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A PROTECTIVE FUNCTION OF SUPEROXIDE DISMUTASE DURING RESPIRATORY CHAIN ACTIVITY

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SUMMARY

(1) Aerobic incubation of heart muscle submitochondrial particles in phosphate buffer after treatment with NADH causes a progressive and substantial inhibition of the NADH oxidation system. Succinate oxidation remains almost unaffected by NADH treatment.

(2) The loss of NADH oxidase activity is due to an inhibition of the respiratory chain-linked NADH dehydrogenase. This inhibition of the enzyme is very similar to that caused by combination of the organic mercurial mersalyl with NADH dehydrogenase.

(3) The inhibition of NADH oxidation is largely prevented by compounds that are known to react with superoxide ions ($O_2^{-\cdot}$), including superoxide dismutase, cytochrome *c*, tiron and Mn^{2+}. EDTA also has a protective effect, but a number of other metal chelating agents, and several proteins, including catalase, are without effect.

(4) It is concluded that the inhibition of NADH oxidation is due to the action of superoxide ions formed during respiratory chain activity.

(5) The inhibition of NADH oxidation by superoxide ions or by mersalyl is reversible and is therefore not due to the loss of oxidoreduction components from the respiratory chain or to an irreversible change in protein conformation.

(6) The function of mitochondrial superoxide dismutase is discussed in relation to the key role of NADH dehydrogenase in energy-conserving reactions and the formation of hydrogen peroxide during mitochondrial oxidations.

INTRODUCTION

McCord et al. [1] suggested that the general physiological function of superoxide dismutase is to protect aerobic cells against the potentially harmful effects of the superoxide free radical anion, ($O_2^{-\cdot}$), by catalysing the reaction

$$2O_2^{-\cdot} + 2H^+ \rightarrow O_2 + H_2O_2$$

Superoxide ions are generated during oxidoreduction reactions catalysed by certain

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enzyme preparations including xanthine oxidase [2], the NADPH oxidation system of liver microsomes [3, 4] and ferredoxins [5]. Superoxide ion formation has also been detected in heart submitochondrial particles treated with antimycin and succinate [6]. Although both non-haem iron and flavin groups have been proposed as sites of oxygen reduction to $O_2^{-\cdot}$, non-haem iron sites appear to be far more effective reductants than are sites containing flavin [7].

Part of the superoxide dismutase of isolated mitochondria is located in the matrix space [8, 9]. In the present paper it is shown that treatment of submitochondrial particles with NADH causes a strong inhibition of the NADH oxidation system and that this inhibition is prevented by compounds known to react with superoxide ions, including superoxide dismutase. The inhibition of NADH oxidation is similar to that occurring when the organic mercurial mersalyl combines with type III thiol groups of the respiratory chain-linked NADH dehydrogenase [10–12]. The results suggest that one important function of the superoxide dismutase present in the matrix space is to protect NADH dehydrogenase against the harmful effect of superoxide ions formed during respiratory chain activity.

METHODS

Enzyme preparations

Ox heart mitochondria were obtained by the method of Crane et al. [13]. Submitochondrial particles (electron transfer particle preparation) were prepared by the fragmentation of frozen and thawed mitochondria in alkaline sucrose (Preparation I of Crane et al. [13]) using mitochondria that had been stored at − 20°C. A stock suspension of submitochondrial particles containing about 30 mg of protein/ml was stored in 2-ml aliquots in glass-stoppered tubes at − 20 °C for periods of up to 3 months. The contents of a tube were thawed as required and kept at 1 °C until use. Samples of particles were taken from the tube for enzyme assays within a period of 2 days after thawing.

Bovine erythrocyte superoxide dismutase was purified by the method of McCord and Fridovich [2]. The material obtained after the acetone precipitation step was dissolved in water and dialysed overnight against water. The solution obtained was centrifuged at $5000 \times g$ for 5 min and the supernatant containing the dismutase was further purified by fractional adsorption on alumina C γ gel, fractional precipitation by acetone and crystallization by the method of Mann and Keilin [14]. The final product contained 0.34 μg of copper/mg of protein and 3000 dismutase units/mg of protein when assayed by the method of McCord and Fridovich [2], in which one unit of dismutase activity is defined as the amount required to inhibit by 50 % the rate of aerobic reduction of added cytochrome *c* during xanthine oxidase activity.

