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DETECTION OF THE KINETICS OF BIOCHEMICAL REACTIONS WITH OXYGEN USING EXCHANGE BROADENING IN THE ESR SPECTRA OF NITROXIDE RADICALS

J. M. BACKER[a], V. G. BUDKER[b], S. I. EREMENKO[a] and YU. N. MOLIN[a]

[a]*Institute of Chemical Kinetics and Combustion, Siberian Branch of the Soviet Academy of Sciences, Novosibirsk 630090, and* [b]*Institute of Organic Chemistry, Siberian Branch of the Soviet Academy of Sciences, Novosibirsk 630090 (U.S.S.R.)*

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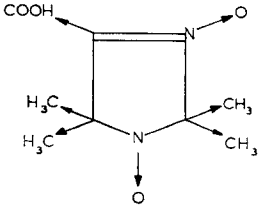
SUMMARY

To detect changes in the oxygen concentration during biochemical reactions, the exchange broadening in the ESR spectra of nitroxide radicals caused by the dissolved oxygen, has been used. The measurements have been carried out using changes in the width either of the proton hyperfine structure components or of the nitrogen hyperfine structure line with an unresolved proton structure. Detection of mitochondrial respiration in a volume of about 10^{-3} cm^3 and respiration for 100 ± 5 liver cells in a volume of about 10^{-4} cm^3 has been carried out.

In the present paper we propose to use the ESR method for detection of biochemical processes accompanied either by absorption or deposition of oxygen. This is possible due to the fact that in solutions the oxygen broadens the hyperfine structure components in the ESR spectra of the radicals to about 0.15 Oe for airated solutions where $[O_2] \approx 4 \cdot 10^{-4}$ M [1]. As it has been shown by Eastman et al. [2], for solutions of nitroxide radicals the spin-exchange broadening is much greater than the dipole-dipole broadening. It is undoubtedly so for the broadening caused by oxygen because oxygen has a very short electron spin-lattice relaxation time.

The methods for exchange broadening detection in mitochondrial and cell respiration are described below. The mitochondria were isolated from rat liver according to ref. 3, the rat liver cells were extracted according to ref. 4. The cell and mitochondrial respiration conditions were similar to those described in ref. 3. Use



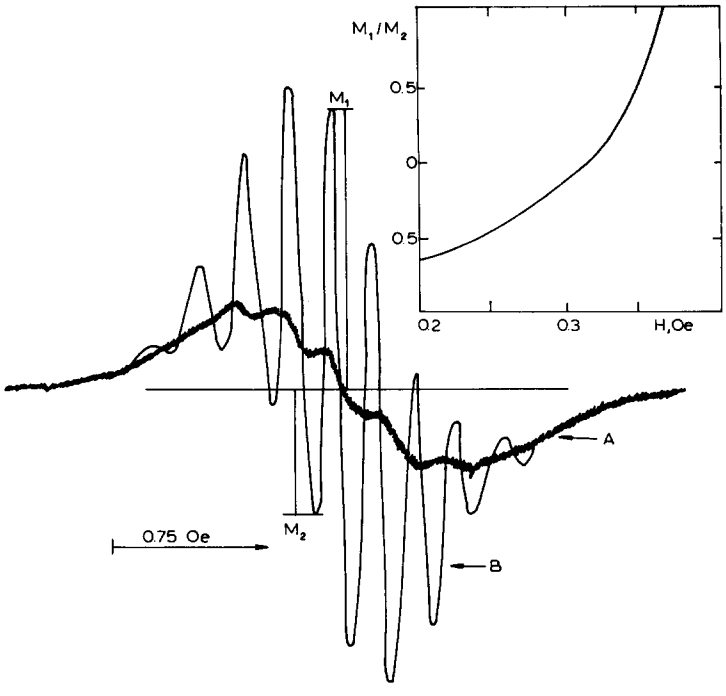


Fig. 1. Transformation of the nitroxide radical Hyperfine structure central component due to reduction of the dissolved oxygen during mitochondrial respiration. [R] = $7 \cdot 10^{-4}$ M, M_1 and M_2 are amplitudes relative to the zero line. In the computer simulation use is made of a hyperfine interaction constant with methyl protons of 0.21 Oe. The reaction mixture composition in all the experiments is: 0.125 M saccharose; $2 \cdot 10^{-2}$ M Tris · HCl pH = 7.4; $2 \cdot 10^{-2}$ M KH_2PO_4; $2 \cdot 10^{-3}$ M EDTA; $5 \cdot 10^{-3}$ M $MgSO_4$; 10^{-2} M succinate; 0.7 mg/ml of mitochondrial protein. (A) the radical spectrum central component before incubation with mitochondria, (B) in 5 min after incubation with mitochondrias.

was made of the nitroxide radical*) I (2,2,5,5-tetramethyl-Δ^3-imidazoline-3-oxide-1-oxyl-4-carbonic acid) whose proton hyperfine structure results from interaction with 12 equivalent methyl protons with $A = 0.21$ Oe [5].

