ESR SPIN TRAPPING STUDY OF SODIUM AND TETRAMETHYLAMMONIUM MERCAPTOUNDECAHYDRO-*closo*-DODECABORATES(2–) AND SODIUM TETRABORATE AQUEOUS SOLUTIONS IRRADIATED BY UV LIGHT AT ROOM TEMPERATURE

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The superoxide anion radicals $O_2^{\bullet-}$ were identified and determined in aqueous solutions of sodium and tetramethylammonium salts of mercaptoundecahydro-*closo*-dodecaborate(2–) (mercaptoborate), and sodium tetraborate, irradiated by high pressure mercury discharge lamp at room temperature by the of ESR spin trapping method. The quantity of the generated $O_2^{\bullet-}$ depends on the initial oxygen concentration in the irradiated solutions. The radical generation can play a crucial role in the application of mercaptoborate in boron neutron capture therapy (BNCT) of brain tumours.

Recently, we have found the superoxide anion radical $O_2^{\bullet-}$ generation in the UV irradiated frozen solution of tetramethylammonium mercaptoborate[1] in the presence of oxygen. Most recently, we have studied the superoxide anion radicals generation by the neutron irradiation of mercaptoborate solution[2]. Here, we used the spin trapping ESR method to verify our previously published results[1] for room temperature experiments using Tiron as an $O_2^{\bullet-}$ spin trap or spin detector. Tiron is a very useful spin trap for $O_2^{\bullet-}$ identification and its determination in aqueous solutions in biological research[3,4]. The aim of this work is a study of mercaptoborate $(B_{12}H_{11}SH)^{2-}$ effect on the superoxide anion radicals generation from oxygen at temperature, i.e. under the conditions similar to those of Boron Neutron Capture Therapy (BNCT).

EXPERIMENTAL

Materials

All used chemicals were analytical grade of purity (Lachema, The Czech Republic), tetramethylammonium mercaptoborate $[N(CH_3)_4]_2(B_{12}H_{11}SH)$ and sodium mercaptoborate

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$Na_2(B_{12}H_{11}SH)$ were synthesized and characterized at the Institute of Inorganic Chemistry, Academy of Sciences of the Czech Republic, Rez near Prague.

Instruments and Experimental Arrangement

ESR spectra were taken on an ESR-220 Instrument (Academy of Sciences, Berlin, Germany) operating in the X-band with a magnetic field modulation of 100 kHz. The magnetic field was measured with ^{1}H NMR magnetometer (Radiopan, Poland) and the microwave frequency was measured using a C3-54 frequency counter (Russia). The calibration was made with the aid of DPPH (g = 2.0037) and Mn(II)/ZnS standards. The samples were irradiated in the ESR resonator by an HBO 200 high pressure mercury lamp (Osram, Germany). The irradiation time was 20 min (it corresponds practically to the maximum of ESR signals of generated Tiron radicals).

Procedures

The solutions were prepared by mixing and stirring the components within about 10 s. The concentration of mercaptoborate was 2 mmol dm^{-3} and concentration of Tiron was approximately 20 mmol dm^{-3}. The solution was placed in a flat cell and directly measured and irradiated in the ESR resonator. The different concentrations of oxygen in solutions were realized by the bubbling of oxygen, air and by the mixture of 1% (v/v) oxygen in nitrogen, respectively. The concentrations of dissolved oxygen were calculated according the Henry law. The phosphate buffer pH 7.2 was used in all experiments.

RESULTS

Tetramethylammonium Mercaptoborate

Tetramethylammonium salt of mercaptoborate is a suitable model compound for the study of mercaptoborate dianion behaviour. Nonirradiated mercaptoborate solution (dark reaction) with Tiron at pH 7.2 shows no signals of Tiron radicals. The UV irradiated solution shows ESR spectra of Tiron radicals which indicate the presence of photogenerated superoxide anion radicals. The type of this spectra has been shown on the Fig. 1. The intensity of spectra depends on the initial oxygen concentration in the sequence from high to low oxygen concentration. The numerical relations are shown on the Table I.

Sodium Mercaptoborate

The amorphous hygroscopic sodium salt of mercaptoborate is the really used drug for BNCT. Similarly as tetramethylammonium salt, the solution of this drug does not generate superoxide anion radicals at ambient temperature without irradiation. The UV irradiated solutions show the ESR spectra of Tiron radicals formed by the reaction with unstable $O_2^{\bullet-}$ radicals. The concentration of these radicals depends proportionally on the initial oxygen concentration in the solution, see Fig. 1 and Table I.

Sodium Tetraborate

The generation of organic radicals was described[5] in neutron irradiated solutions of sodium tetraborate. We tested here $O_2^{\bullet-}$ generation in UV irradiated sodium tetraborate solution. No radicals were generated by the thermal reaction and the photogeneration of $O_2^{\bullet-}$ radicals is significantly lower then that one with sodium and tetramethylammonium mercaptoborates. The concentration of photogenerated $O_2^{\bullet-}$ depends on initial oxygen concentration similarly as in the case of the above described experiments, see Table I.

