The Two Visual Pigments of the Gecko: The Labile Behavior

Frederick Crescitelli*
Department of Biology, University of California, Los Angeles, California 90024, USA

Accepted March 5, 1980

Summary. This report describes five selected experiments that describe the labile behavior of pigment-521 of the Tokay gecko and the relatively more stable properties of the second photopigment, pigment-467, of the same retina. Prepared in the chloride-deficient state, P521 is sensitive to mild temperature increases, is destroyed by NH₂OH and NaBH₄ in the dark, responds to p-hydroxymercuribenzoate by a spectral shift to shorter wavelengths, exchanges some of its 11-cis retinal for the 9-cis isomer in the dark, and reacts to added chloride and nitrate by spectral shifts to longer and shorter wavelengths, respectively. Dissolved in Triton-X-100 it is irreversibly destroyed by only moderate increases in temperature. In all these responses, chloride ions act specifically to protect the pigment. Pigment-467, in contrast, is less sensitive to temperature, is not bleached by NH₂OH and NaBH₄ in the dark, does not exchange its prosthetic group and responds neither to chloride nor to nitrate by the typical P521 effects. With regard to molecular stability and access to the chromophoric structure there appears to be a dual system in the gecko retina with P521 showing similarities to the cone pigment iodopsin; P467 to rhodopsin. It is pointed out that this dual system may be associated with certain responses of the gecko retina that indicate physiological duality. This is the case even though there are no rods and cones, in the classical sense, in the gecko retina.

Introduction

Rhodopsin, especially of cattle, has been the most thoroughly studied visual pigment, and most of our ideas on the properties of these proteins are based on these studies. It is generally agreed that rhodopsin is a Schiff base, probably protonated, in which 11-cis retinal is covalently bound to the epsilon-amino group of a lysine residue. Schiff bases are typically unstable and subject to attack by various reagents, especially at the pH used in the studies. Nevertheless, rhodopsin is unusually stable when solubilized in a detergent such as digitonin and is neither a pH indicator, nor is it bleached in the dark by NH₂OH or NaBH₄. It is also resistant to elevations of temperature up to 25–30 °C. The reason for this relative stability is assumed to be the protection against the aqueous environment offered to the aldimine bond by its being imbedded within an hydrophobic segment of the opsin (Bownds, 1967).

A biologically broader approach to this subject leads one to the suspicion that there may be other aspects to this problem of stability that remain undetected. There are indications, for example, that even for the rhodopsin system, stability is not as it is for cattle and frog rhodopsins. Extracted into digitonin, the rhodopsin of the Arctic cod (Arctogadus borisovi) has a high thermolability, comparable to that of the fish as a whole (Crescitelli, 1977a). The Arctic cod has apparently become acclimated to the temperature of the Arctic waters in which it lives and it has been found feeding in the water from the ice flow at temperatures of 0 °C and lower. Then there are the porphyropsins which are more thermolabile than rhodopsins (Bridges, 1967; Williams and Milby, 1968). These are from fishes living at the usual biological temperatures of the Northern latitudes. One pigment that has been studied extensively by several investigators that is unstable is the chicken cone pigment iodopsin. This is temperature sensitive, requiring low temperatures in its extraction; it is attacked in the dark by NaBH₄ and NH₂OH; and its prosthetic group (11-cis retinal) is able to exchange in the dark with 9-cis...

* This work was supported by grant EY-02178 from the National Institutes of Health

Abbreviations: PMB, p-hydroxymercuribenzoate; DTT, dithiothreitol, Cleland’s reagent
retinal in the environment (Bliss, 1946; Wald et al., 1955; Fager et al., 1975; Matsumoto et al., 1975). Perhaps the most interesting property of iodopsin, in relation to lability, is the shift of its spectrum according to the absence or presence of chloride ions in the extract (Fager and Fager, 1979). The aldimine bond of iodopsin is accessible to the environment, and, unlike that of cattle rhodopsin, it responds in predictable manner to specific changes in this environment. The reason for the difference in behavior of cattle rhodopsin and iodopsin is unknown.

