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SHORT COMMUNICATION

The Influence of Alkylhydroxybenzenes on Electron Stabilization Processes in the Quinone Acceptor Portion of the Reaction Centers of the Bacterium *Rhodobacter sphaeroides*

P. P. Knox[a,1], E. P. Lukashev[a], A. V. Simanova[a], Yu. F. Krupyanskii[b], N. G. Loiko[c,2], G. I. El'-Registan[c], and A. B. Rubin[a]

[a] *Faculty of Biology, Moscow State University, Moscow, Russia*
[b] *Semenov Institute of Chemical Physics, Russian Academy of Sciences, Moscow, Russia*
[c] *Winogradsky Institute of Microbiology, Russian Academy of Sciences, Moscow, Russia*

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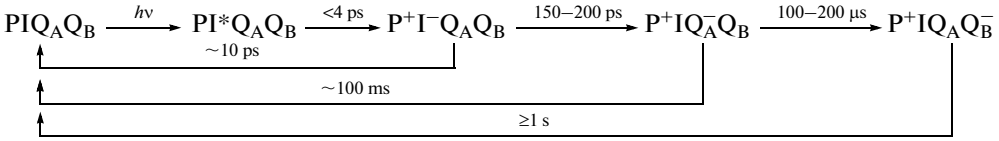
The membrane pigment–protein complexes of photosynthetic reaction centers (RCs) are unique natural macromolecular structures that enable us to study the mechanisms of highly efficient electron transfer in biological systems and the role of molecular dynamics in the regulation of these processes. RCs are convenient and informative models for investigating the relationship between the functional properties of a protein and its structural dynamics because (i) the electron transport activity of RCs can be monitored using modern spectral kinetics assays and (ii) various electron transfer stages within the structure of the transmembrane proteins of RCs are accomplished on time scales ranging from hundreds of femtoseconds to seconds [1–3]. The pigment–protein complexes of the RCs of the bacterium *Rhodobacter sphaeroides* consist of three subunits with a total molecular mass of about 100000. They include the following protein-embedded components: the bacteriochlorophyll dimer P, the primary electron donor; two molecules of monomeric BChl (B_A and B_B) located in the active (A) and in the inactive (B) branch of the electron transfer system; two bacteriopheophytin molecules (H_A and H_B); and two quinone acceptor (ubiquinone-10) molecules (Q_A and Q_B).

The sequence of events taking place in isolated bacterial RC is schematically represented below:



The RC photoactivation is followed by electron transfer from the excited form P* (bacteriochlorophyll dimer) of the primary donor to a bacteriopheophytin molecule (I) that is accomplished in 3–4 ps. The electron from I passes in ~200 ps to the primary acceptor molecule (Q_A) and then in 150–200 μs, to a molecule of the secondary acceptor pool (ubiquinones-10). In the preparations lacking external electron donors for the light-oxidized P, the time of the dark backward transfer of the electron to P^+ (with the subsequent stages of forward electron transport blocked) is ~10 ns, ~100 ms, and ~1 s for the recombination of P^+ and I^-, P^+ and Q_A^-, and P^+ and Q_B^-, respectively. Efficient electrostatic stabilization of the light-mobilized electron on the quinone acceptors that accounts for the above times of dark backward electron transfer to P^+ is due to proton shifts in the protein environment of the acceptors. These processes involve protonated amino acid residues in the vicinity of the quinone cofactors and amino acids located within a distance of up to 15–17 Å.

The molecular dynamics of the protein is influenced by its interactions with chemical chaperones (CCs) [4], i.e., low molecular weight compounds that are capable of correcting the structural and dynamic state of proteins, including those responsible for the development of a number of health problems (Alzheimer's and Parkinson's diseases and prion infections) [5]. Particularly well characterized CCs include polyatomic alcohols (glycerol), sugars (trehalose), trime-

[1] Corresponding author; e-mail: knox@biophys.msu.ru
[2] Corresponding author; e-mail: loikonat@mail.ru

AHB influence on the characteristic time of P^+ and Q_A^- recombination in the RC of *Rhodobacter sphaeroides* (the samples contained *o*-phenanthroline (10^{-2} M)

AHB concentration, mg/ml	Characteristic time of P^+ and Q_A^-, recombination, ms (% of control)				
	T	2MR	5MR	5ER	4HR
Control	79 (100)	79 (100)	79 (100)	79 (100)	79 (100)
0.5	82 (104)	85 (108)	84 (106)	84 (106)	94 (119)
1.0	82 (104)	85 (108)	86 (109)	89 (113)	102 (129)
1.5	82 (104)	86 (109)	89 (113)	91 (115)	108 (137)
2.0	83 (105)	88 (111)	92 (116)	95 (120)	114 (144)
3.0	83 (105)	89 (113)	93 (118)	98 (124)	*
4.0	87 (110)	90 (114)	100 (127)	102 (129)	*

* Micelle formation and the opacification of the samples occurred.

thylamine-N-oxide (TMAO), dimethyl sulfoxide, amino acids (glycine and betaine), and alkylated hydroxybenzenes (AHBs), which can enhance the functional and operational stability of a number of enzyme proteins (hydrolases) [6, 7]. AHBs are synthesized by microorganisms and plants as a mixture of isomers and homologues that differ in the number and position of substituents in the aromatic nucleus and in alkyl radical length. These differences affect the reactivity and biological activity of AHBs [8]. The AHB effect on multisubunit proteins has not yet been investigated.

