily due to free radical scavenging. This is supported by Mishra and Srivastava (1981)[6], and Ambanelli et al. (1981)[10]. The apparent sensitization by MPG for lower doses of radiation was due to circumstantial interaction of MPG with Fe^{++}/Fe^{+++}. However, this dual behaviour of MPG (protection as well as sensitization) must be carefully considered in radiotherapy involving MPG, for the most effective use of this drug.

Additional work is in progress in this laboratory to study the interaction of MPG with Fe^{++}/Fe^{+++} using stopped flow spectroscopy to elucidate the chemical nature of this interaction.

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