The ESR spectra were recorded by a Varian E-3 spectrometer (U.S.A.) at 23 °C in glass capillaries of 80 μm and 300 μm in diameter. In the experiments with cells the number of cells was calculated microscopically on termination of the reaction kinetics.

The oxygen concentration changes were detected using the exchange broadening in magneto-diluted solutions of radical I. i.e. at $[R] < 10^{-3}$ M. In these solutions the ESR caused by the interaction with protons was observed in every nitrogen hyperfine structure component. The resolution of the proton hyperfine structure increased with decreasing oxygen concentration. Fig. 1 gives an example of this transformation in the radical ESR spectrum caused by mitochondrial respiration. To plot the kinetic curve by a set of the ESR spectra we employed the relation-

* The radical was kindly supplied by Dr. L. Volodarsky (Institute of Organic Chemistry, Novosibirsk).

ship of the characteristic parameter M_2/M_1 on the proton hyperfine structure component width. Fig. 1 shows this relationship obtained by a computer simulation of the proton hyperfine structure with $A = 0.21$ Oe.

Using this dependence one can obtain the width of the proton hyperfine structure H component for every spectrum and, on termination of the process, the proper line width H_0. Since the exchange broadening $\delta H = H - H_0$ depends linearly on the dissolved oxygen concentration, the kinetic curve is convenient to represent as

$$\frac{\delta H}{\delta H_{max}} = \frac{[O_2]}{[O_2]_0} \propto t$$

The above relationship (practically linear) is given in Fig. 2 as a dotted curve.

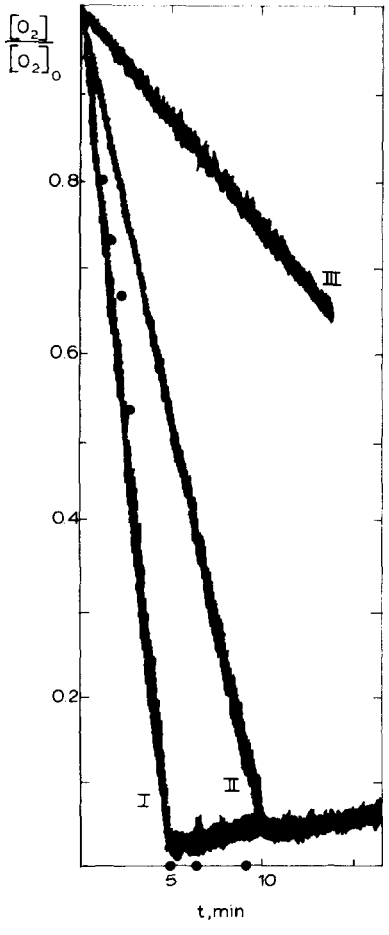


Fig. 2. Continuous kinetic curves for the process of mitochondrial respiration; I, [ADP] = $2 \cdot 10^{-2}$ M; II, no ADP; III, in the presence of [NaN_3] = 10^{-3} M; [R] = $1.2 \cdot 10^{-2}$ M. The dots are obtained by processing a set of the spectra obtained in the experiments with magneto-diluted radical solutions.

One more detection method allowed continuous kinetic curves to be obtained using extremely small volumes of reaction mixtures. For this purpose use was made of magneto-concentrated radical solutions ($R = 10^{-2}$ M) in which spin exchange between the radicals themselves led to disappearance of the proton hyperfine structure in the nitrogen hyperfine structure components. In this case the spin exchange between radicals and dissolved oxygen resulted in an additional broadening of the whole component of the nitrogen hyperfine structure by the factor of δH and in a corresponding decrease in the peak intensity δI. As the integral intensity P, the line width H and the peak intensity I are connected through $P \propto H^2 I$, it is easy to get δI as a function of δH: $\delta I = I \cdot [(H^2/(H-\delta H)^2) - 1]$ where H is the line width and I is the peak intensity before the reaction starts. This dependence evidently converts into $\delta I \approx 2 \cdot \delta H \cdot I/H \propto [O_2]$ at $\delta H \ll H$, i.e. it becomes linear with respect to the dissolved oxygen concentration.