The goal of this work was to investigate the effect of AHBs on the operation of the photosynthetic RCs of purple bacteria and assess the possible application of RCs for the purpose of selecting the biologically active substances influencing the structural and dynamic state of functional proteins.

The bacterium *Rhodobacter sphaeroides* (Collection of Microorganisms of the Moscow State University, no. 284) was cultivated in liquid Ormerod medium under anaerobic conditions in a luminostat at 30°C for 3 days. To isolate RC from the photosynthetic membranes, a chromatophore suspension was supplemented with 0.5% solution of the detergent lauryldimethylamine oxide (LDAO) and incubated for 30 min at 20°C. Membrane debris was removed by centrifugation (144000 *g*, 60 min, 4°C). The proteins were precipitated with ammonium sulfate (22%). The resulting RC-containing clot was dissolved in phosphate buffer containing 0.05% LDAO and dialyzed against the same buffer to remove ammonium sulfate (24 h, 4°C). The RC preparation was purified by chromatography on a granulated oxyapatite (or aluminum oxide) column eluted with 0.01 M phosphate buffer (pH 7.2). The RC concentration in these studies was 18 μM (measured at $\lambda = 800$ nm, $\varepsilon = 294$ mM^{-1} cm^{-1}). The photochemical activity of the samples was measured with a single-beam differential spectrophotometer. The kinetics of light-induced changes in bacteriochlorophyll P absorbance in RC samples was recorded in the Q_y absorbance band (870 nm). One-electron transfer between the photoactive bacteriochlorophyll and the quinone acceptors was monitored (the preparations lacked a physiological electron donor for the light-oxidizable P such as cytochrome *c* or exogenous reductants). After the flash excitation of photoreactions, three or four signals were accumulated and the kinetic curves of dark P^+ reduction were resolved into exponents using the Marquardt method of nonlinear regression analysis [9].

The following AHB were used as structural modifiers of the RC protein complex: 5*n*-methylresorcinol (5MR), 4*n*-hexylresorcinol (4HR), and 2-(4-hydroxyphenyl)ethanol (tyrosol, T) (Sigma), as well as 2*n*-methylresorcinol (2MR) and 5*n*-ethylresorcinol (5ER) (VKG, Estonia). The photochemical activity was recorded 5 min after supplementing an RC sample with aqueous solution of AHB at a concentration of 0.5–4.0 mg/ml (not exceeding 2 mg/ml for 4HR because micelle formation occurred at higher 4HR concentrations). One RC molecule corresponded to 200–1600 molecules of AHB (to 150–600 molecules for 4HR). Our measurements demonstrated that the dark P^+ reduction kinetics did not change upon increasing the time of RC and AHB incubation to 40 min.

In order to investigate the influence of structurally different AHBs on the temporary stabilization of the electron on the primary quinone acceptor of RCs, the tested samples were preincubated with *o*-phenanthroline (10^{-2} M) that blocks the electron transfer from Q_A^- to Q_B. The characteristic time of dark P^+–Q_A^- recombination was about 80 ms in the control samples. The addition of all tested AHB to RC preparation resulted in an increase in characteristic time of the P^+–Q_A^- recombination (table). The extent of the effect produced by them depended on both the AHB concentration and structure. It increased in the sequence T, 2MR, 5MR, 5ER, and 4HR, which corre-

lated with the hydrophobicity degree of these AHB. The maximum effect was caused by 4HR, which had the longest alkyl radical. The modification of hydrolases by the tested AHBs within a wide range of concentrations resulted in an increase in their catalytic activity (for 4HR, this only occurred at low concentrations) [6, 7]. In contrast, the AHB effect on RC preparations slowed down the tested reaction, while the efficient concentrations of AHB were an order of magnitude above those for hydrolases. These differences may be due to the different structural features of the two systems and dieefernces in their different hydrophobicity. Unlike water-soluble hydrolases, the tested hydrophobic membrane multisubunit protein was solubilized in an aqueous system by a ring composed up of ~150 LDAO molecules. For RC crystals, the estimated thickness of the ring is 25–30 Å (perpendicular to the plane of the photosynthetic membrane). The increase in characteristic time of P^+ and Q_A^- recombination upon addition of the tested AHB to RC preparations may be due to the functioning of the alkyl radicals in the AHBs' aromatic nucleus and their hydroxyl groups. The hydroxyl groups obviously exert an influence on the RC system of hydrogen bonds the state of which affects the functional activity of the RCs [2, 3]. This is consistent with the higher efficiency of 5MR compared to 2MR, the alkyl radical of which screens the hydroxyl groups. These findings are in line with the fact that the electron transfer in the RC structure is decelerated by the triatomic alcohol glycerol, the addition of which to RC at a concentration of 75% (vol/vol) increased the characteristic reaction time 1.2-fold. However, the manifest dependence of the modifying effect of exogenous substances on the hydrophobicity degree of their molecules suggests a decisive role of hydrophobic interactions between AHB and the RC protein.

