

Superoxide Scavenging Activity of Plastoquinone Derivative 10-(6'-Plastoquinonyl)decyltriphenylphosphonium (SkQ1)

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Abstract—The plastoquinone derivative 10-(6'-plastoquinonyl)decyltriphenylphosphonium (SkQ1) has the ability to scavenge superoxide anion radical. This ability is manifested both *in vitro* and *in vivo* in experiments with the bacterium *Escherichia coli*. The protective effect of SkQ1 *in vivo* significantly exceeds that of ascorbic acid.

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The lipophilic cation 10-(6'-plastoquinonyl)decyltriphenylphosphonium (SkQ1) is the most effective geroprotector known today [1]. The ability of SkQ1 to reduce the intensity of pathological processes connected with ROS generation forms the basis of this activity. The activity has been found in tens of models of different degrees of complexity, both *in vitro* and *in vivo*. Nevertheless, the biochemical mechanisms forming the basis of the antioxidant activity of SkQ1 are not completely studied. In particular, data on the ability of SkQ1 to “intercept” superoxide anion radical ($O_2^{\cdot-}$) are quite fragmentary. It is known that $O_2^{\cdot-}$ is the primary free-radical product generated during respiratory chain functioning. Its production turns the mitochondria into the “dirty places in a cell” and starts the processes causing phenoptosis [2]. The purpose of this work was to study the ability of SkQ1 to “intercept” $O_2^{\cdot-}$.

Superoxide scavenging activity of SkQ1 was determined *in vitro* by means of a standard test system (SOD determination kit 19160; Sigma, USA). Optical density values were measured using the microplate reader of an Alisei automatic analyzer (Radim, Italy). Superoxide dismutase (SOD) at concentrations 1-200 units/ml

(Sigma) was used for calibration. Superoxide scavenging activity of SkQ1 was calculated by the method of Fridovich [3].

In vivo superoxide-scavenging activity was evaluated according to the decrease in *E. coli* Sox-operon induction caused by exposure of the bacteria to pure oxygen with 0.3 MPa for 1 h. *Escherichia coli* MG1655 (pSoxS-lux) strain, provided by I. V. Manukhov (State Scientific Center Genetika, Moscow) was the object of the study. Cells of this strain are a lux-biosensor of Sox-operon induction by luminescence intensification [4]. For luminescence measurements, an LM-01A automatic microplate luminometer (Immunotech, Czech Republic) was used. Measurements were started 10 min after taking samples out of the pressure chamber, and then were carried out every 10 min for 120 min. For evaluation of the influence of the studied factors on Sox-operon expression, the induction factor (I^s) was calculated according to the formula:

$$I^s = (L_e/L_k) - 1, \quad (1)$$

where L_k and L_e are luminescence intensities of control and experimental samples, respectively.

A statistically significant excess of L_e over L_k estimated with the *t*-criterion was considered as a sign of significant influence on the induction effect.

Protective activity (P , %) was calculated taking induction into account in the presence of corresponding protector concentrations:

Abbreviations: HBO, hyperbaric oxygenation; $O_2^{\cdot-}$, superoxide anion radical; SkQ1, plastoquinone derivative 10-(6'-plastoquinonyl)decyltriphenylphosphonium; SOD, superoxide dismutase.

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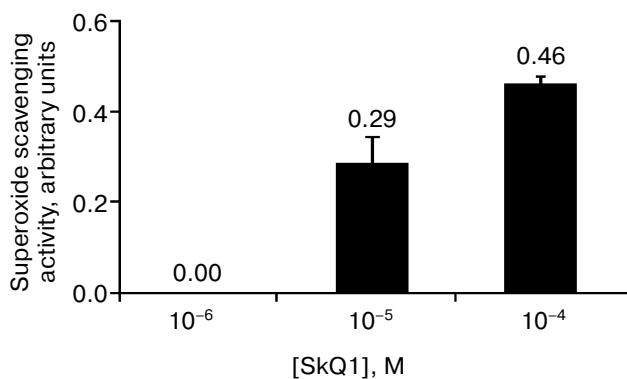


Fig. 1. Superoxide scavenging activity of SkQ1 *in vitro* at 10⁻⁶-10⁻⁴ M concentrations.

$$P = (1 - (I_a/I_p)) \cdot 100\%, \quad (2)$$

where I_a and I_p are induction factors of the SOS-response with the investigated influence in the presence of protector and in the control sample, respectively.