The protein content of the enzyme preparations was determined by the biuret method [15] using samples treated with 0.2 % (w/v) sodium cholate.

Assays

Respiration rates were recorded at 25 °C by the polarographic technique with a Clark oxygen electrode. The reaction mixture (3.2 ml) contained 0.1 M potassium phosphate buffer, pH 7.4, submitochondrial particles (0.4–0.6 mg of protein) and other compounds specified in the figure legends. Oxidase activities were assayed with

either 0.25 mM NADH or 15 mM potassium succinate. NADH-ferricyanide reductase activity, measured in 40 mM triethanolamine-HCl buffer, pH 7.8, and NADH-acetylpyridine adenine dinucleotide transhydrogenase activity were assayed at 20 °C by the methods of Minakami et al [16]. Stock solutions of acetylpyridine adenine dinucleotide, cysteine-HCl, ferricyanide, ferrocyanide, NAD^+, NADH (in 1 % w/v $NaHCO_3$), mersalyl and metal-chelating agents except EDTA were prepared on the day of use. After incubation of the electron transfer particles in phosphate buffer, the oxidase activities of untreated (control) samples were 0.92–1.24 and 0.5–0.84 μmol of substrate oxidized/min per mg protein for NADH oxidation and succinate oxidation respectively. Values presented in the Table and Figures are the mean values of triplicate samples and are similar to values obtained with electron transfer particle preparations from at least three different hearts.

Nomenclature

The different types of thiol groups that can be distinguished in the respiratory chain-linked NADH dehydrogenase have been numbered by Estabrook et al. [17] and by Singer [18]. Unfortunately, the numbers used in these two papers do not correspond. The numbering system of Singer is used in the present paper, because the system has been discussed fully in a review article [18] that is generally available.

Chemicals

The following compounds were obtained from the Sigma Chemical Co. (St. Louis, Mo., USA): antimycin, catalase (purified powder from bovine liver), cytochrome *c* (type III from horse heart), gramicidin, oligomycin, 1, 10-phenanthroline hydrate, sodium bathocuproine sulphonate, sodium bathophenanthroline sulphonate and sodium mersalyl (sodium O-(3-hydroxymercuri-2-methoxypropyl) carbamoylphenoxyacetate). Acetylpyridine adenine dinucleotide, NAD^+, NADH and triethanolamine-HCl were from the Boehringer Corp. (London) Ltd. (London, W5). Crystallized bovine plasma albumin, rotenone and 2-thenoyltrifluoroacetone were from Armour Pharmaceutical Co. Ltd (Eastbourne, Sussex), S. B. Penick and Co. (New York, N.Y., U.S.A.) and Eastman Kodak Co. (Rochester, N.Y., U.S.A.) respectively. Diphenyl-*p*-phenylene diamine and α-tocopheryl acetate were obtained from Koch-Light Laboratories Ltd (Colnbrook, Bucks). Other reagents including EDTA, potassium ferricyanide, potassium ferrocyanide and tiron (1,2-dihydroxybenzene-3,5-disulphonic acid, sodium salt) were from British Drug Houses Ltd. (Poole, Dorset). Analytical reagent grade chemicals were used whenever possible.

The enzyme used was NADH: (acceptor) oxidoreductase (EC 1.6.99.3; trivial name, NADH dehydrogenase).

RESULTS

Inhibition of NADH oxidase activity

When heart muscle submitochondrial particles were suspended in aerobic phosphate buffer and treated with NADH, a rapid oxidation of NADH occurred during the first minute of incubation. If the incubation was then continued before polarographic assay with a second addition of NADH, it was observed that a strong

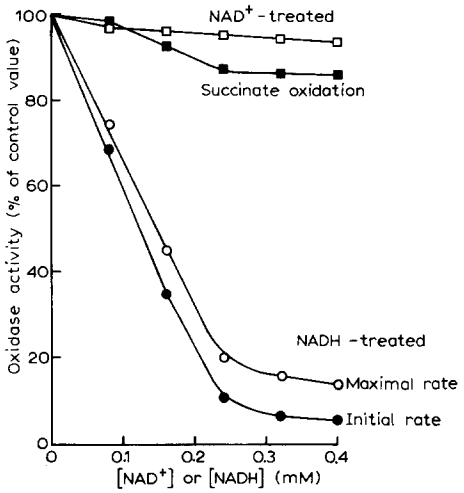

Fig. 1. Effect of incubation with NADH or NAD^+ on the oxidase activities of submitochondrial particles. Samples of a suspension of submitochondrial particles were treated with the concentrations of NADH or NAD^+ indicated and incubated for 40 min before the assay of oxidase activities. ●, ○, ■, particles treated with NADH. ● and ○ indicate the initial and maximal rates of NADH oxidase activity respectively; ■, succinate oxidase activity; □, NADH oxidase activity after treatment with NAD^+.

