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INTRACELLULAR LOCALIZATION OF SUPEROXIDE DISMUTASE AND ITS RELATION TO THE DISTRIBUTION AND MECHANISM OF HYDROGEN PEROXIDE-PRODUCING ENZYMES

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SUMMARY

Superoxide dismutase has been localized exclusively in the soluble fraction of rat liver homogenates. In view of this result, the generation of O_2^- by different H_2O_2-producing enzymes has been examined. It appeared that O_2^- is generated only by xanthine oxidase, which is localized in the same compartment as the dismutase. Other enzymes, which are localized in other subcellular fractions, do not produce O_2^-, but H_2O_2 directly.

INTRODUCTION

It has been recently shown[1] that catalase and a number of H_2O_2-producing enzymes are localized in the liver cell inside a special cytoplasmic body named "peroxisome", which would, therefore, be the major site of production and inactivation of H_2O_2. The basic assumption underlying this idea is the toxicity of H_2O_2, which is overcome by the association of the systems responsible for its production with catalase. It should, however, be pointed out that H_2O_2 is harmful since it yields extremely reactive species such as singlet oxygen[2] or OH radicals[3] on decomposition. Moreover not all H_2O_2-producing enzymes are located in peroxisomes. One of them, xanthine oxidase, which is present exclusively in the soluble fraction from liver homogenates[4], has been shown[5,6] to produce H_2O_2 *via* univalent steps, *i.e. via* a superoxide anion radical (O_2^-). This radical has been supposed to be a damaging agent for molecules and cells, but also in this case the toxic action is probably due to the secondary production of singlet oxygen or OH radicals. In fact, in aqueous solution O_2^- dismutates spontaneously quite rapidly ($k = 2.1 \cdot 10^5$ M^{-1} s^{-1} at pH 7.12; see ref. 7) producing H_2O_2 and singlet oxygen[8], and can also react with H_2O_2 *via* the reaction

$$H_2O_2 + O_2^- + H^+ \longrightarrow OH + H_2O + O_2 \qquad (1)$$

In the presence of the enzyme superoxide dismutase[10], the O_2^- dismutation rate is much faster ($k \simeq 2 \cdot 10^9\ M^{-1}\ s^{-1}$; refs 11 and 12), so that a source of the damaging OH radical is effectively removed. Moreover the enzyme-catalyzed dismutation does not give rise to singlet oxygen[13] and can thus fulfil a protective role by this additional mechanism. The aim of the present work was to study the intracellular localization of superoxide dismutase and to relate it to that of H_2O_2- and O_2^--producing enzymes.

METHODS AND MATERIALS

Tissue fractionation of rat liver was performed according to de Duve *et al.*[14], using cytochrome oxidase, acid phosphatase, urate oxidase and glucose-6-phosphatase as markers for mitochondria, lysosomes, peroxisomes and microsomes, respectively.

RESULTS AND DISCUSSION

The distribution pattern of superoxide dismutase is shown in Fig. 1. It is apparent that the enzyme is entirely recovered in the supernatant fraction.

Many H_2O_2-producing enzymes have already been located in different cell compartments. It would be interesting to know if they produce O_2^- during their catalytic action. This is possible by assaying their ability to catalyze the aerobic reduction of cytochrome *c* (ref. 5) and the inhibition of this reduction by superoxide dismutase. Such an approach has already been used to test O_2^- production by many systems, in particular flavoenzymes[15]. Xanthine oxidase has been confirmed by this method to be the best O_2^--producing enzyme system, while D-amino acid and glycolate oxidases do not show any appreciable generation of O_2^- (ref. 15).

Diamine oxidase is a H_2O_2-producing enzyme which has been demonstrated to be localized in the microsome fraction of rat liver[16,17]. It has been shown to reduce cytochrome *c* when oxidizing cadaverine in slightly alkaline solution (pH 9.4): at neutral pH the cytochrome *c* absorption was bleached instead. These results were interpreted as an indirect evidence for O_2^- production by diamine oxidase, and cytochrome bleaching at neutral pH was explained as due to the oxidizing action of H_2O_2 which prevailed over the reducing effect of O_2^- because of the very short life of the radical at this pH. The cytochrome *c* reductase activity of diamine oxidase purified according to Mondovi *et al.*[19] was assayed with cadaverine as substrate at pH 9.4, in the presence and absence of bovine superoxide dismutase or catalase. Dismutase had no effect on cytochrome *c* reduction, while catalase completely prevented it. At pH 7.4, in the presence of either catalase or both dismutase and catalase, neither reduction nor bleaching of cytochrome *c* was observed during the catalytic action of diamine oxidase. These results indicate that the enzyme-produced H_2O_2 is the cytochrome *c* reducing species in alkaline solution and that, if O_2^- is produced during the catalytic action of diamine oxidase, it is not present in appreciable amounts as a free species in solution.

