QUENCHING OF CHEMILUMINESCENCE OF SINGLET OXYGEN IN THE PRESENCE OF CARNOSINE

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Among the factors causing the development of human diseases (malignant degeneration, atherosclerosis, cataract) one of the most important is intensification of the production of active forms of oxygen and, in particular, of singlet oxygen — $^1O_2$ [8, 10]. It can be formed as a result of photosensitization of psoralenes, phenothiazines, and dyes [5] or in the chemical reaction: H_2O_2 + NaClO [6]. Most investigations have been undertaken with the aid of systems of photoproduction of singlet oxygen [9]. Screening new preparations calls for simple, economical, and rapid methods of evaluating their quenching action relative to $^1O_2$.

The aim of this investigation was to develop such a method. For this purpose, for the first time we used a chemical method of generating singlet oxygen and we studied a number of known drugs and carnosine, a dipeptide which possesses several properties from which it can be considered to be a promising therapeutic agent [2].

EXPERIMENTAL METHOD

Singlet oxygen was formed by the reaction H_2O_2 + NaClO. Its production was monitored by measuring chemiluminescence, recorded by means of a chemiluminometer, model 1251 (LKB-Wallach, Finland). The signal was recorded and analyzed by computer (M19, Olivetti, Italy). The substances tested were introduced into a cuvette containing 300 μl of 1.5% H_2O_2 solution. During continuous mixing, 300 μl of NaClO solution was added to the cuvette through a dispenser. For each series of experiments the same solutions of H_2O_2 and NaClO were used; the latter was obtained electrochemically by means of an EDO-3 apparatus [1]. Several drugs were investigated: Catalin* ("International," India; "Takeda," Japan); Baineiting (Wu-han Pharmaceutical Combine, China); Quinax ("Alcon," Belgium), and Voltaren ("Pliva," Yugoslavia; "Ciba-Geigy," Switzerland). Carnosine was obtained from the Leningrad Pharmaceutical Enterprise Attached to the Meat Combine, and generously provided for study by the staff of the Biochemistry Department, M. V. Lomonosov Moscow State University. Amino acids used in the work were of Soviet origin, and of the analytically pure grade.

EXPERIMENTAL RESULTS

A typical curve of chemiluminescence obtained by interaction of H_2O_2 with NaClO is given in Fig. 1. Clearly the development of a flash lasts only a few seconds, and it is inhibited by typical quenchers of singlet oxygen, namely NaN_3 and methionine. Conversely, the HO·-radical scavenger ethanol did not affect the kinetics of luminescence (not shown in the figures).

*Alternative name for Catalin is Pirenoxine.

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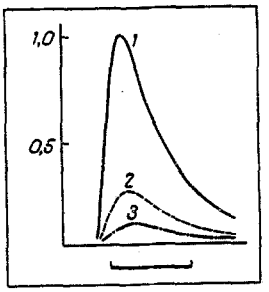

Fig. 1. Chemiluminescence of reaction of H_2O_2 + NaClO. Abscissa, time (in sec); ordinate, intensity of chemiluminescence (in conventional units). 1) Control, 2) 5 mM methionine, 3) 5 mM NaN_3.

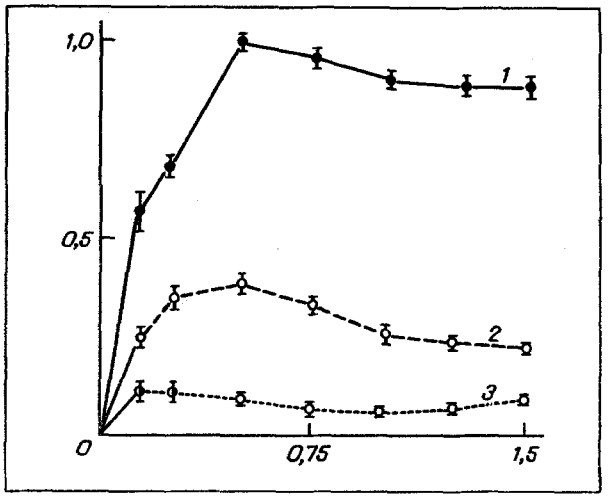

Fig. 2. Dependence of intensity of chemiluminescence on H_2O_2 concentration. Abscissa, H_2O_2 concentration (in %); ordinate, intensity of chemiluminescence (in conventional units). 1, 2, and 3) In presence of 13, 6, and 3 mM NaClO, respectively.

It will be clear from Fig. 2 that the intensity of chemiluminescence depends on the H_2O_2 and NaClO concentrations in the system. When low concentrations of the peroxide were used, the signal was weak, and for subsequent experiments we chose a final H_2O_2 concentration of 1.5%. The molar concentration of NaClO in the cuvette under these circumstances was 33 times smaller than that of H_2O_2, which ensured its utilization completely without a remainder.