inhibition of NADH oxidase activity had developed during the incubation period (Fig. 1). The maximal rate of NADH oxidation by inhibited particles was observed only after a pronounced lag in oxygen uptake at the start of the NADH oxidase assay. Treatment with NAD^+ instead of NADH caused only a slight decrease (about 10%) in NADH oxidase activity, indicating that the strong inhibition observed was not due to incubation of the particles with NAD^+ formed during the first period of NADH oxidation. No loss of activity was observed when particles were incubated anaerobically with NADH before assay by dilution into aerobic buffer, showing that oxygen was required for inactivation to occur. The loss of activity was considerably greater when particles were suspended in phosphate or arsenate buffer compared with Tris-HCl, triethanolamine-HCl, borate or bicarbonate buffers or solutions of potassium chloride, citrate or sulphate. When samples of particles suspended in 50 mM Tris-HCl and containing various concentrations of phosphate buffer were treated with NADH, the presence of 20 mM potassium phosphate caused a near-maximal decrease in NADH oxidase activity.

The results of experiments similar to those presented in Fig. 1 were unaffected by the addition of the uncoupling agent carbonylcyanide *m*-chlorophenylhydrazone (1.6 μM) and/or gramicidin (2 μg/ml) or by oligomycin (2 μg/ml), indicating that inhibition of NADH oxidase activity was not dependent on energy-conserving reactions. No loss of NADH or succinate oxidase activity was observed when particles were treated with 0.25 mM succinate. NADH treatment caused a marked change in the affinity of NADH dehydrogenase for artificial electron acceptors. Using untreated particles, the ferricyanide reductase and transhydrogenase activities at V were 23.3 and 1.1 μmol NADH oxidized/min per mg of protein. The corresponding values observed

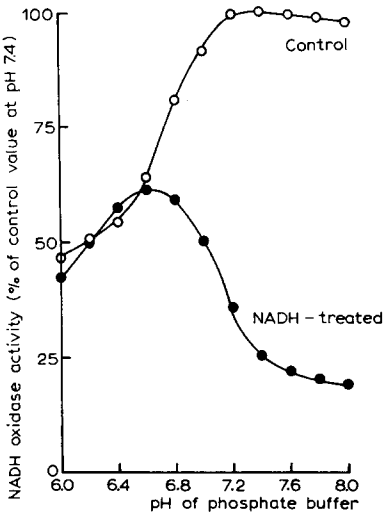

Fig. 2. Effect of pH on NADH oxidase activity. Samples of submitochondrial particles, suspended in 0.1 M potassium phosphate buffer at the pH indicated, were treated with either 0 or 0.25 mM NADH and incubated for 40 min before the assay of NADH oxidase activity. ○, no NADH added; ●, incubated with NADH.

with NADH-treated particles were 1.8 and 0.32. These results indicate that the loss of NADH oxidase activity (Fig. 1) is due to an effect at, or close to the substrate-binding site of the dehydrogenase. No loss of NADH oxidase activity occurred when submitochondrial particles were treated with NADH in phosphate buffer below pH 6.6. Above this pH, the inactivation became more pronounced as the alkalinity of the buffer was increased (Fig. 2).

Reagents preventing the loss of NADH oxidase activity

Two metal-chelating agents, tiron and EDTA, were found to prevent most of the inactivation of NADH oxidation caused by NADH. Cysteine and other reagents containing a free thiol group, including dithiothreitol, had a similar effect (Fig. 3). However, a variety of other metal-chelating agents including bathophenanthroline, bathocuproine, dithizone, 8-hydroxyquinoline, *o*-phenanthroline and 2-thenoyltrifluoroacetone were without effect when each was tested in concentrations up to 0.8 mM.