Urate oxidase is generally used as the marker enzyme for peroxisomes. The cytochrome *c* reductase activity of urate oxidase was assayed only at pH 7.4, since cytochrome *c* was reduced by urate itself at pH 9.4. Neither reduction nor bleaching of cytochrome *c* was observed in the presence of catalase, while in the absence of catalase bleaching of cytochrome *c* occurred. This result is identical to that obtained

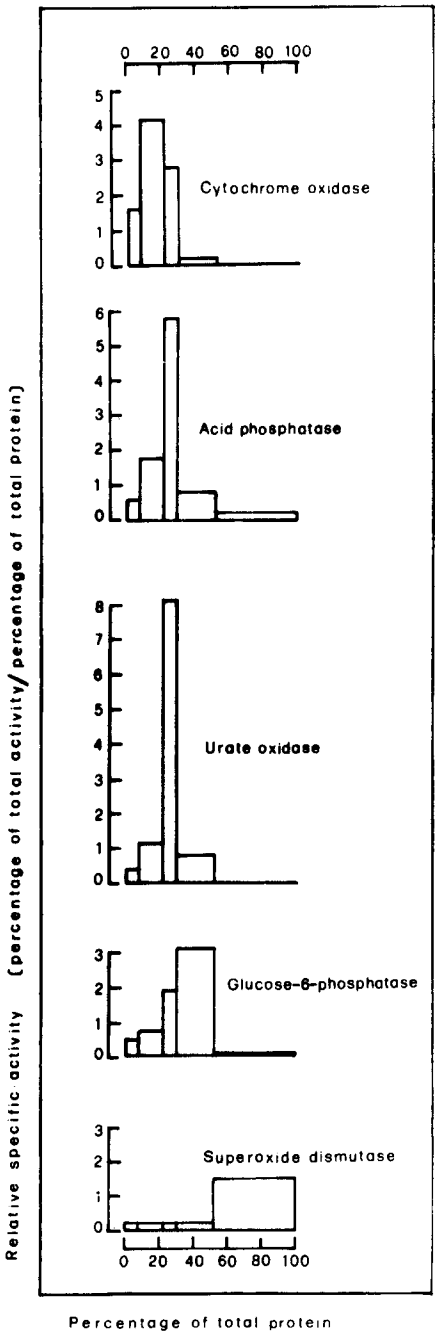

Fig. 1. Distribution patterns of superoxide dismutase and marker enzymes in rat liver. Fractions are represented on the abscissa scale by their relative nitrogen content, in the order in which they are isolated, *i.e.* from left to right: nuclei, mitochondria, peroxisomes, microsomes and final supernatant.

with diamine oxidase, and suggests that also in this case H_2O_2 is produced without a superoxide intermediate which dissociates from the enzyme.

All these data seem to indicate that a relation exists between the localization of H_2O_2-producing enzymes in rat liver cells and their ability to generate O_2^- and that this relation can in turn be explained by the localization of the major scavenging systems for O_2^- and H_2O_2. In fact only the cytoplasmic enzyme xanthine oxidase produces O_2^- as a species capable of interacting with cytochrome *c*, while the peroxisomal enzymes urate oxidase, glycolate oxidase D-amino acid oxidase and the microsomal enzyme diamine oxidase do not. Superoxide dismutase is localized in the same compartment as xanthine oxidase, and this is a further evidence for its physiological role. In fact it competes with the H_2O_2 produced by dismutation for O_2^- and ,therefore, inhibits OH generation according to Eqn 1; moreover it prevents singlet oxygen formation. On the other hand H_2O_2 inhibits the dismutase itself[20], but the presence of glutathione peroxidase in the soluble fraction from rat liver homogenates[21] can protect it against H_2O_2 produced *via* O_2^- dismutation.

It could be suggested that the two different classes of H_2O_2-producing enzymes–*i.e.* those capable of aerobic cytochrome *c*-reductase activity inhibitable by superoxide dismutase and those which do not show this activity–reduce oxygen by two different mechanisms, that is *via* one electron or two electron donation steps. However, it is more likely that the mechanical difference among the different H_2O_2-producing enzymes is the nature of the intermediate which dissociates from the enzyme. In other words in such enzymes as xanthine oxidase, the form which dissociates from the enzyme is O_2^-, and dismutation to H_2O_2 occurs in solution with production of singlet oxygen in the absence of dismutase. In other enzymes, such as peroxisomal enzymes and diamine oxidase O_2^- could remain bound to the enzyme, and the peroxide be the form which dissociates. In both cases H_2O_2 will be the final product but with different rates and steady-state concentrations, which may fit the catalytic properties of the peroxide-removing enzymes of different compartments, *i.e.* glutathione peroxidase and catalase[22]. Microsomal enzymes may find a particular situation for the utilization of peroxide and superoxide in the metabolism of endogenous lipids and xenobiotics.

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