SUPEROXIDE RADICAL GENERATION DURING BIOGENIC AMINE OXIDATION CATALYZED BY MITOCHONDRIAL MONOAMINE OXIDASE

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During oxidation of biogenic amines catalyzed by monoamine oxidase (MAO) its coenzyme flavine-adenine dinucleotide (FAD) is reduced. At the second stage of the monoamine oxidase reaction FAD is oxidized by atmospheric oxygen with the formation, in particular, of H_2O_2. It was postulated in 1972 that semiquinone radicals of FAD may probably be formed in oxidative reactions [6], indicating the possibility that the superoxide anion radical ($O_2^{\bullet}$) may be generated as a result of single-electron reduction of O_2 by the semiquinone radical of FAD. In 1981 the suggestion was put forward that $O_2^{\bullet}$ may be formed in the monoamine oxidase reaction, and some data confirming this possibility indirectly were obtained.

It is interesting to test this hypothesis because we know that although $O_2^{\bullet}$ is a radical with low reactivity, at physiological and lower pH values it is readily converted into the $HO_2^{\bullet}$ radical, which has far greater activity and possesses, in particular, the property of inducing lipid peroxidation (LPO) in biomembranes [12]. Under certain conditions $O_2^{\bullet}$ may be the precursor of the active hydroxyl radical ($OH^{\bullet}$) [1]. It can be tentatively suggested that the known phenomenon of qualitative modification (transformation) of the properties of MAO is based on partial oxidation of thiol groups important for activity [2], which takes place with the participation of active oxygen ($HO_2^{\bullet}$ or $OH^{\bullet}$).

No direct data on generation of $O_2^{\bullet}$ or other oxygen radicals in the monoamine oxidase reaction has yet been obtained. Accordingly, the aim of this investigation was to attempt to find $O_2^{\bullet}$ in the course of monoamine oxidase oxidation of biogenic amines, using a highly sensitive chemiluminescence method.

EXPERIMENTAL METHOD

Human placental mitochondria isolated by differential centrifugation of 30% tissue homogenate in isotonic sucrose solution (0.25 M) with EDTA ($5 \cdot 10^{-4}$ M), made up in K^+-phosphate buffer, pH 7.4 (10^{-2} M), followed by washing twice with medium of the same composition [4], were used as the source of MAO.

MAO activity was determined by a chemiluminescence method [11], using 2-phenylethylamine (2-PEA) as the substrate. Chemiluminescence was recorded on an EMI 96535QB instrument, programmed to record current.

The medium for determination of chemiluminescence contained K^+,Na^+-phosphate buffer, pH 7.4 (10^{-2} M), a suspension of mitochondria (0.5 mg/ml), horseradish peroxidase (1.25 unit/ml), luminol (10^{-4} M), and 2-PEA ($5 \cdot 10^{-5}$ M). The total volume of the sample was 3.3 ml.

To determine xanthine oxidase activity, xanthine oxidase (10^{-2} unit), acetaldehyde (10^{-3} M), catalase (300 units), and superoxide dismutase (SOD, 100 units) were used. The source of the reagents was as follows: luminol (5-amino-2,3-dihydro-1,4-phthalazinedione) was from Koch-Light, England; horseradish peroxidase (25 units/ml) was from Reanal, Hungary; catalase 6500 units/mg) and xanthine oxidase (0.4 unit/mg) were from Boehringer, West Germany. Before use

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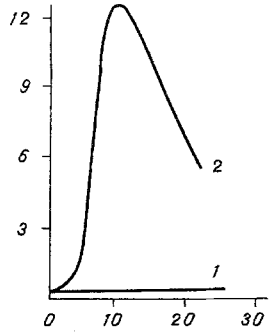

Fig. 1

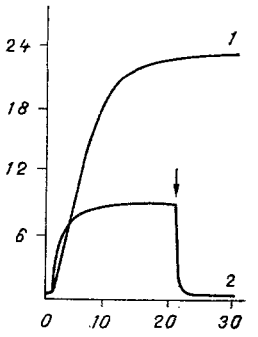

Fig. 2

Fig. 1. Typical curve of change in chemiluminescence during monoamine oxidase reaction in the absence (1) and presence (2) of peroxidase. Here and in Fig. 2: abscissa, reaction time (in min); ordinate, amplitude of chemiluminescence (in impulses/sec$\cdot 10^{-2}$).

Fig. 2. Typical curve of change in chemiluminescence during reaction of oxidation of acetaldehyde by xanthine oxidase in the absence (1) and presence (2) of catalase (3000 units). Arrow indicates time of addition of SOD (100 units) to the cuvette.

the catalase was passed through a column with Sephadex G-50 to remove thymol. All other reagents were of Soviet manufacture. SOD (2800 units/mg) was obtained by the method in [10]. Protein was determined by Lowry's method [9].

EXPERIMENTAL RESULTS

In the system used to determine MAO activity the luminol can be directly oxidized by O_2 [7], and this took place, for example, during oxidation of xanthine by xanthine oxidase. Under these circumstances the chemiluminescence arising on oxidation of luminol is inhibited by SOD; H_2O_2 also induces luminescence of luminol, not only in a reaction catalyzed by peroxidase [5].