The loss of NADH oxidase activity was also prevented by the addition of ferricyanide just after the brief period of rapid NADH oxidation during NADH treatment, whereas ferrocyanide was without effect (Fig. 4). When a sample of submitochondrial particles was incubated with ferricyanide, sedimented by centrifugation and resuspended in fresh phosphate buffer before treatment with NADH, no protective effect of ferricyanide was found. These results suggest that the protective action of ferricyanide in the experiment of Fig. 4 is due to the ability of ferricyanide to oxidize a component reduced or exposed by NADH during the brief initial period of NADH oxidation. Acetylpyridine adenine dinucleotide was without effect, suggesting that the component oxidized by ferricyanide contains non-haem iron or other

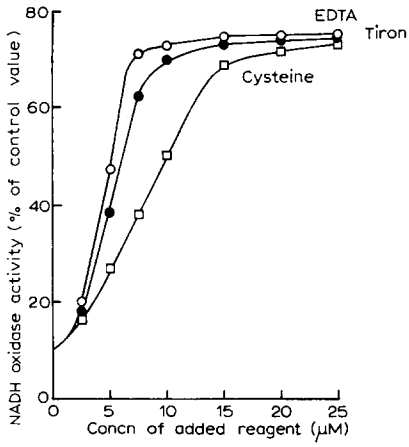

Fig. 3. Effect of metal-chelating agents and cysteine on NADH oxidase activity. Samples of a suspension of submitochondrial particles containing the concentrations of reagents indicated were treated with 0.25 mM NADH and incubated for 40 min before the assay of maximal NADH oxidase activity. ○, EDTA present; ●, tiron present; □, cysteine present. The NADH oxidase activities of control samples incubated with the reagents in the absence of NADH were 95–115 % of the activity of samples incubated only in phosphate buffer.

oxidoreduction groups but not reduced FMN. Inactivation was also prevented by the following compounds: by 0.75 mM sodium sulphite (but not by sodium sulphate); by 0.5 mM $MnCl_2$ (but not by $MgCl_2$ or $CaCl_2$); and by sodium ascorbate (2 μM). The antioxidant compounds, diphenyl-*p*-phenylenediamine and α-tocopheryl acetate had no protective effect. No evidence was obtained that the inhibition of NADH oxidation was due to lipid peroxidation reactions. In a typical experiment, in which lipid

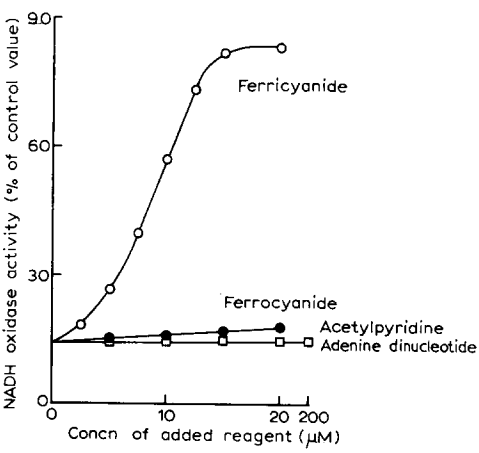

Fig. 4. Effect of oxidizing agents on NADH oxidase activity. Samples of a suspension of submitochondrial particles in phosphate buffer were treated with 0.25 mM NADH at zero time, followed 2 min later by the addition of the concentrations of reagent indicated. The samples were then incubated for 40 min before the assay of maximal NADH oxidase activity. Reagents added were ○, ferricyanide; ●, ferrocyanide; □, acetylpyridine adenine dinucleotide.

peroxide formation was measured by the TBA method [19] the absorbance values obtained at 532 nm were: buffer only, zero reading; buffer+ETP, 0.046; buffer+NADH, 0.062; buffer+ETP+NADH, 0.090. Thus, the protective effect of Mn^{2+} on NADH oxidation appears not to be related to the potent inhibitory effect of Mn^{2+} on lipid peroxidation reactions [20]. It is possible that free Mn^{2+} catalyse superoxide dismutation since the mitochondrial matrix superoxide dismutase is a mangano-protein [8].

Protein preparations preventing the loss of NADH oxidase activity

Native erythrocyte superoxide dismutase was found to prevent the loss of NADH oxidase activity induced by NADH, and both the protective effect and the dismutase activity were abolished by boiling the dismutase. Superoxide dismutase had a similar protective action when liver submitochondrial particles were used instead of ox heart particles. The protective effect of superoxide dismutase suggests strongly that the loss of NADH oxidase activity was due to the action of superoxide ions generated in the incubation mixture. NADH treatment caused a similar inactivation of NADH oxidation when a suspension of particles was treated and assayed in a spectrophotometer cell, showing that $O_2^{-\bullet}$ generated at the surface of an oxygen electrode [21] were not responsible for the loss of activity.

