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Structural Study of the Cytochrome-containing Reaction Centre Complex of the Bacteria *Chromatium minutissimum* in Solution and Langmuir-Blodgett Films

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The problem of photosynthetic reaction centers has been a subject of intensive study during recent years. The interest in this subject is due to the high quantum and energetic efficiency of transforming light energy into the electrochemical potential. Up until now, grown crystals, suitable for structural investigation, provided information for only a few types of reaction centers. The reaction centers of multiheme cytochrome C complex from *Rhodopseudomonas viridis*[1,2] and *Rhodobacter sphaeroidas* R-26[3,4] are the most convenient for study.

METHODS AND MATERIALS

Cytochrome containing reactions centres (RC) complexes were isolated from photosynthetic membranes of purple bacteria *Chromatium minutissimum* by treatment with detergent Triton X-100 and sodium cholate. Fractionation was done on hydroxyapatite column.[5] According to polyacrylamide gel electrophoresis data, the complex consists of three RC protein and two cytochrome subunits with total molecular mass of 120 ± 10 kDa.

The cytochrome has at least four heme: two high potential and two low potential ones. The hydrophobic surfaces of the complex are screened from the water mol-

ecules by the detergent. The RC samples were in 0.05 M sodium-phosphate buffer, pH 7.0, containing 0.5% Triton X-100. The complex concentration was varied from 1 to 7 mg/ml. Samples with higher concentration could be used because of their tendency toward aggregation.

X-ray small-angle scattering intensities $I(s)$ (where $s = 4\pi \sin \Theta/\lambda$), 2Θ-scattering angle, λ-wavelength) were registered on an automatic diffractometer with a linear position-sensitive detector.[6] The monochromatic radiation with $\lambda = 1.542$ Å (CuKα) was used. The solutions were placed in thin-walled quartz capillary tubes with internal diameter 1 mm. Measurements were carried out at room temperature in the scattering range $0.0007\ \text{Å}^{-1} \leqslant s \leqslant 0.32\ \text{Å}^{-1}$.

The experimental data treatment was performed on NORD-100 computer using the programs included smoothing the scattering curves (from samples and solvents), collimation correction and a correction for the termination effects. Then small-angle scattering invariants were calculated.

A Joyce Loeble Langmuir trough 4 was used for monolayers formation, and was previously reported as a technique for RC (*Rhodobacters sphaeroides*) monolayer formation.[7,8] This technique includes the successive transferring of monolayers to substrate by Langmuir-Shaefer method (horizontal lift). This technique was found not to be useful for fabrication of LB films of RC *Ch. minutissimum*. Interaction of RC with water was higher than with the substrate. This made impossible a remotion of the protein monolayers from the water surface. More complicated technique was designed for LB films of the complex monolayer from the water surface is due to a charge interaction between hydrophilic parts of the protein components and water dipoles. So we should deposit the monolayer of the complex onto a charged surface. For this purpose we used the following procedure. First

