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The effect of the chromophoric group modification on the optical properties of retinal proteins

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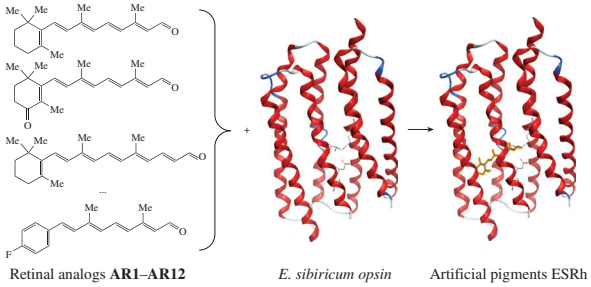
The effects of chromophoric group structures on the functional properties of bacteriorhodopsin (BR) and proteorhodopsin from *E. sibiricum* (ESRh) were compared. ESRh retinal binding site was found as preserving the similar stereo- and spatial restrictions on the chromophore structure during the retinal protein reconstitution process (except for C25-analog AR8). It was revealed that the structure peculiarities of the chromophore analog molecules affect the optical parameters of ESRh and BR pigment families in similar ways.



Light-induced changes in retinal proteins are associated with charge redistribution in the excited retinal chromophore and are driven by the isomerization of chromophore moiety around a 'critical' double bond. Chromophoric group modifications allow one to tune the spectral properties and other characteristics of microbial rhodopsin, providing their optimization for biomedical and nanotechnological applications.[1–9] In this work, the following retinal proteins were studied: (1) bacteriorhodopsin (BR), the well-known light-driven proton pump from the extremely halophilic microorganism *H. salinarum*, for which a whole arsenal of modern methods for determining structure–function relationships has been developed in past 30 years[1,2,10] and (2) a new member from the retinal protein family, the unique proteorhodopsin, from microorganism *E. sibiricum* (ESRh), which was isolated from permafrost aged about three million years. Their chromophoric groups are bound to the apoproteins *via* the protonated aldimine bond with the ε-amino group of Lys residue. The photocycle and proton transport mechanisms of retinal-proteins ESRh and BR are different in many aspects.[2–9] The light driven proton pump ESRh from the psychrotrophic bacterium *E. sibiricum* utilizes Lys96 as a proton donor to a Schiff base that distinguishes it from related retinal proteins, wherein the donor function is performed by a carboxyl side chain. Similarly to other members of the proteorhodopsin family, but unlike BR, the proton acceptor in ESRh, Asp85, is tightly coupled with His57, which exerts a profound influence upon the properties of the acceptor.[5] From 19 amino acid residues, which form close contacts with retinal in BR, only three of them (S141, T142, and M145) are mutated in ESRh. These differences stimulated the structural and functional studies of ESRh in comparison with other retinal proteins making them the interesting objects. To study the effect of chromophoric group nature on the structure and function of the retinal proteins, we developed synthetic methods for the preparation of retinal analogs (**AR2**–**AR12**) and conducted the comparative study of their interaction with apoproteins: ESRh opsin from *E. sibiricum*[3–9] and bacterioopsin from *H. salinarum*[2] (Table S3, see Online Supplementary Materials).

In the present study, to estimate the influence of the chromophoric group structure on the functional properties of recombinant ESRh expressed in *E. coli*, we employed the following modifications of natural all-*E*-retinal molecule **AR1** (retinal analogs **AR2**–**AR12**): modified at the ring 3,4-didehydroretinal **AR2**, 4-oxoretinal **AR3**, 5,6-dihydro-5,6-epoxyretinal **AR5**, 4-fluorophenylretinal **AR10**; analog modified at the polyenic chain (13-desmethylretinal **AR4**); analogs with altered length of polyenic chain (C22, C25, and C15) **AR7**–**AR9**, respectively; the acyclic retinal derivative (without the ionone ring) **AR6**; analogs with altered terminal polar group (retinoic acid and retinonitrile **AR11**–**AR12**), which are different in electronic and conformational properties [Figure 1(*a*,*b*)]. We used the set of experimental procedures developed earlier in our laboratory for the synthesis of the already known retinal analogs **AR2**–**AR6**, and **AR10**.[10] Novel retinoid derivatives with alterated polyenic chain length (**AR7**–**AR9**) were obtained *via* a two-step synthetic procedure, which included HWE olefination of the corresponding carbonyl precursor by either C_2-phosphonate or C_5-phosphonate with the following DIBAL/CH_2Cl_2 reduction of intermediate nitriles. All retinoid samples were of 98–99% purity according to HPLC

