LETTERS TO THE EDITORS

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Bleaching of Rhodopsin by Light and by Heat

Ever since Mirsky[1] suggested that the bleaching of rhodopsin by light involves the reversible denaturation of its protein moiety, opsin, analogies have been drawn between bleaching and denaturation of the visual pigments. This notion derives its strongest experimental support from the apparent agreement between the minimum size of quantum required to bleach frog or cattle rhodopsin with light[2] (48,500 cal./mole) and the Arrhenius activation energy (E_a) for the thermal bleaching of frog rhodopsin[3] (44,000 cal./mole). We find that this analogy breaks down on closer examination, and that light and heat bleach rhodopsin by different mechanisms, yielding different products.

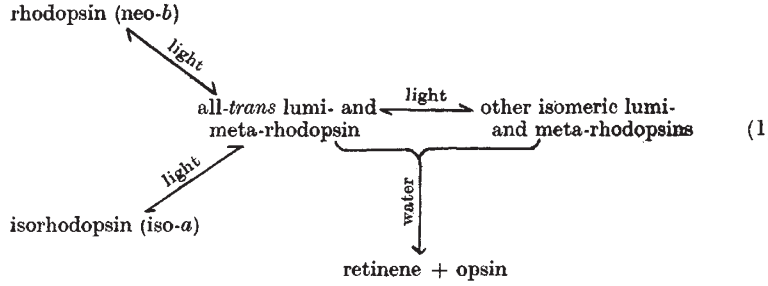
This is shown most convincingly by examining both rhodopsin and isorhodopsin. These two photosensitive pigments differ only in the geometrical configurations of their chromophores, rhodopsin being derived from neo-*b* (11-*cis*) retinene, and isorhodopsin from iso-*a* (9-*cis*) retinene[4]. Both bleach in the light to all-*trans* retinene and opsin[4]. The mechanism of photochemical bleaching is discussed more fully elsewhere[5]. Briefly, we find that light bleaches rhodopsin and isorhodopsin by isomerizing their chromophores to the all-*trans* configuration, yielding as first products the chromoproteins, all-*trans* lumi- and meta-rhodopsin. These in turn can be isomerized by light to a steady-state mixture of chromophores, all still attached to opsin: neo-*b* (= rhodopsin), iso-*a* (= isorhodopsin), and all-*trans*, neo-*a*, etc. (= the stereoisomeric lumi- and meta-rhodopsins). (It should be noted that we are defining lumi- and meta-rhodopsin as those stereoisomeric chromoproteins which are readily hydrolysed to retinene and opsin; or said another way, all possible stereoisomers of the chromophore except neo-*b* and iso-*a*. In our experiments so far, however, lumi- and meta-rhodopsin have always been predominantly in the all-*trans* configuration.) The meta-rhodopsins are relatively unstable and hydrolyse to retinene and opsin (bleaching); the neo-*b* and iso-*a* fractions of the steady state mixture constitute regenerated rhodopsin and isorhodopsin[6,7]. After exhaustive irradiation with white light, the final mixture usually consists of about 1 part rhodopsin and isorhodopsin, and 1 part retinene and opsin[6,7].

Table 1. Minimum Size of Quantum required to bleach Rhodopsin with Light, and the Arrhenius Activation Energy (E_a) for Thermal Bleaching

Rhodopsin	Minimum quantum energy (cal./mole)	Ref.	Arrhenius energy (cal./mole)	Ref.
Frog	48,500	2	44,000	3
Cattle	48,500	2	100,000	8
Squid	Not measured		71,800	9

The effect of light on rhodopsin and isorhodopsin can therefore be summarized as in mechanism (1).

Whereas light bleaches rhodopsin by isomerizing the chromophore, heat bleaches rhodopsin by denaturing the opsin. Opsins, like other proteins, differ from one species to another. It is therefore not surprising that the activation energies (E_a) for thermal bleaching differ for different rhodopsins (Table 1). It can readily be seen from Table 1 that the agreement found earlier between the energies required to bleach frog rhodopsin with light and with heat was accidental; cattle rhodopsin displays no such correspondence.



(1)

The products of thermal bleaching are denatured opsin and retinene, virtually unisomerized from its configuration in the chromophore. The thermal bleaching of rhodopsin or isorhodopsin yields about 70 per cent of the retinene in the original neo-*b* or iso-*a* configurations[8,9]. The process of thermal bleaching can therefore be summarized as:

$$\text{rhodopsin} \xrightarrow{\text{heat}} \text{neo-}b\text{ retinene} + \text{denatured opsin}$$

$$\text{isorhodopsin} \xrightarrow{\text{heat}} \text{iso-}a\text{ retinene} + \text{denatured opsin} \qquad (2)$$

The complementary nature of mechanisms (1) and (2) is emphasized by the fact that the products of bleaching by light regenerate rhodopsin or isorhodopsin upon addition of the appropriate isomer of retinene[4]; the products of bleaching by heat, if one adds opsin[8].

There is by now considerable evidence that the integrity of rhodopsin depends upon a close steric fit between the retinene chromophore and the opsin surface[10]. Light and heat both bleach rhodopsin by destroying this fit, light by attacking the configuration of the chromophore, heat by attacking the configuration of the opsin.

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