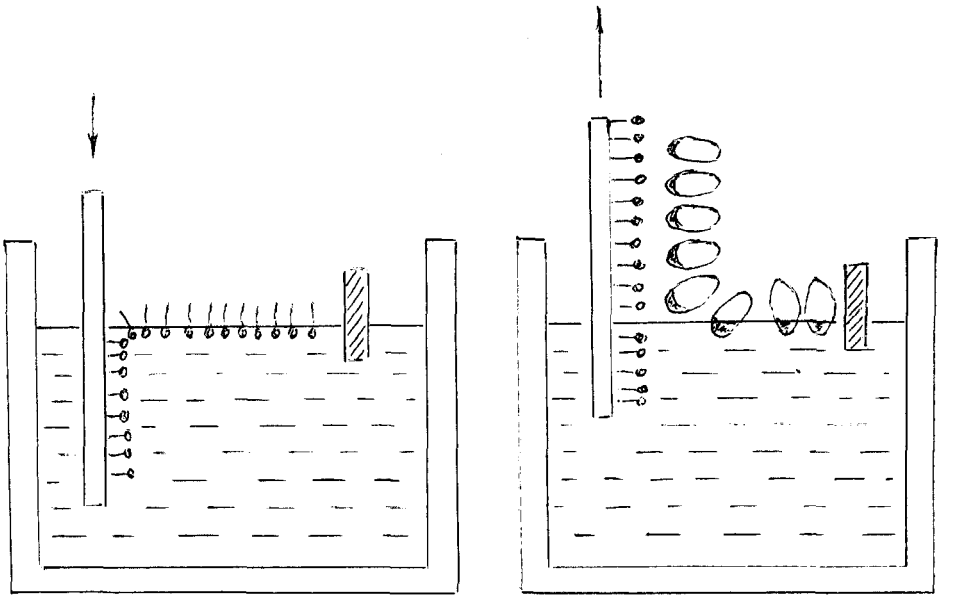

FIGURE 1 The scheme of the protein-containing superlattice deposition process.

the monolayer of deprotonated arachidic acid was formed at the clean water surface (pH 7.0, surface pressure π = 28 mN/m). A silicon substrate with a hydrophobizated surface was moved downward through this monolayer. In the bottom position the movement was stopped. The arachidic acid monolayer was removed from the water surface by a water-stream pump. A RC-cytochrome complexes monolayer was then formed on the cleaned surface (π = 45 mN/m). The substrate then was moved upward. The scheme of the process is presented in Figure 1. Repeating the procedure a protein-containing superlattice was created (in our case it was consisted in 15 alternation). For superlattice spacing, we used small-angle X-ray scattering. The curves were registered within the range $0.5° \leq 2\Theta \leq 7.0°$ in the swing geometry.

RESULTS AND DISCUSSION

The X-ray scattering curve for the cytochrome complex is presented in Figure 1, in coordinates log $I(s)$ versus s^2. The radius of gyration (R_g) was calculated from the slope of the inner part of the curve (within the range s from 0.02 to 0.04 $Å^{-1}$). The dependence of R_g from concentration was not taken into account, because the concentration was low enough (1–5 mg/ml). The value of R_g was calculated as an average from the several series of measurements at the different concentration. Thus the value R_g was found to be 35.5 ± 1.0 Å. The distances distribution function $p(r)$ obtained for the sample with c = 7 mg/ml containing about 0.5% Triton-100 is presented in Figure 2. The largest dimension in particle (L_{max}) can be estimated directly from function $p(r)$ (crossing of the function with abscissa (r, A), that is

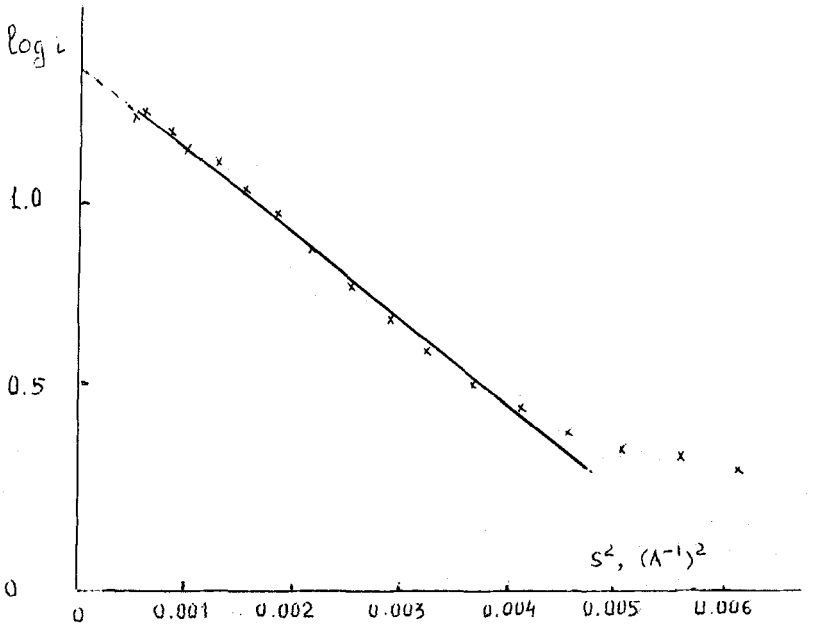

FIGURE 2 The dependence of small-angle X-ray scattering intensity $I(s)$ with respect to scattering angle $s = 4\pi \sin \Theta/$ for RC of *Chr. minutissimum* (Concentration c = 5 mg/ml).