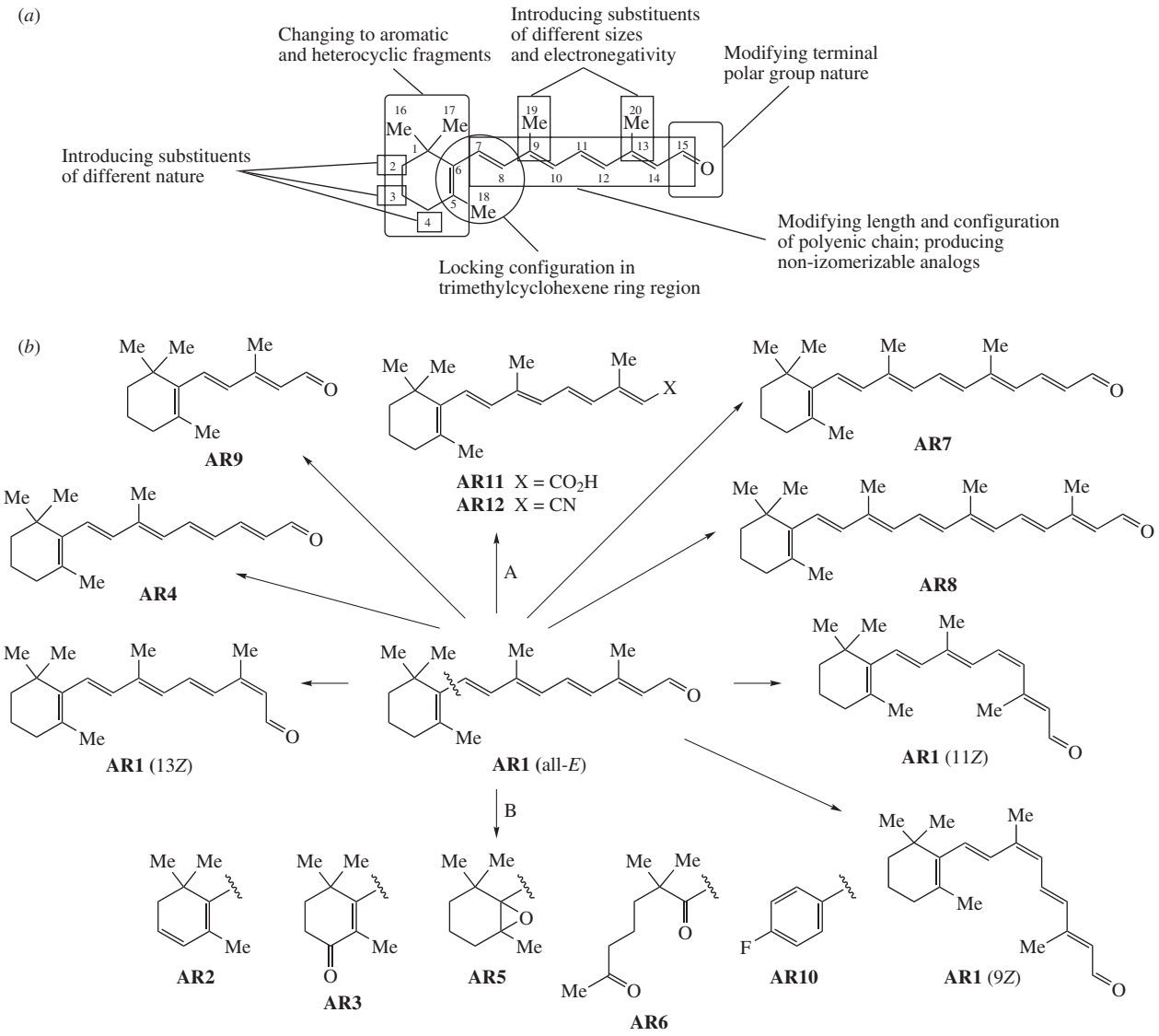


Figure 1 Basic sites in the strategy of retinal molecule modifications: (*a*) polyenic chain (the double bond configurations, the chain length, the nature of substituents, and terminal group types) and (*b*) the trimethylcyclohexene ring (its modification and replacement with aromatic fragments).

and their structures were confirmed by the modern methods of analysis (NMR and other data are given in Tables S1 and S2, and in Figures S2–S9, see Online Supplementary Materials).

The reconstitution of pigments was conducted by addition of the analog solution in methanol to a suspension of apomembranes in buffer (50 mM MES, 5 mM EDTA, pH 6.5) for BR analogs or, in case of ESRh, after addition of 5 μM all-*E*-retinal or its analog solution in ethanol to the membrane fraction in buffer (50 mM NaH_2PO_4, 200 mM NaCl, 0.2% DDM, 300 mM imidazole, pH 8.0). It was found that the formation of pigments has been completed within 0.5–2 h for BR and 1–2 min for ESRh. We have shown that ESRh easily forms a photoactive artificial pigments from the recombinant ESRh membrane fraction and retinals **AR1**–**AR7**, and **AR10** in dodecylmaltoside micelles (Figures 2–3 and data in Table S3). Analogs **AR11**, **AR12** without terminal conjugated formyl group did not form the artificial pigments with apoproteins during the incubation. The same phenomenon was also demonstrated by the 9*Z*- and 11*Z*-isomers of natural retinal **AR1**. This indicates that ESRh retinal binding site preserves the restriction in stereospecificity for the recognition only in the case of all-*E*- and 13*Z*-retinal isomers. A drastical alteration of polyenic chain length in chromophore molecule also blocks the reconstitution process (compare **AR7** with **AR8** and **AR9**).

