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On the Photocycle of 4-Ketobacteriorhodopsin

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Abstract—The artificial pigment 4-ketobacteriorhodopsin is an interesting analog of bacteriorhodopsin. Arguments concerning the scheme of the photocycle of 4-ketobacteriorhodopsin are discussed.

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Bacteriorhodopsin (BR) is a light-dependent generator of $\Delta\bar{\mu}_{H^+}$ [1-5]. It is isolated from the bacterium *Halobacterium salinarum* (*halobium*) as purple membranes (PM). The chromophore of the pigment consists of the Schiff base of retinal and the ε-amino group of a lysine residue of the protein. The transfer of H^+ occurs during a cyclic transformation of the pigment (the photocycle, see table). Chromophore modification is an approach to the study such pigments. The 4-ketoanalog combines high efficiency and a significant deceleration of the photocycle, and this is of importance for both scientific and applied problems [12-15]. Unfortunately, the unsuccessful scheme of the 4-ketoBR photocycle in a series of publications (table, item 7; [9-11]) leads to delay in discussion of new experimental data. The purpose of the present work is to show apparent errors in those publications.

A group of biophysicists has proposed in 1991 a scheme of 4-ketoBR functioning [9], and the authors defended it later [10, 11], whereas a group from Pushchino supported it also in 2009 in interviews. These authors consider three peaks in the spectrum of M-intermediate of 4-ketoBR detected at −180°C (table, item 6) to be a specific feature of 4-ketoBR and conclude that these peaks indicate three different M-containing cycles (table, item 7), whereas the 13*Z*-form enters one of the cycles and the *E*-conformer is a precursor of two other cycles. The authors of [9-11] include the recorded K_{570} into all three cycles of the scheme, do not notice the L-intermediate, doubt its presence in 4-ketoBR, and indicate a branch of cycle "shortening" from M to the initial state. However, there is no doubt that on prolonged illumination a rapid return is recorded considering that the photosensitivity of virtually all intermediates has been described long before [5, 16]; in particular, such transition (the so-called "blue inhibition") is a well-studied phenomenon [16, 17]. Moreover, the photosensitivity of M in the cycle of the analog is not sufficient to indicate the branching M → (analog) $BR_{\text{in steady-state}}$ in the dark.

The vibronic structure of the M-intermediate of BR containing the natural retinal in low-temperature spectroscopy is also known [5-7] (table, item 3), but it does not suggest parallel cycles. Moreover, such ideas about the functioning mechanisms became out of date after publication of [2], which established the reversibility of the majority (or all) stages of the BR cycle. Maxima of low temperature peaks of BR and 4-ketoBR are rather close (table, items 3 and 6), and even more peaks have been found in BR (possibly because of increase resolution).

In works [9, 10] the accumulation of a photostationary long-lived M_{440} is an additional confirmation of the scheme, but phototransformations of intermediates are diverse [5], and most long-lived product will be accumulated, independent of its relation with a single turnover of the cycle.

As differentiated from the proton transfer cycle of *E*-BR, the *Z*-cycle under usual conditions has neither an M-intermediate nor H^+ transfer, and the life of K-like intermediates is longer [1, 18, 19]. It was shown by Kaulen's group [20-22] that at high pH the M-intermediate and proton transfer appear in the *Z*-cycle, but the decay of K

Abbreviations: BR, bacteriorhodopsin; PM, purple membrane; *Z*-BR and *E*-BR, BR with retinal chromophore in 13-*cis*- and *all-trans*-configurations, respectively; λ_{max}, maximum of the major band in the absorption spectrum of the chromophore; the lower index of an intermediate of the cycle (e.g. M_{412}, K_{570}) is its differential maximum.