TABLE I
The ESR signal intensities of Tiron radicals generated by the reaction of Tiron with $O_2^{\bullet-}$ anion radicals at different concentrations of oxygen in the presence of mercaptoborates and sodium tetraborate

System	Oxygen concentration, mmol dm^{-3}		
	128.0	26.6	1.33
$Na_2(B_{12}H_{11}SH)$	6.50	6.00	1.00
$[N(CH_3)_4]_2(B_{12}H_{11}SH)$	3.15	1.20	1.00
$Na_2B_4O_7$	1.60	0.95	0.70

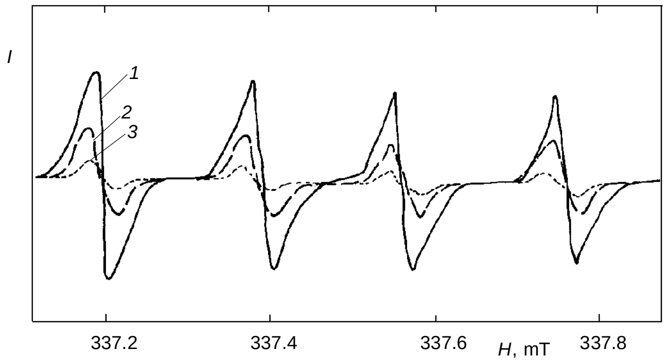

FIG. 1
The ESR spectra of Tiron radicals (g_{iso} = 2.005, $a_{H(o)}$ = 0.193 mT, $a_{H(p)}$ = 0.361 mT) generated by the UV irradiation of sodium mercaptoborate solution (2 mmol dm^{-3}) at pH 7.2. Solutions were saturated by: *1* oxygen, *2* air, *3* mixture of 1% oxygen (v/v) and nitrogen. Microwave power 50 mW, modulation amplitude 0.02 mT, microwave frequency 9.472 GHz, room temperature

The Comparison of the Mercaptoborates and Tetraborate Effects

The effects of used compounds on the $O_2^{\bullet -}$ generation decrease in the sequence sodium mercaptoborate > tetramethylammonium mercaptoborate > sodium tetraborate, see Fig. 2. This generation is decreasing in the sequence of oxygen concentration in the following steps: oxygen > air > 1% oxygen in nitrogen saturation in the solution. The effect of oxygen concentration on to the superoxide anion-radicals generation is expressed by the logarithmic equation of type: $A_{pp} = a \ln ([O_2]) + b$; ($A_{pp}$ intensity of radicals signals, $[O_2]$ concentration of dissolved oxygen, a, b calculated constants of statistically most significant curve), see Table II.

TABLE II
Values of constants a, b in the equation $A_{pp} = a \ln ([O_2]) + b$ describing effects of oxygen concentration on to the superoxide anion-radicals generation

System	Constants	
	a	b
$Na_2(B_{12}H_{11}SH)$	1.017	6.505
$[N(CH_3)_4]_2(B_{12}H_{11}SH)$	0.373	2.486
$Na_2B_4O_7$	0.201	1.432

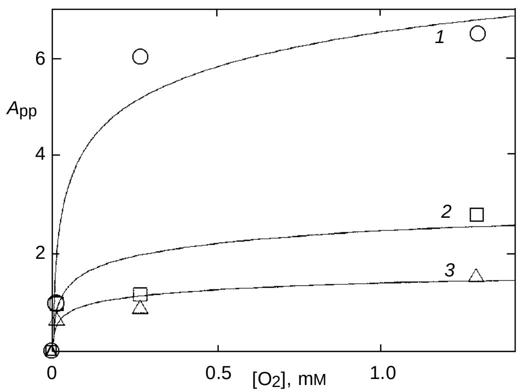

FIG. 2
The effect of initial oxygen concentration on the signal intensity of Tiron photogenerated radicals which corresponds the $O_2^{\bullet -}$ radicals for different sensitizers: *1* sodium mercaptoborate, *2* tetramethylammonium mercaptoborate, *3* sodium tetraborate

DISCUSSION

As it was determined by the spin trapping/detection method using Tiron as an $O_2^{\bullet-}$ trap, mercaptoborate shows a high ability for oxygen photochemical generation of $O_2^{\bullet-}$ anion radicals at room temperature. This is an interesting fact because the number of senzitizers for the photochemical generation of $O_2^{\bullet-}$ is rather limited. Such compounds must form a reactive intermediate complex with oxygen molecule in which transport of one electron to the oxygen molecule is probable. Previously described[5] organic radicals generation by sodium tetraborate is probably the product of the reaction between primary generated oxygen radicals and presented organic substances. The similar reaction mechanism could play an important role in the breakdown of tumours in the BNCT in which particles are generated from 10-Lithium by the neutron irradiation. The differences in the effects of a variety of complexes (aqueous solutions of sodium and tetramethylammonium salts of mercaptoundecahydro-*closo*-dodecaborate(2–) (mercaptoborate), and sodium tetraborate) is difficult to explain. Our findings indicate the possibility to test the biological mercaptoborate effects of these irradiations under combined neutron and ultraviolet irradiations and probable use of generated radicals to the tumours breakdown. The described generation of high reactive intermediate radicals could bring useful contribution to the knowledge of BNCT mechanism and pharmacokinetics.

REFERENCES

1. Stopka P., Strouf O.: Collect. Czech. Chem. Commun. *56*, 657 (1991).
2. Burian J., Marek M., Rataj J., Mares V., Stopka P., Strouf O. in: *Preclinical Tests for BNCT on the LVR-15 Reactor. Advances in Neutron Capture Therapy* (A. H. Soloway et. al., Eds), p. 25. Plenum Press, New York 1993.
3. Ledenev A. N., Konstantinov A. A., Popova E., Ruuge E. K.: Biochem. Int. *13*, 391 (1986).
4. Ledenev A. N., Konstantinov A. A., Popova E., Ruuge E. K.: Biol. Membr. (U.S.S.R.) *3*, 360 (1986).
5. Carmichael A. J., Arroyo D. M., Moore M. L.: Radiat. Phys. Chem. *32*, 227 (1988).