To characterize the protective activity of the studied concentration of the substance, the mean value of P during the whole duration of measurements was used.

As seen in Fig. 1, SkQ1 *in vitro* at 10⁻⁵ and 10⁻⁴ M concentrations shows superoxide scavenging activity (0.29 and 0.46 arbitrary unit). It should be pointed out that the estimation method forming the basis of the test system registers “interception” of superoxide anion radical generated in the xanthine oxidase–xanthine system. This generation system is one of the most reliable according to reproducibility of results and, therefore, it is widely used. However it is meant for detection of high activity, characteristic for SOD enzyme. The sensitivity of the test

was found to be insufficient for detection of superoxide scavenging activity of ascorbic acid (data not shown), which is reliably registered with more sensitive resonance methods [5]. Thus, the superoxide scavenging activity of SkQ1 according to the test *in vitro* can be characterized as high.

Processing of bacteria with oxygen under pressure causes a strong induction of the Sox-operon (Fig. 2), which is considered to be the consequence of growth of the intercellular level of superoxide anion radical [4]. Introduction of SkQ1 at 10⁻⁵ M concentration considerably reduces Sox-induction (on average by 61.3%) (Fig. 2a). Statistically significant effects are registered even for concentrations as low as 10⁻¹⁴ M (Fig. 2b). The comparative data on the ability of different concentrations of ascorbic acid and SkQ1 to suppress O₂⁻ generation caused by hyperbaric oxygenation (HBO) are shown in Fig. 3. This figure shows higher superoxide scavenging activity is characteristic for SkQ1 *in vivo*. Minimum concentrations of SkQ1 and ascorbate that show the effect are 10⁻¹⁴ and 10⁻¹¹ M, respectively. Maximum protective effect reached with SkQ1 and ascorbate is 61.3 and 30.2%, respectively. Superoxide scavenging activity of SkQ1 is manifested *in vitro* at concentrations much higher than those *in vivo*. This is apparently connected, on one hand, with accumulation of the substance in bacterial cells by means of the electrochemical mechanism described in detail, and, on the other hand, with formation of oxidation–reduction cycles conjugated with the respiratory chain enzymes. As a result, the balance of the oxidized and reduced forms remains constant without rigid dependence on concentration [1].

Thus, SkQ1 is able to intercept superoxide anion radical. This ability is demonstrated both *in vitro* and *in vivo* in experiments with aerobic bacteria. Similar processes apparently occur in mitochondria. This is indi-

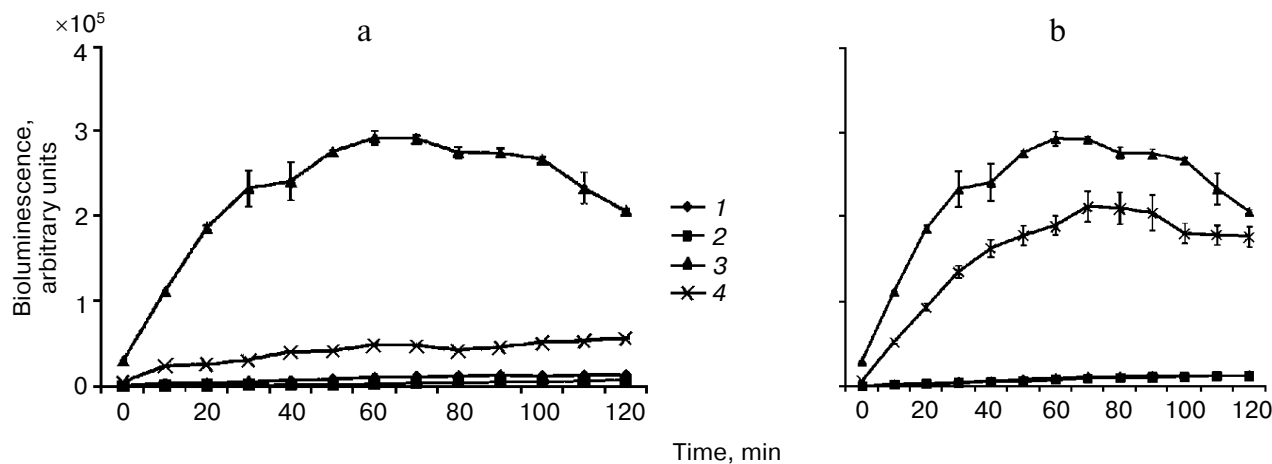


Fig. 2. *Escherichia coli* MG1655 (pSoxS-lux) strain bioluminescence after exposure to hyperbaric oxygenation (HBO) of 0.3 MPa for 1 h in the presence of SkQ1 at 10⁻⁵ M (a) and 10⁻¹⁴ M (b) concentrations: 1) control; 2) SkQ1; 3) HBO; 4) HBO + SkQ1.