Oxidized cytochrome *c* partly prevented the loss of NADH oxidase activity when added before NADH treatment, but was less effective compared to superoxide dismutase and the other protective reagents mentioned above. Cytochrome *c* had no effect when added to inactivated particles just before the assay of NADH oxidase activity. The amount of added cytochrome c required for maximal protection (60 nmol/ml) was about 500 times the amount of endogenous cytochrome *c* present in the particles, showing that this effect was not related to the function of cytochrome *c* in the respiratory chain. The effect of added cytochrome *c* is probably due to its $O_2^{-\bullet}$-trapping activity [2].

A number of other purified proteins were without effect, including catalase (15 μg/ml), myoglobin (0.2 mg/ml) and bovine plasma albumin (1 mg/ml). The failure of catalase to prevent the loss of activity suggested that the inhibition was due to superoxide ions and not to the formation of the hydroxyl radical (·OH) formed [22] according to the following equation:-

$$O_2^{-\bullet} + H_2O_2 \rightarrow O_2 + OH^- + \cdot OH$$

This conclusion is supported by the observation that several · OH radical trapping agents, including benzoate, formate and ethanol [23], had no protective effect.

Although EDTA, tiron, cysteine and superoxide dismutase provided considerable protection against inactivation, the NADH oxidase activity obtained in the presence of these compounds was never fully equal to that observed with untreated control samples. This finding is only partly explained by the slight inhibition caused by NAD^+ formed during NADH treatment.

Kinetics of inactivation

The rate of inactivation of the NADH oxidase system is shown in Fig. 5. At 25 °C, half-maximal inactivation occurred after about 7 min of incubation. The kinetics of inactivation were similar in air-saturated and oxygen-saturated buffer. Copper sulphate (0.2–1.0 μM) or ferrous sulphate (1–10 μM) had no significant

effect on the kinetics or extent of inactivation, suggesting that formation of $O_2^{-\cdot}$ by trace metal impurities [24] is not concerned in the mechanism of inactivation. The addition of protective agents such as EDTA, cysteine or superoxide dismutase during the incubation blocked any further loss of activity but was unable to reverse the inhibition (Fig. 5). When the experiment of Fig. 5 was repeated using ferricyanide, the inhibition was not only blocked but also reversed (Fig. 6A). Ferrocyanide reversed the inhibition only after a pronounced lag period, during which a slow oxidation of ferrocyanide to ferricyanide was observed, as shown by an increase in extinction of the suspension at 420–450 nm. Acetylpyridine adenine dinucleotide was again without effect. The NADH oxidation pathway restored by incubation with ferricyanide was fully blocked by rotenone, antimycin or cyanide and therefore appeared to be identical to that present in untreated submitochondrial particles.

Tyler et al. [11] found that the inhibition of NADH oxidation by mersalyl was reversed only slightly by cysteine or other thiol-group reagents. In the present study, it was observed that ferricyanide was able to reverse the inhibition caused by mersalyl (Fig. 6B) and that the kinetics of reversal were similar to those found in the experiment of Fig. 6A. Cysteine was found to remove virtually all mersalyl-Hg^{203} after the labelled compound had combined with type III thiol groups of NADH dehydrogenase in submitochondrial particles (Tyler, D. D. and Dumont, J. E., unpublished). Since ferricyanide reversed the inhibition by mersalyl only when cysteine was added after mersalyl treatment, the effect of ferricyanide is evidently dependent on the removal of mersalyl from the particles by cysteine. The results of Fig. 6 show that the inhibition of NADH oxidation caused by treatment with NADH, either in the presence or absence of mersalyl, is not due to a loss of respiratory chain components or an irreversible change in protein conformation.