The results of chemiluminescence measurements showed that some drugs, created for the conservative treatment of cataract, are good quenchers of singlet oxygen in the reaction described above. The most active preparations in this respect were the Japanese Catalin and the Chinese Baineiting. The therapeutic form Quinax induced a weak effect, although its therapeutic action, like the effect of Catalin, is associated with ability to prevent photooxidation of amino-acid residues of lens proteins [4]. Thus among the anticataract preparations studied by chemiluminescence analysis, an undoubted effect of quenching of singlet oxygen was discovered for Catalin and Baineiting (Fig. 3a). The antirheumatic agent Voltaren also demonstrated its quenching action against singlet oxygen (Fig. 3b).

The effect of several amino acids and of the dipeptide carnosine was studied separately. The latter was found to have almost the same action as histidine, a known quencher of singlet oxygen. β-alanine, one of the components of the dipeptide, did not possess these properties (Fig. 3b). We now know that carnosine can effectively cure several inflammatory diseases which are accompanied by activation of free-radical oxidation [2]. One of the therapeutic mechanisms of carnosine may perhaps be its ability to block the damaging action of singlet oxygen [3, 7].

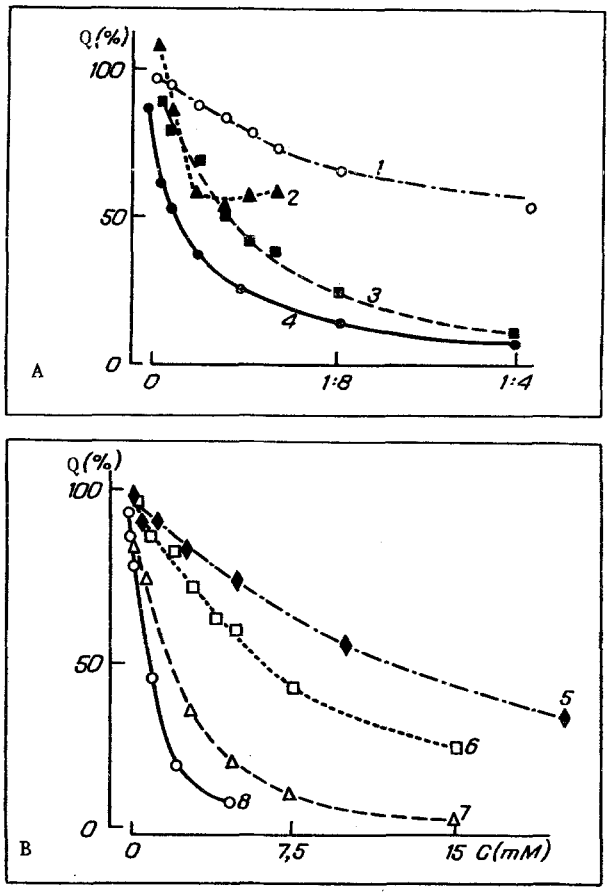

Fig. 3. Quenching of chemiluminescence of H_2O_2 + NaClO reaction in the presence of various substances. Quenching (Q, %) of chemiluminescence in the presence of different concentrations (C) of Quinax (1), Catachrome – OFTAN (2), Baineiting (3), and Catlin (4) (A), Voltaren (5), alanine (6), histidine (7), and carnosine (8) (B). In the series A curves, the content of the liquid therapeutic form is shown in the final dilution.

The suggested method can thus be used to evaluate certain therapeutic preparations on the basis of their ability to quench singlet oxygen, and carnosine was shown to possess this action.

LITERATURE CITED

1. Yu. B. Vasil'ev, V. I. Sergienko, V. A. Grinberg, et al., Élektrokhimiya, **23**, No. 5, 661 (1987).
2. A. A. Boldyrev, Int. J. Biochem., **48**, 244 (1989).
3. T. A. Dahl, R. W. Midden, and P. E. Hartman, Photochem. Photobiol., **47**, No. 3, 357 (1988).
4. K. A. Eyada, Metab. Pediat. Ophthal., **5**, 99 (1981).
5. E. M. Jackson (ed.), Photobiology of the Skin and the Eye, New York (1986).
6. A. U. Khan, J. Am. Chem. Soc., **103**, 6516 (1981).
7. R. Kohen, Y. Yamamoto, K. C. Cundy, and B. N. Ames, Proc. Nat. Acad. Sci. USA, **85**, 1747 (1988).
8. G. Moreno, R. H. Pottier, and T. G. Truscott, Photosensitization: Molecular, Cellular and Medical Aspects, Berlin (1988).
9. G. Tissie, V. Guillermet, E. Latour, et al., Ophthal. Res., **20**, 27 (1988).
10. H. H. Wasserman and R. W. Murray, Singlet Oxygen, New York (1979).