OXYGEN CONSUMPTION BY PERITONEAL MACROPHAGES MEASURED BY ELECTRON PARAMAGNETIC RESONANCE

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Activation of the phagocyte by both corpuscular and soluble agents is known to be accompanied by a "respiratory burst," which includes an increase in the rate of O_2 consumption, activation of the pentose monophosphate pathway of glucose oxidation, and increased production of active forms of O_2 (O_2^-, $OH^\cdot$, H_2O_2...) and so on [1, 3, 5-8]. The "respiratory burst" is one of the principal manifestations of reactivity of the phagocyte and reflects its functional state and its microbiocidal potential. For an objective evaluation of the state of the phagocyte, it is therefore important not only to use traditional methods (calculating the phagocytic number and phagocytic index), but also to record the rate of O_2 consumption by the phagocytes and their production of active forms of O_2. To record the rate of O_2 consumption in biological systems polarography with an oxygen electrode is the method usually adopted [7, 8]. The nonelectrode method suggested by Backer et al., is of great interest, because it can be used with small volumes or even single cells [4]. The essence of the method lies in measurement of the line width of the electron paramagnetic resonance (EPR) spectrum of the nitroxyl radical, which depends on exchange interaction of the radical with dissolved O_2.

The aim of this investigation was to study the possibility of using the method mentioned above to measure the rate of O_2 consumption by macrophages when activated.

EXPERIMENTAL METHOD

Peritoneal exudate cells, consisting chiefly of peritoneal macrophages, were obtained by the method in [2] without preliminary stimulation. Noninbred albino rats were used: Immediately before decapitation they were given an intraperitoneal injection of 20 ml of balanced Hanks' salt solution. After abdominal massage for 2-3 min the contents of the peritoneal cavity were withdrawn by means of a Pasteur pipet, filtered through two layers of Kapron tissue, and then centrifuged at 400g and 4°C for 10 min. The supernatant was removed and the residue resuspended in 5 ml of medium and then again separated by centrifugation. The cells were resuspended again in 0.5 ml of medium, counted in a Goryaev's chamber, and the concentration adjusted to 10^7 cells/ml. The viability of the cells in suspension was determined by the trypan blue test. Suspensions containing at least 75% of viable cells were used. The cells were kept at the temperature of melting ice, and the rate of O_2 uptake was recorded at 37°C.

The O_2 concentration in the solutions for calibration was measured on an "Aquachek-3" instrument (Hungary). EPR spectra of the TEMPON nitroxyl probe used in the work (1-oxyl-2, 2,6,6-tetramethyl-4-piperidone) were recorded on a "Varian E-4" radiospectrometer (USA).

DQ_{12} silica dust particles (International Standard, West Germany), ground in an agate mortar immediately before preparation of the suspension, were used as the activating agent.

EXPERIMENTAL RESULTS

The authors of [4] suggested using measurement of the width of the individual component (H) of the superfine structure (SFS) of the EPR spectrum of the nitroxyl radical as a result of interaction with O_2 molecules (which are biradicals) to determine the rate of O_2 consumption in biological systems. Additional widening of the spectrum δH under the conditions of

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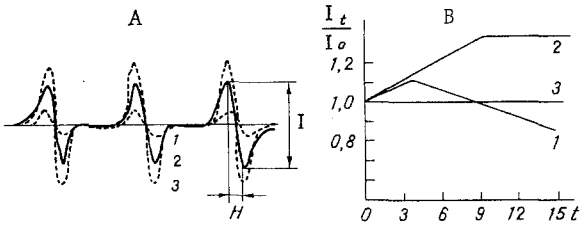

Fig. 1. Typical curves of change in amplitude of EPR spectrum of TEMPON. A) General appearance of spectrum at different times (incubation time of specimen for 1, <2, <3); B) change in amplitude of high-field component of spectrum with time for specimens containing 3×10^7 cells in 1 ml and 10^{-3} M TEMPON (1), the same plus 10^{-3} M $K_3Fe(CN)_6$ (2), and specimen with inactivated cells or without cells (3). I) Amplitude of component of spectrum at time t; I_0) amplitude at initial moment of time.

the present experiments is the result of radical—radical and radical—O_2 exchange interactions. Dependence of δH on O_2 concentrations is expressed by the equation $\delta H = K[O_2]$. It follows from this equation that with a decrease in O_2 concentration in the environment of the probe the width of the spectrum will diminish. It can be tentatively suggested that the amplitude of the spectrum will change as a linear function of O_2 concentration. In fact, the area below the curve of the spectrum, reflecting the number of paramagnetic particles in the specimen, is given by the expression:

$$S \sim \alpha H^2 I,$$

where H is the line width, I the amplitude, and α a coefficient of proportionality. After removal of O_2 from the specimen the same area will be defined by the expression:

$$S \sim \alpha (H - \delta H)^2 (I + \delta I),$$

where δI denotes the change in amplitude of the spectrum. Since

$$H^2 I = (H - \delta H)^2 (I + \delta I),$$

where

$$\delta I = I\left[\frac{H^2}{(H - \delta H)^2} - 1\right].$$

As was shown above, under the experimental conditions $\delta H \ll H$, and for that reason

$$\delta I \approx I \cdot \frac{2\delta H}{H} \sim [O_2].$$

The graph between coordinates of δI_{rel} (abscissa) and $[O_2]$ (ordinate) is thus a straight line which can be used for calibration. To construct the calibration curve 3 points were used: a specimen saturated with pure O_2, a specimen saturated with air at 22°C, and a specimen from which O_2 had been removed to 10^{-2} mm Hg (Fig. 1A). Provided that the specimens for measurement initially contained equal O_2 concentrations, namely 4×10^{-4} M, the quantity of O_2 consumed could be determined from the change in amplitude of the spectrum within a known time interval, and the rate of O_2 consumption by peritoneal exudate cells could then be calculated.