Features of BR and its 4-ketoanalog

No.	Preparation*	Isomer	Temperature, °C	Features	Reference
1	BR	*E*	>0**	cycle $BR_{570} \rightarrow K_{590} \rightarrow L_{550} \rightarrow M_{412} \rightarrow N_{560} \rightarrow O_{640} \rightarrow BR_{570}$	[3]
2	BR	*E*	>0	$\lambda_{max\ of\ M\text{-}intermediate}$ = 412 (410-412) nm	[1-5]
3	BR	*E*	−180	$\lambda_{max\ of\ vibronic\ structure\ of\ M\text{-}intermediate}$ = 375, 398, 419 and 442 nm	[5-7]
4	4-ketoBR	*E*	>0	λ_{max} = 527 nm	[8]
5	4-ketoBR	*Z*	>0	λ_{max} = 504 nm	[8]
6	4-ketoBR	*E* + *Z*	−180	$\lambda_{max\ of\ M\text{-}intermediate}$ = 395, 420 and 440 nm	[9]
7	4-ketoBR	*E* + *Z*	>0	λ_{max} = 506 nm cycle assigned to *Z*, $BR_{504} \rightarrow K_{570} \rightarrow L? \rightarrow M_{395} \rightarrow O \rightarrow BR_{504}$ cycle assigned to *E*, $BR_{504} \rightarrow K_{570} \rightarrow L? \rightarrow M_{420} \rightarrow O \rightarrow BR_{527}$ └→ ... → $M_{440} \rightarrow O_{595} \rightarrow$ ┘	[9-11]

* For BR data are presented for PM, preparations of 4-ketoBR are membranes resulting on the interaction of apo-membranes and 4-ketoretinal.
** Above 0°C and below the temperature of the first signs of thermal denaturation of the preparation (usually below 30°C).

accelerates to times typical for the *E*-cycle. Thus, depending on conditions, BR and its analogs either have in their cycles an M-like intermediate or specific long-lived long-wavelength intermediates [21-24]. We compared the kinetics of spectral transformations of individual forms of *E*- and *Z*-4-ketoBR and isomerized preparations containing a mixture of these forms [8], and we found that the *E*-cycle contains no long-wavelength intermediates, and all signals recorded in this region on uncontrolled preparations are a summary of K-like long-lived intermediates of the *Z*-cycle and a decrease in the optical density in the main band of the *E*-cycle specific absorption. Therefore, the assignment of M_{395} of 4-ketoBR to the *Z*-cycle under conditions discussed in [9, 10] (table, item 7) and K_{570} to all cycles of the *E*- and *Z*-forms of the analog seems to be unreasonable. An unclear situation with L is caused by a high amplitude of K_{570} which is specific just for the *Z*-cycle (a similar case has been analyzed in detail for phenyl analogs of BR [25]). We have recorded similar long-wavelength kinetics in the cycles of individual *Z*-forms of 4-ketoBR [8] and of other analogs [23-26], but no such kinetics have been recorded in the cycle of the individual *E*-form of 4-ketoBR [8]. The comparison of λ_{max} (table, items 4, 5, and 7) shows that in preparations of the authors of the scheme of the cycle [9-11] *Z*-4-ketoBR is prevalent (we have also studied similar mixed preparations [8, 12, 14]). The maximum of the differential spectrum of the *E*-form K-intermediate is further to the red than that of the *Z*-form [5, 18]. Therefore, it is reasonable to assign K_{570} only to the *Z*-cycle and exclude it from the scheme of the *E*-4-ketoBR cycle.

Thus, the scheme of the 4-ketoBR proposed and discussed in works [9-11] is not experimentally consistent and contradicts many findings.

REFERENCES

1. Stoeckenius, W., Lozier, R. H., and Bogomolni, R. A. (1979) *Biochim. Biophys. Acta*, **505**, 215-278.
2. Chernavskii, D. S., Chizhov, I. V., Lozier, R. H., Murina, T. M., Prokhorov, A. M., and Zubov, B. V. (1989) *Photochem. Photobiol.*, **49**, 649-653.
3. Kalaidzidis, I. V., Kaulen, A. D., Radionov, A. N., and Khitrina, L. V. (2001) *Biochemistry (Moscow)*, **66**, 1220-1233.
4. Dioumaev, A. K., and Lanyi, J. K. (2009) *J. Phys. Chem. B*, **113**, 16643-16653.
5. Balashov, S. P., and Litvin, F. F. (1985) *Photochemical Transformations of Bacteriorhodopsin* [in Russian], MGU Publishers, Moscow.
6. Balashov, S. P., and Litvin, F. F. (1981) *Biofizika*, **26**, 557-570.
7. Karneeva, N. V., Balashov, S. P., and Litvin, F. F. (1982) *Dokl. Akad. Nauk SSSR*, **263**, 725-729.
8. Khitrina, L. V., and Lazarova, Ts. R. (1989) *Biokhimiya*, **54**, 136-139.
9. Brown, L. S., Druzhko, A. B., Lukashev, E. P., and Chamorovsky, S. K. (1991) *Biol. Membr. (Moscow)*, **8**, 460-467.

