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Modification of the Radiation Effect on Catalase by Selenium[1]

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A radioprotective effect of selenoamino acids on amino acids, alcohol dehydrogenase, and ribonuclease has recently been reported (1). The selenium compounds were found to be more effective than analogous sulfur compounds. Scavenging of radiation-produced free radicals, as well as repair mechanisms, are thought to be responsible for protective action. It is the purpose of this communication to show that even the inorganic selenium compound, Na_2SeO_4, protects catalase against x-irradiation through direct action with the enzyme.

MATERIALS AND METHOD

Two ml (in a thin layer) of lyophilized beef liver catalase solution (8.8×10^{-8}M, 0.05M phosphate buffer, pH 7.0; Worthington Biochemical Corp., Freehold, New Jersey) were irradiated in an open Lucite container with an exposure of 4.5×10^5 R. The exposure rate of the beryllium-window x-ray tube (100 kV, 12 mA, HVL 0.065 mm Al; Philips Electronics Inc., Mt. Vernon, New York) was about 9×10^4 R/min. The x-ray tube was calibrated with an air-wall ionization chamber. Na_2SeO_4 (Fisher Scientific Co., Fair Lawn, N. J.) was dissolved in the enzyme solution prior to irradiation. The selenium concentrations used varied between 0.26 and 24.0 mM.

Since selenium inactivates catalase, the time course for the activity change of the controls was studied. In order to study the protective effect of selenium at various times after preparing the enzyme-selenate solutions, the samples were irradiated with a constant dose immediately before determining the enzyme activity. The catalase activity was determined spectrophotometrically (at 240 mμ; Cary 14 Spectrophotometer; Applied Physics Corps., Monrovia, California) using the method of Beers and Sizer (2). Details of the procedure were described previously (3). All measurements and irradiations were done at room temperature.

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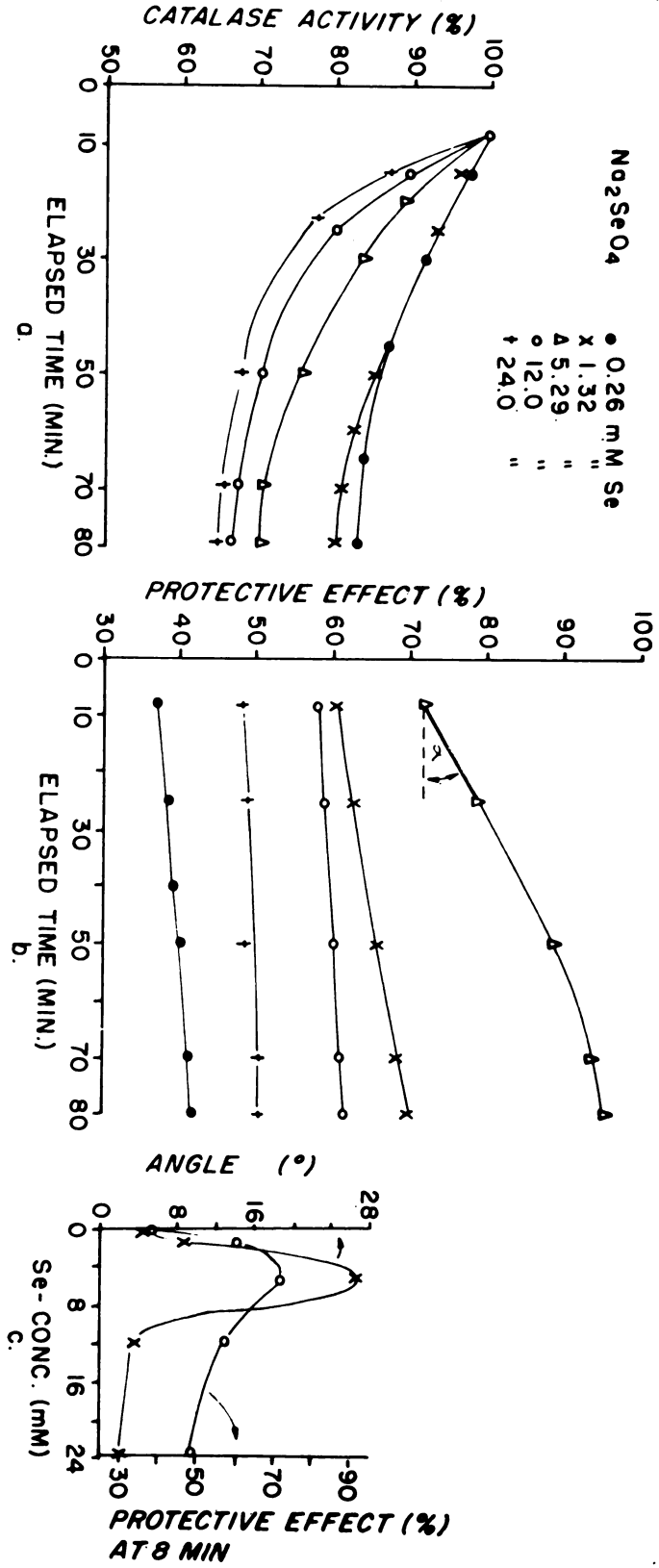

Fig. 1. The influence of different concentrations of Na_2SeO_4 on catalase activity: a.) controls (rel. values) b.) radioprotective effect; details see text c.) radioprotective effect (o) and angle α (x) (see b.) vs. Se-concentration.

RESULTS AND DISCUSSION

The inactivation of catalase by Na_2SeO_4 is shown in Fig. 1. For comparison, the value obtained for the activity eight min after preparing the enzyme-selenate solution was set as 100 per cent. With the highest selenium concentration used (24 mM), the relative value for the catalase activity 70 min after preparing the solution is about 65.8%; in absolute units a value of about 57% was obtained. The absolute units were used for calculating the protective effect.

Previous investigations have shown (3) that an exposure of 4.5×10^5 R completely inactivates this catalase solution. In order to determine the radioprotective effect of selenium, samples were irradiated with this exposure immediately before activity determination (at various times after preparing the enzyme-selenate solutions). The values obtained were compared with those of the controls determined at the same time after preparation of the solution. These absolute values of the control samples were set as 100% for the time under investigation. The per cent values, obtained in this manner, representing the radioprotective effect, are shown in Fig. 1-b. As can be seen, selenium protects the enzyme quite well. It can be noted that the protective effect increases with time elapsed between preparing the solution and irradiation. Moreover, this increase seems to depend on the selenium concentrations used. With increasing selenium concentration, the rate of increase in protective effect (see Fig. 1-c) is initially very fast followed by a sharp decline; that is, the protective effect exerted by the highest selenium concentration used varies only slightly with time.

A similar result was obtained for the absolute radioprotective effect. Using the 8 min values (see Fig. 1-c), the protective effect increases from 37% to 72% followed by a decline to 48% with increasing selenium concentration. The inactivation of catalase by inorganic selenium, as can be seen in the control values (Fig. 1-a), demonstrates the close interaction of the selenate ion with the enzyme. It is of interest that the radioprotective effect of the selenate ion is greater than its evident enzyme-inhibiting action, and the question of radiation-induced changes in enzyme inhibition by selenate ion arises.

SUMMARY

The change in radiosensitivity of catalase by selenium (as Na_2SeO_4) was investigated using a spectrophotometer. The irradiation dose was 4.5×10^5 R in each case. The protective effect seems to be due to a complex formation between selenium and the enzyme molecule rather than to radical scavenging.

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