The results of one typical experiment are given in Fig. 1. On incubation of a specimen containing 10^7 cells in 1 ml and 10^{-3} M TEMPON, an increase in amplitude of the spectrum was observed during the first minutes (Fig. 1B). Later, however, the amplitude of the signal steadily decreased and ultimately disappeared completely. The cause of the decline in the signal is reduction of molecules of the spin probe by peritoneal exudate cells, with the formation of nonparamagnetic particles. Addition of ferricyanide to the specimen in an equimolar ratio with the probe prevents reduction (Fig. 1B).

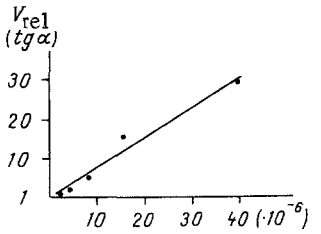

Fig. 2

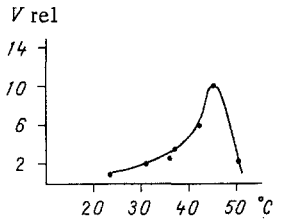

Fig. 3

Fig. 2. Dependence of rate of removal of O_2 from specimen on cell concentration.

Fig. 3. Dependence of rate of O_2 consumption by peritoneal macrophages on incubation temperature.

It can be postulated that the change in the EPR signal of TEMPON in the presence of ferricyanide will be entirely determined by the change in O_2 concentration as the result of functioning of the cells. To test this hypothesis the cells were inactivated by heating to 70-80°C. In specimens treated in this way the amplitude of the signal remained constant throughout the period of recording (Fig. 1B). Further evidence in support of this hypothesis is given by dependence of the rate of O_2 consumption on the cell concentration in the specimen (Fig. 2) and on the incubation temperature (Fig. 3).

The change in amplitude of the signal is thus determined by a change in O_2 concentration as a result of cell function, and it can be used to record the rate of O_2 consumption by peritoneal macrophages. The next experiments were undertaken to choose optimal conditions of measurement to ensure the highest sensitivity of the method. The reason is that additional widening may arise both from radical–O_2 interaction and from interaction of probe molecules with one another. To find the optimal TEMPON concentration, the change in amplitude (I/I_0) was measured for different concentrations of the probe (from 10^{-5} to 10^{-2} M), on saturation of the specimen with O_2 and after removal of O_2 from it down to 10^{-2} mm Hg. The greatest decrease in amplitude of the signal when the specimen was saturated with O_2 was observed within the range of concentrations of TEMPON from 5×10^{-4} to 10^{-3} M. The maximal increase in amplitude after removal of O_2 also was observed within the same range. The presence of ferricyanide in the specimen in an equimolar ratio with the probe had virtually no effect on the maximal change in signal amplitude. We therefore used 5×10^{-4} M TEMPON and ferricyanide in the experiments.

The rate of O_2 consumption in our method was estimated as the tangent of the angle of slope of the straight line between coordinates of δI and time. The experimental points lay on this straight line with a coefficient of correlation $r = 0.97$-0.99. If all the measurements were made under identical conditions, for every value of the tangent a corresponding rate of O_2 consumption can be ascribed. Calculations showed that intact peritoneal exudate cells consume O_2 at the rate of about 0.1 nanomole $O_2/10^6$ cells/min. The rate of O_2 consumption by cells from animals of different groups differed. The rate of O_2 consumption of the cell suspension fell during keeping.

Activation of cells by silica particles measuring $\leqslant 3$ μ in a concentration of 2 mg/ml per 10^7 cells increased the rate of O_2 consumption by the cells three- to fourfold. The measured values were close to those given in the literature.

LITERATURE CITED

1. B. T. Velichkovskii, L. G. Korkina, and T. B. Suslova, Gig. Tr., No. 5, 31 (1983).
2. I. Ya. Uchitel', Macrophages in Immunity [in Russian], Moscow (1978).
3. I. A. Carr, The Macrophage: A Survey of Its Ultrastructure and Function [Russian translation], Moscow (1978).
4. J. M. Backer et al., Biochim. Biophys. Acta, 460, 15 (1977).
5. P. Bellative et al., J. Reticuloend. Soc., 29, 47 (1981).
6. G. Hath et al., Environ. Res., 29, 121 (1980).
7. C. Linda et al., J. Clin. Invest., 67, 710 (1981).
8. M. Reiss and D. Roos, J. Clin. Invest., 61, 480 (1978).