10. Brown, L. S., Druzhko, A. B., Lukashev, E. P., and Chamorovsky, S. K. (1992) *Biofizika*, **37**, 79-85.
11. Druzhko, A. B., and Chamorovsky, S. K. (1995) *Biosystems*, **35**, 133-136.
12. Drachev, A. L., Drachev, L. A., Evstigneeva, R. P., Kaulen, A. D., Lazarova, Ts. R., Laikhter, A. L., Mitsner, B. I., Skulachev, V. P., Khitrina, L. V., and Chekulaeva, L. N. (1984) *Biol. Membr. (Moscow)*, **1**, 1125-1142.
13. Crouch, R. K. (1986) *Photochem. Photobiol.*, **44**, 803-807.
14. Khodonov, A. A., Eremin, S. V., Lokshin, J. L., Shvets, V. I., Demina, O. V., Khitrina, L. V., and Kaulen, A. D. (1996) *Bioorg. Khim.*, **22**, 745-776.
15. Vanhanen, J., Leppanen, V. P., Jaaskelainen, T., Parkkinen, J. P. S., and Parkkinen, S. (1999) *Opt. Mater.*, **12**, 473-480.
16. Karvaly, B., and Dancshazy, Z. (1977) *FEBS Lett.*, **76**, 36-40.
17. Dancshazy, Z., Drachev, L. A., Ormos, P., Nagy, K., and Skulachev, V. P. (1978) *FEBS Lett.*, **96**, 59-63.
18. Sperling, W., Carl, P., Rafferty, Ch., and Dencher, N. A. (1977) *Biophys. Struct. Mech.*, **3**, 79-94.
19. Drachev, A. L., Drachev, L. A., Kaulen, A. D., Skulachev, V. P., and Khitrina, L. V. (1988) *Biokhimiya*, **53**, 707-713.
20. Zorina, V. V., and Kaulen, A. D. (1988) *Biol. Membr. (Moscow)*, **5**, 1135-1144.
21. Kaulen, A. D., Drachev, L. A., and Zorina, V. V. (1990) *Biochim. Biophys. Acta*, **1018**, 103-113.
22. Drachev, L. A., Dracheva, S. V., and Kaulen, A. D. (1993) *FEBS Lett.*, **332**, 67-70.
23. Drachev, L. A., Kaulen, A. D., Khitrina, L. V., Eremin, S. V., Khodonov, A. A., Shvets, V. I., and Chekulaeva, L. N. (1993) *Biochemistry (Moscow)*, **58**, 545-550.
24. Khitrina, L. V., Drachev, L. A., Eremin, S. V., Kaulen, A. D., and Khodonov, A. A. (1992) in *Proc. V Int. Conf.: Structures and Functions of Retinal Proteins (Dourdan)* (Rigaud, J. L., ed.) Vol. 221, Collogue INSERM/John Libbey Eurotext Ltd., Montrouge, France, pp. 167-170.
25. Drachev, A. L., Zorina, V. V., Mitsner, B. I., Khitrina, L. V., Khodonov, A. A., and Chekulaeva, L. N. (1987) *Biokhimiya*, **52**, 1559-1569.
26. Danshina, S. V., Drachev, A. L., Drachev, L. A., Kaulen, A. D., Mitsner, B. I., Khitrina, L. V., and Khodonov, A. A. (1989) *Bioorg. Khim.*, **15**, 307-312.