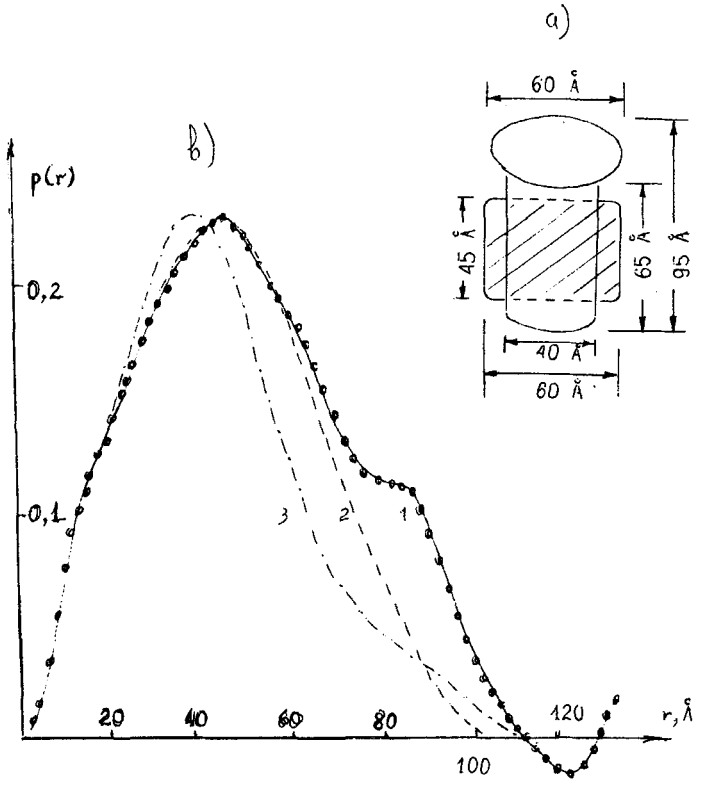

FIGURE 3 (a) A model of RC-cytochrome complex in a solution. (b) The distance distribution function $p(r)$, (1) experimental, (2) for the model a, (3) for a model with parallel long axis of RC and cytochrome.

$p(r) = 0$ for $r = L_{max}$). The measurements of scattering curves in a limited region of angles resulted in periodic oscillations of the $p(r)$ function (termination effects), the strongest in $r > L_{max}$. To diminish distortions we used averaging on a period of a termination wave.[9] It was found for RC complex $L_{max} = 110 \pm 10$ Å. Analysing the behaviour of $p(r)$ function it is possible to make some conclusions about anisometry of the scattering particle.[9] The symmetrical shape of the main peak ($r_m/L_{max} = 0.4$, where r_m is the position of maximum of $p(r)$ function) suggests that the particle shape has a small deviation from spherical one.

A number of models were considered based on the X-ray small-angle scattering data and taking into account structural data from literature. The radius of gyration of the models was postulated to be equal to experimental one $R_g = 35.5$ Å. A mutual arrangement of the protein and the cytochrome subunit was varied. The best coincidence between the experimental and calculated models was found for the model presented in Figure 3. In this model the ellipsoidal cytochrome subunit is perpendicular to the main axis of RC, and the sizes of components are indicated in the Figure 3. Experimental (1) and calculated (2) $p(r)$ functions for this model are presented at the same figure. One can see a good correspondence of experimental and calculated curves. A model with the long axis of cytochrome subunit

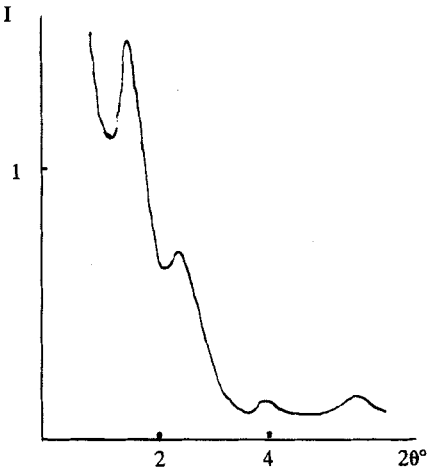

FIGURE 4 Small-angle X-ray intensity curve for protein containing superlattice (15 periods of alternation).

parallel to the main axis of RC results in a worse correspondence (Figure 3). The maximum distance in the best model turned out to be 100 Å.