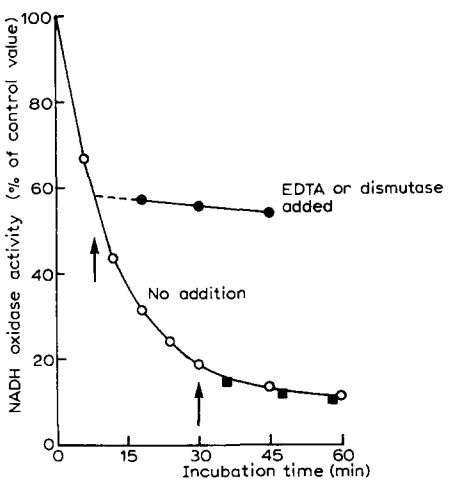

Fig. 5. Effect of EDTA and superoxide dismutase on the kinetics of inhibition of NADH oxidase activity. A suspension of submitochondrial particles was treated with 0.25 mM NADH at zero time and incubated. EDTA (20 μM) or erythrocyte dismutase preparation (3 μg/ml) was added to portions of the suspension at the times indicated by the arrows, before the assay of maximal NADH oxidase activity. ○, no further addition: EDTA or dismutase were added either 7 min (●) or 30 min (■) after NADH.

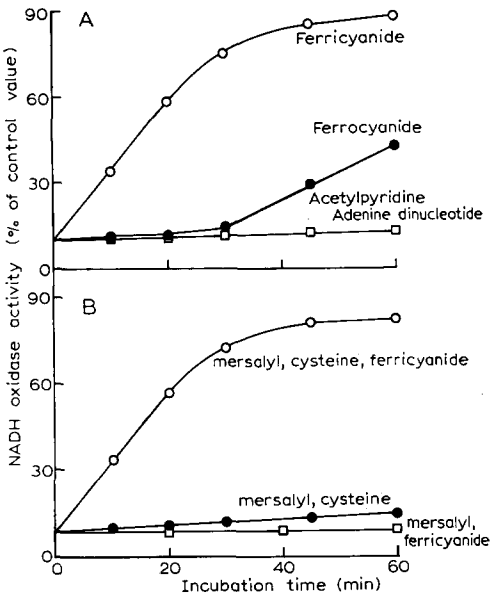

Fig. 6. Effect of ferricyanide on the inhibited NADH oxidase activity of submitochondrial particles. (A) A suspension of submitochondrial particles was treated with 0.25 mM NADH and incubated for 40 min. The suspension was then divided into portions and reagents were added as indicated below. The incubation was continued and samples withdrawn for the assay of maximal NADH oxidase activity. ○, with ferricyanide (30 μM); ●, with ferrocyanide (30 μM); □, with no further addition or with acetylpyridine adenine dinucleotide (0.2 mM). (B) A suspension of submitochondrial particles was treated with 0.25 mM NADH at zero time and with 15 μM mersalyl after a 2 min incubation. The suspension was divided into 2 portions and 0.15 mM cysteine was added to one portion 1.5 min after the addition of mersalyl. The particles were sedimented by centrifugation at 100 000 $\times g$ for 30 min and resuspended in fresh phosphate buffer. Samples of this suspension were then incubated in the absence or presence of 30 μM ferricyanide before the assay of maximal NADH oxidase activity at the times indicated. ○, particles treated with mersalyl, cysteine and ferricyanide; ●, particles treated with mersalyl and cysteine; □, particles treated with mersalyl and ferricyanide. The oxidase activities are expressed relative to those of control samples treated in a similar manner but without the addition of NADH, mersalyl, cysteine or ferricyanide.

DISCUSSION

Origin of superoxide ions

The ability of superoxide dismutase to protect NADH dehydrogenase against aerobic NADH-induced inactivation strongly suggests that the inactivation depends on the formation of superoxide free radical ions during respiratory chain activity. Although the capacity of NADH-treated particles to form $O_2^{-\bullet}$ is probably very limited after most, if not all, the NADH used to treat the particles has been oxidized, the NADH oxidase activity is dependent on extremely small amounts of NADH dehydrogenase. Heart muscle submitochondrial particles contain about 30 pmol of NADH dehydrogenase/mg of protein [25] and thus the amount of dehydrogenase in the samples used is about 15 pmol. Only very small amounts of $O_2^{-\bullet}$ are therefore required to cause a pronounced inhibition. NADH treatment of submitochondrial particles induces the formation of stable reduced components including a cytochrome