To detect δI as a function of time in the course of the reaction, we maintained the external field so that the recorder pen was kept on the minimum of the nitrogen hyperfine central component. In this case a shift of the recorder pen along the Y axis during the process was unambiguously identified by the signal peak intensity changes.

The set of curved thus obtained for mitochondrial and cell respiration is given in Figs. 2 and 3. The data for magneto-diluted solutions are seen from Fig. 2 to be an attractive fit to those for magneto-concentrated ones. This means that

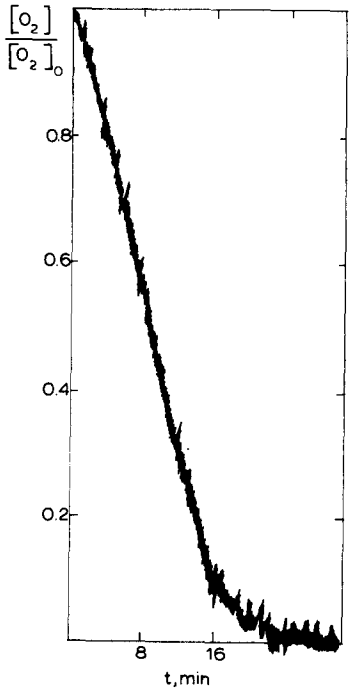


Fig. 3. A continuous kinetic curve for the process of cell respiration. $[R] = 1.2 \cdot 10^{-2}$ M. There were 100 ± 5 cells in the sample with $V \approx 10^{-4}$ cm^3.

starting from $H \gtrsim 1$ Oe changes in the hyperfine structure component peak intensity depend linearly on oxygen concentration.

The above method is seen from Fig. 2 to be sensitive enough to detect standard effects on mitochondrial respiration (acceptor control, inhibitor influence). Note that the radical reduction observed on termination of the respiration (detected via decreasing the signal intensity) occurs much slower than the oxygen reduction during respiration. However, sometimes it may be necessary to take into account a possible influence of this process on the observed kinetics. Note that the radical concentrations being used did not influence the respiration rate of the mitochondrias and cells which were defined by the polarographic method.

The results given in Figs. 1 and 2 were obtained on samples of $\approx 10^{-3}$ cm^3 in volume. The kinetic curve in Fig. 3 was obtained on a sample of $\approx 10^{-4}$ cm^3 in volume containing 100 ± 5 cells. In the present work we did not aim at detecting respiration of one or several cells in a volume of about 10^{-5}–10^{-6} cm^3, but we think that this level could be achieved via selection of radicals and using oxygenated solutions.

Indeed, as it has been shown above, the absolute value of the effect $\delta I \approx 2 \cdot \delta H \cdot (I/H)$ where $\delta H = q[O_2]$, $H = H_0 + K[R]$, $I = A \cdot V \cdot [R] \cdot (M/H^2)$. Here V is the volume of the sample, A is a constant determined by the ESR spectrometer sensitivity, q and K are rate constants of the radical-oxygen and radical-radical spin exchange, respectively, M is the modulation amplitude of the magnetic field, H_0 is the line width in the absence of spin exchange.

Bearing in mind that $M \approx H$ it is easy to show that δI reaches its maximum at $[R] = H_0/K$. Using this optimum radical concentration we have: $\delta I = (A \cdot V \cdot q \cdot [O_2])/(2 \cdot K \cdot H_0)$.

It is thus clear that by increasing $[O_2]$ and using radicals with minimum K and H_0 one can obtain an essential decrease in the indispensable sample volume V.

REFERENCES

1 Hausser, K. H. (1961) Z. Electrochem. 65, 636–638
2 Eastman, M. P., Kooser, R. G., Das, M. P., and Freed, J. H. (1969) J. Chem. Phys. 51, 2690–2709
3 Mosolova, I. and Gorskaya, I. (1975) in Methods in Modern Biochemistry, (in Russian) "Nauka", Moscow
4 Hommes, F. A., Draigma, M. I. and Molenaar, I. (1970) Biochim. Biophys. Acta 222, 361–367
5 Sadikov, R. A. (1976) Ph.D. Thesis, Kazan State University