The protein containing superlattice spacing was calculated from the X-ray pattern (Figure 4) obtained in a swing geometry. The absence of a first maximum on the X-ray pattern is due to the fact that its position is close to initial beam and it is impossible to resolve it. The angle distance between the second and the third reflexes is equal to 0.77° and it corresponds to the spacing $d = 115 \pm 1$ Å. It should be mentioned that the X-ray pattern of the superlattice is more informative with respect to the monocompound LB films of RC from *Rb. Sphaeroides*.[7,8] This fact seems to be due to an ordering influence of arachidic acid interlayers. The value of the spacing corresponds to monolayers of the complex and arachidic acid alternation.

The length of one arachidic acid is equal to 27 Å.[10] So the length of the complex in the superlattice is about 90 Å. It means that arachidic acid and RC-cytochrome complex molecules are inclined slightly with respect to the perpendicular to a film plane.

The obtained cytochrome containing RC complex sizes (*Chr. minutissimum*) differ from the sizes of analogous RC-cytochrome complex of *Rps. viridis* is equal to $L_{max} = 130$ Å. This situation can indicate the different structure of such complexes in various bacteria. But we can not exclude the possibility that this difference is due to the state of the substance (crystal and solution). The fact that the molecular masses of components and similarity of the protein and chromophore composition in RC of both bacteria confirms the latter idea. But if the supposition really takes place, the topology of the hemes and photoactive bacteriochlorophyll in cytochrome-RC complexes in a native state can differ from mutual configuration of hemes and bacteriochlorophyll in crystals. This fact is very important for understanding the functional interaction between the components of the electron-trans-

port chain, and it should be taken into account when making a suitable model of electron transferring processes in the cytochrome-RC system.

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References

1. J. Deisenhofer, O. Epp, R. Huber and H. Michel, *Nature*, **318**, 618–624 (1985).
2. J. Deisenhofer and H. Michel, *EMBO J.*, **8**, 2149–2170 (1989).
3. J. P. Allen, G. Feher, T. O. Yeates, H. Koiya and D. S. Rees, *Proc. Natl. Acad. Sci. USA*, **84**, 5730–5734, 6162–6166 (1987).
4. T. O. Yeates, H. Komiya, D. C. Rees, J. P. Allen and G. Feher, *Proc. Natl. Acad. Sci. USA*, **84**, 6438–6442 (1987).
5. Ya. Sabo, N. I. Zakharova, N. Ya. Uspenskaya, S. K. Chamorovski and A. A. Kononenko, *Dokladi Akademii Nauk SSSR* (in Russian), **305**, 732–735 (1989).
6. L. Yu. Mogilevski, A. T. Dembo, D. I. Svergun and L. A. Feigin, *Crystallography* (in Russian), **29**, 587–591 (1984).
7. V. V. Erokhin, L. A. Feigin, R. L. Kayushina, Yu. M. Lvov, A. A. Kononenko, P. P. Knox and N. I. Zakharova, *Stud. Biophys.*, **122**, 231–236 (1987).
8. V. V. Erokhin, R. L. Kayushina, Yu. M. Lvov and L. A. Feigin, *Stud. Biophys.*, **132**, 97–104 (1989).
9. L. A. Feigin and D. I. Svergun, *Structure Analysis by Small-Angle and Neutron Scattering*, Plenum Press, N.Y., (1987).
10. Yu. M. Lvov and L. A. Feigin, *Crystallography* (in Russian), **32**, 802–817 (1987).