b-type pigment [26] and an iron-sulphur component of NADH dehydrogenase [27]. The latter component is the more likely site of oxygen reduction, because the effect of $O_2^{-\bullet}$ is largely confined to the NADH branch of the respiratory chain. The small amounts of H_2O_2 formed during NADH oxidation by submitochondrial particles [28] and during the oxidation of substrates by mitochondria [29] may originate from the dismutation of $O_2^{-\bullet}$ generated in the respiratory chain. The conclusion that the respiratory chain NADH dehydrogenase is capable of reducing oxygen to $O_2^{-\bullet}$ is consistent with recent work [30–32] suggesting that the E'_0 value of the $O_2/O_2^{-\bullet}$ couple is about -0.3 V. Since the matrix space of mitochondria contains superoxide dismutase but no catalase [9] the relatively stable H_2O_2 molecules presumably diffuse from mitochondria to the cytosol before destruction by catalase. In contrast, a simple diffusion mechanism would be inadequate for the disposal of highly reactive free radical $O_2^{-\bullet}$ formed in mitochondria and the presence of superoxide dismutase is therefore essential.

Mechanism of inhibition

The inhibition by $O_2^{-\bullet}$ shares several features in common with the inhibition by mersalyl [10] and it is therefore probable that inactivation is due to the oxidation or some other modification of the type III thiol groups of NADH dehydrogenase by $O_2^{-\bullet}$. Both the kinetics of inactivation and the restoration of activity by ferricyanide are extremely slow compared with the rate of NADH oxidation by the respiratory chain. The slow kinetics of inactivation may be due to the slow rate of formation or release of $O_2^{-\bullet}$ and it seems probable that slow conformation changes in the NADH dehydrogenase molecule are also concerned in the loss and restoration of activity. The importance of conformational changes in the inhibition of both the particulate and soluble forms of the enzyme have been emphasized by Singer [18]. Rossi et al. [33] found that the aerobic incubation of soluble purified NADH dehydrogenase with NADH caused fragmentation of the enzyme and an extensive loss of ferricyanide reductase activity. This phenomenon may also be due to the formation and action of $O_2^{-\bullet}$. The chlorella enzyme NADH: nitrate oxidoreductase (EC 1.6.6.1) is inactivated by NADH treatment and can be re-activated by ferricyanide [34] but the possible role of $O_2^{-\bullet}$ formation has not been investigated.

The requirement for phosphate buffer during the inactivation of NADH dehydrogenase may be related to the fact that the univalent reduction of O_2 by Fe^{2+} is specifically favoured in phosphate-buffered solutions [35] and the stronger inactivation at high pH values can be attributed to the greater stability of $O_2^{-\bullet}$ in alkaline solution [36]. Of the two metal-chelating agents that protected the NADH oxidation system, the effect of tiron can be explained by its action as an $O_2^{-\bullet}$-trapping agent [37]. It is possible that the effect of EDTA on free radical reactions is unrelated to metal chelation [4, 38].

Physiological significance

The presence of superoxide dismutase in the matrix space of mitochondria [8, 9], in close proximity to NADH dehydrogenase situated on the matrix face of the inner mitochondrial membrane [39], shows that mitochondrial superoxide dismutase is sited most efficiently to protect the dehydrogenase. Other sources of $O_2^{-\bullet}$ may exist in mitochondria, particularly the metalloflavoproteins and quinones that they contain.

The failure of succinate oxidation to cause inactivation suggests that the formation of $O_2^{-\cdot}$ during cytochrome oxidase activity [3] is unlikely to produce harmful effects on the respiratory chain. Type III thiol groups are present in submitochondrial particles isolated from rat liver and kidney as well as ox heart [40]. It is therefore probable that the NADH dehydrogenase of many mammalian cells is sensitive to inhibition by $O_2^{-\cdot}$. By protecting the relatively small quantities of this enzyme present in cells against the harmful effects of $O_2^{-\cdot}$, mitochondrial superoxide dismutase ensures that the key role of dehydrogenase in citric acid cycle activity and energy conservation reactions is maintained in the presence of oxygen. Tosteson et al. [41] found that when an electric potential of 60 mV was applied across a thin lipid bilayer membrane separating KCl solution, $O_2^{-\cdot}$ was formed and acted as a charge carrier in the membrane. Experimental estimates of the electrical component of the protonmotive force generated across the inner mitochondrial membrane during respiratory chain activity indicate a value considerably greater than 60 mV [42]. It is therefore possible that the presence of superoxide dismutase would also be required for efficient energy conservation to occur by the chemisomotic coupling mechanism.

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