To solve a quite complicated problem of the quantitative estimation of retinal analog structure influence on chromophore–protein interaction character in retinal proteins, the following parameters were proposed: 'Opsin shift' (OS, cm^{-1}) and 'Percent red-shift' (PRS, %). They were calculated according to the equations:

$$OS = 1/\lambda_{max}(SBH^+) - 1/\lambda_{max}(pigment^{LA}) \text{ (see refs. 11,12),}$$

$$PRS = \frac{1/\lambda_{max}(retinal) - 1/\lambda_{max}(pigment^{LA})}{1/\lambda_{max}(retinal)} \times 100 \text{ (see refs. 13,14).}$$

We calculated these parameters for the series of BR and ESRh analogs (see Table S3, Online Supplementary Materials). The opsin shift is a measure of chromophore–protein interactions at the binding site. The OS values for retinal proteins were notable, indicating the significant influence of the protein environment on the absorption maxima of these pigments. The PRS has recently been introduced as a new estimate of the extent of charge delocalization in chromophores. A PRS threshold of approximately 34% is strongly suggestive to the participation of a highly charge-delocalized cation.[13,14] The PRS values for native BR and ESRh and their analogs are given in Table S3. It was found that the electronic and conformational properties of the chromophore analog molecules affected the optical parameters of ESRh and BR pigments families in the similar way. The Schiff base protonated

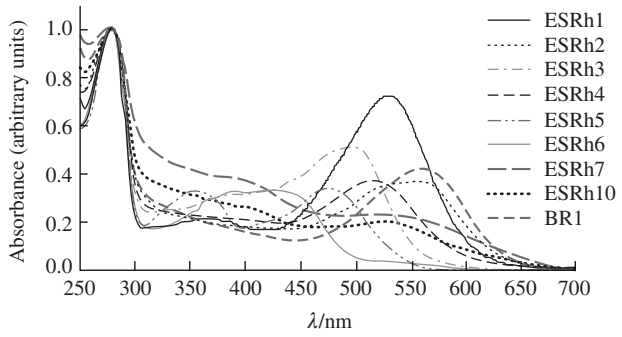


Figure 2 UV–VIS spectra of artificial pigments ESRh 1–7, 10, and BR1. The artificial pigment numbering corresponds to that of retinal analogs.

aldimine bond between the retinal analog and Lys residue at the binding site of ESRh is moderately stable against hydrolysis by hydroxylamine in the dark, but very slowly undergoes a replacement by the excess of all-*E*-retinal (except for **AR6**, **AR7**, and **AR10**). Artificial pigments ESRh1–5, 7, 10 have demonstrated cycles of photoconversions (the details of these processes will be published elsewhere). Both BR6 and ESRh6 analogs underwent the irreversible destruction under the light illumination. Some of artificial pigments (ESRh4, 5, 7, 10) preserve the light-dark adaptation ability (see data in Table S3).

Therefore, our results have clearly confirmed that the use of synthetic retinal analogs is an efficient approach to the direct modification of the spectral properties of microbial rhodopsins, which can be applied in a development of the new optogenetic tools with improved characteristics.[15–17]

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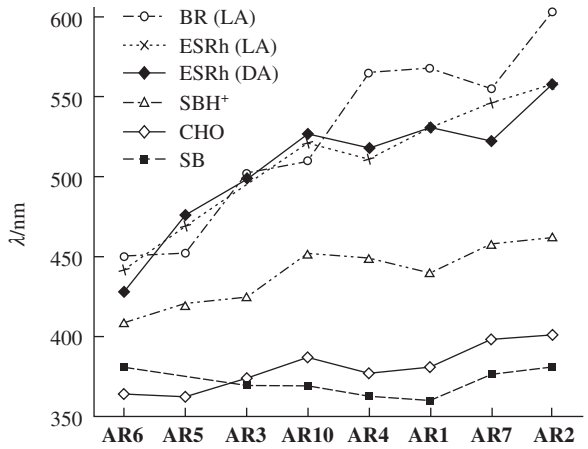

Figure 3 Effect of chromophoric group structure on the spectral properties of ESRh 1–7, 10 and BR series (see Table S3, Online Supplementary Materials).

Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2018.07.022.

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