Thus, this work demonstrated for the first time that AHBs exert a regulatory influence on the functional activity of the multi-subunit protein complex of the photosynthetic RCs of purple bacteria. The biological goal of the regulatory influence of AHBs that function as adaptogens [8] may be a more efficient stabilization of the light-induced charge separation between the bacteriochlorophyll and the quinone acceptor. This stabilization decreases the potential probability of generating reactive oxygen species from bacteriochlorophyll triplets that result from the dark recombination of separated charges.

The results obtained indicate that the RC of purple bacteria can be used as a convenient model system for the primary testing of biologically active substances for the purpose of assessing their potential employment as efficient modifiers of the functional activities of biopolymers.

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REFERENCES

1. Goushcha, A.O., Manzo, A.J., Scott, G.W., Christophorov, L.N., Knox, P.P., Barabash, Yu.M., Kapustina, M.T., Berezetska, N.M., and Kharkyanen, V.N., Self-Regulation Phenomena Applied to Bacterial Reaction Centers 2. Nonequilibrium Adiabatic Potential: Dark and Light Conformations Revisited, *Biophys. J.*, 2003, vol. 84, pp. 1146–1160.
2. Krasilnikov, P.M., Mamonov, P.A., Knox, P.P., Paschenko, V.Z., and Rubin, A.B., The Influence of Hydrogen Bonds on Electron Transfer Rate in Photosynthetic RCs, *Biochim. Biophys. Acta*, 2007, vol. 1767, pp. 541–549.
3. Krasilnikov, P.M., Knox, P.P., and Rubin, A.B., Relaxation Mechanism of Molecular Systems Containing Hydrogen Bonds and Free Energy Temperature Dependence of Reaction of Charges Recombination within *Rhodobacter sphaeroides* RC, *Photochem. Photobiol. Sci.*, 2009, vol. 8, pp. 181–195.
4. Krupyanskii, Yu.F., Korotina, O.A., Esin, S.V., Mikhailyuk, M.G., Rodin, D.S., Dembo, K.A., Volkov, V.V., Nikolaev, Yu.A., and El'-Registan, G.I., Effects of the Interaction of Chemical Chaperons with Enzymes: Changes in Structure, Dynamics, and Functional Activity, in *Tez. Dokl. VI natsional'noi konferentsii po primeneniyu rentgenovskogo, sinkhrotronnogo izluchenii, neitronov i elektronov dlya issledovaniya materialov* (Proc. 6th Natl. Conf. of Application of X-Rays, Synchrotron Radiation, Neutrons, and Electrons in Material Sciences), Moscow, 2007, p. 46.
5. Cohen, F.E. and Kelly, J.W., Therapeutic Approaches to Protein-Misfolding Diseases, *Nature*, 2003, vol. 426, pp. 905–909.
6. Martirosova, E.I., Karpekina, T.A., and El'-Registan, G.I., Enzyme Modification by Natural Chemical Chaperons of Microorganisms, *Mikrobiologiya*, 2004, vol. 73, no. 5, pp. 708–715 [*Microbiology* (Engl. Transl.), vol. 73, no. 5, pp. 609–615].
7. Petrovskii, A.S., Deryabin, D.G., Loiko, N.G., Mikhailenko, N.A., Kobzeva, T.G., Kanaev, P.A., Nikolaev, Yu.A., Krupyanskii, Yu.F., Kozlova, A.N., and El'-Registan, G.I., Regulation of the Functional Activity of Lysozyme by Alkylhydroxybenzenes, *Mikrobiologiya*, 2009, vol. 78, no. 2, pp. 176–185 [*Microbiology* (Engl. Transl.), vol. 73, no. 5, pp. 144–153].
8. El'-Registan, G.I., Mulyukin, A.L., Nikolaev, Yu.A., Suzina, N.E., Gal'chenko, V.F., and Duda, V.I., Adaptogenic Functions of Extracellular Autoregulators of Microorganisms, *Mikrobiologiya*, 2006, vol. 75, no. 4, pp. 446–456 [*Microbiology* (Engl. Transl.), vol. 75, no. 4, pp. 380–389].
9. Himmelblau, D., *Analiz protsessov statisticheskimi metodami* (Process Analysis by Statistical Methods), Moscow: Mir, 1973.