The results of determination of MAO activity by a chemiluminescence method are given in Fig. 1. Direct addition of luminol (curve 1) did not induce chemiluminescence, but in the presence of peroxidase (curve 2) a chemiluminescent response appeared. These results are evidence that not $O_2^{\bullet}$, but H_2O_2, is formed in the monoamine oxidase reaction.

It might be supposed that if $O_2^{\bullet}$ and H_2O_2 are present simultaneously in the system difficulties will arise in the determination of one of the products. To make sure that H_2O_2 does not interfere with the finding of $O_2^{\bullet}$, we determined chemiluminescence in an acetaldehyde–xanthine oxidase system, in which both $O_2^{\bullet}$ and H_2O_2 are formed [8].

The chemiluminescent response obtained in one such experiment is shown in Fig. 2. Curve 1 corresponds to total chemiluminescence on account of direct oxidation of luminol by $O_2^{\bullet}$ and oxidation of luminol by H_2O_2 in the peroxidase reaction. Curve 2 corresponds to the chemiluminescent response on account of oxidation of luminol by $O_2^{\bullet}$, with removal of H_2O_2 by catalase. On the addition of SOD to this system the amplitude of chemiluminescence falls to zero. This indicates that $O_2^{\bullet}$ and H_2O_2, if formed simultaneously in an enzymic reaction, can be determined independently.

The results indicate that it is impossible to find generation of free $O_2^{\bullet}$ in the monoamine oxidase reaction with 2-PEA as the substrate, by the use of a highly sensitive chemiluminescence method. This result is in agreement with previous data showing that LPO cannot be induced during oxidation of the same substrate, catalyzed by MAO, in the absence of Fe^{++} and in the presence of catalase [3].

LITERATURE CITED

1. I. B. Afanas'ev, Khim.-farm. Zh., 19, 11 (1985).
2. V. Z. Gorkin, Mol. Biol., 10, 717 (1976).
3. V. E. Kagan, A. V. Smirnov, V. M. Savov, and V. Z. Gorkin, Vopr. Med. Khim., No. 1, 112 (1984).

4. V. A. Pekkel' and A. Z. Kirkel', Biokhimiya, 50, 289 (1985).
5. T. M. Freeman and W. R. Seltz, Anal. Chem., 50, 1242 (1978).
6. L. Hellerman, Y. K. Chuang, and D. C. De Luca, Adv. Biochem. Psychopharmacol., 5, 327 (1972).
7. E. W. Hodgson and I. Fridovich, Photochem. Photobiol., 18, 451 (1978).
8. E. W. Kellogg and I. Fridovich, J. Biol. Chem., 252, 6721 (1977).
9. O. H. Lowry, N. J. Rosebrough, A. L. Farr, et al., J. Biol. Chem., 193, 265 (1951).
10. J. Maral, K. Puget, and A. M. Michelson, Biochem. Biophys. Res. Commun., 77, 1525 (1977).
11. S. R. Ribarov and P. G. Bochev, J. Biochem. Biophys. Meth., 8, 205 (1983).
12. R. J. Youngman, Trends Biochem. Sci., 9, 280 (1984).

INTERACTION OF α-TOCOPHEROL WITH PHOSPHOLIPID LIPOSOMES: ABSENCE OF TRANSBILAYER MOBILITY

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Active attempts have been made in recent years to use vitamin E as a therapeutic substance in clinical practice [2-4]. Fundamentally new opportunities are deemed to have been provided by the specific delivery of vitamin E to target organs in phospholipid liposomes [7]. Liposomes circulating in the blood stream clearly interact with the internal medium of the body through their own outer surface, i.e., through their outer monolayer, whereas the inner monolayer of liposomes is in contact only with the internal contents of the liposomes and is inaccessible for macromolecular and cellular components of blood. Hence it follows that compounds built into the two monolayers of liposomes are accessible for target organs only if they possess sufficiently high transbilayer mobility (the so-called "flip-flop"). In the case of vitamin E, the main component of which is α-tocopherol (TP), this problem is of fundamental importance because the limited degree of incorporability of TP into phospholipid vesicles has been demonstrated [4], and this naturally limits the saturation of biological structures (biomembranes, lipoproteins) with vitamin E.

In the investigation described below a method of fluorescence analysis was used to study interaction between TP and liposomes, obtained from saturated and unsaturated phospholipids, and the possibility of its "flip-flop" in them.

EXPERIMENTAL METHOD

Liposomes were obtained from phosphatidylcholines of different nature (from egg, dimyristoyl-, dipalmitoyl-, and dioleyl-phosphatidylcholine). Liposomes were obtained by evaporation of the phosphlipids under argon, followed by shaking in Tris-HCl buffer (50 mM), NaCl (100 mM), pH 7.4, and treatment on an MSE ultrasonic disintegrator (22 kHz) by repeated sonication (12 × 15 sec with intervals of 30 sec). Ultrasonic treatment was carried out at a temperature above the phase transition temperature until the liposomes were completely translucent. The liposomes thus obtained were centrifuged (90,000g, 20 min), and supernatant containing monolayer liposomes [5] was used for the measurements. To incorporate TP into the outer and inner monolayers of the liposomes, solutions of phospholipids and TP in chloroform were mixed in definite proportions. The mixture was evaporated in a current of argon, and liposomes prepared from it. Fluorescence spectra of TP were recorded on "Perkin-Elmer MPF-44B"

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