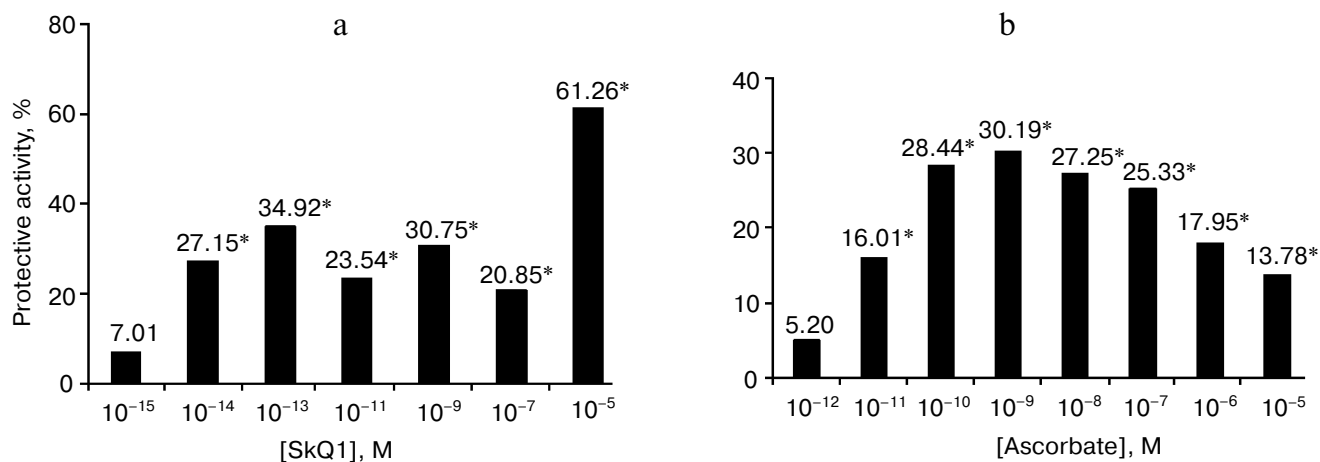


Fig. 3. SkQ1 (a) and ascorbate (b) protective activity at exposure to hyperbaric oxygenation of 0.3 MPa during 1 h. * Statistically significant effects ($p < 0.05$).

rectly proved by numerous data on hydrogen peroxide (which is the main product of superoxide radical dismutation) generation decrease in mitochondria in the presence of SkQ1 [1]. The HBO bacterial model is promising for express screening of new Skulachev ions with antioxidant load.

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REFERENCES

1. Skulachev, M. V., Antonenko, Y. N., Anisimov, V. N., Chernyak, B. V., Cherepanov, D. A., Chistyakov, V. A., Egorov, M. V., Kolosova, N. G., Korshunova, G. A., Lyamzaev, K. G., Plotnikov, E. Y., Roginsky, V. A., Savchenko, A. Y., Severina, I. I., Severin, F. F., Shkurat, T. P., Tashlitsky, V. N., Shidlovsky, K. M., Vyssokikh, M. Y., Zamyatnin, A. A., Jr., Zorov, D. B., and Skulachev, V. P. (2011) *Curr. Drug Targets*, **12**, 800-826.
2. Skulachev, V. P. (2005) *IUBMB Life*, **57**, 305-310.
3. Fridovich, I. (1979) *Free Radicals in Biology* [Russian translation], Mir, Moscow, pp. 190-226.
4. Zavilgelsky, G. B., Kotova, V. Yu., and Manukhov, I. V. (2007) *Mutat. Res.*, **634**, 172-176.
5. Wagner, A. E., Huebbe, P., Konishi, T., Rahman, M. M., Nakahara, N., Matsugo, S., and Rimbach, G. (2008) *J. Agric. Food Chem.*, **56**, 11694-11699.