With regard to the problem of lability, the visual pigment that is most easily and convincingly investigated is the 521-pigment (P521) of the Tokay gecko (Gekko gekko), and in its behavior it appears to be typical of the comparable photopigments of other gecko species (Crescitelli, 1977a). The 521-pigment is known to undergo a reversible thermochromic change in going from 5 °C to about 25 °C (Crescitelli, 1974); to shift its spectrum toward the blue from 521 nm upon the addition of P-hydroxymercuribenzoate (PMB), a shift that is reversed by dithiothreitol (DTT) (Crescitelli, 1975); to be bleached in the dark by NH₂OH and NaBH₄; and to be capable of bathochromic ("red") and hypochromic ("blue") spectral shifts with chloride and nitrate ions, respectively (Crescitelli, 1977b, 1978, 1979a). In its behavior, P521 resembles iodopsin more than cattle rhodopsin even though the cells from which P521 is extracted have large, cylindrical outer segments and can, in truth, be called rods. One suggestion that could account for the origin of this apparently anomalous behavior of the gecko pigments is that the gecko rods are, in reality, cells in process of transmutation from ancestral photopic receptors (Walls, 1934).

In addition to P521 the Tokay gecko has a second photopigment, P467, that has been detected in extracts (Crescitelli, 1963) and within the cells (Crescitelli et al., 1977). This pigment is more difficult to study because it is a minor component (8-10% of the photopigment density) in extracts and it is in the presence of a large moiety of P521. Investigation of P467 is even more onerous than the study of iodopsin in the presence of chicken rhodopsin. Nevertheless, it has been ascertained that P467, unlike P521, is less labile and is more like rhodopsin in its behavior than iodopsin. The gecko retina appears to possess a dual system with respect to the behavior of the only two photopigments that are known for the gecko. In respect to this point the idea of Walls (1942) requires mention, i.e., that in a given retina in process of transmutation of its visual cells, not all the cells are in the same stage of the process; some seem to lag behind others. Walls' conclusion was made especially for the transmuting cones of the ophidian retina and was based only on morphological observations. It is the specific purpose of this paper to examine and to compare the two pigments of the Tokay retina in respect to the property of stability and to demonstrate the differences in the two systems.

Procedure

There is little profit in detailing the methods used since these were identical to those previously reported for this series of studies. The retinas removed from dark adapted lizards were washed once with double distilled water; placed in cold, 4% potassium aluminum sulfate for 2 h; washed three times with the distilled water and once with Tris-maleate buffer (pH 7.3); and finally extracted either with 2% digitonin or 1% Triton-X-100, both detergents in Tris buffer at pH 7.3. Using a Beckman DU spectrophotometer, the spectral absorbances were obtained, the temperatures being maintained at about 5 °C by means of a refrigerated bath (Lauda) which circulated water through the blocks of the spectrophotometer chamber. The same bath was employed when it was desired to raise and maintain the temperature at a higher level. Photic bleaching, when required, was carried out by utilizing light from a monochromator (B & L), the extracts, during bleaching, being kept at 5 °C by a similar circulating system. Additions of reagents to the extracts were made in a dark room illuminated by deep red light, the final concentrations in the extracts being computed by weighings made before and after each addition. NH₂OH was always added as (NH₂O)₂H₂SO₄ an important point, since SO₄⁻, unlike Cl⁻, does not alter the spectral absorbance of pigment-521.

Results

Experiment 1: The PMB "Blue" Shift

Apparently, P521 has sulfhydryl groups that are accessible to such sulfhydryl reagents as PMB and DTT and these sulfhydryls are somehow involved in the structure of the opsin that is implicated in setting the color of the photopigment. This is demonstrated by the experiment in which PMB was shown to produce a significant "blue" shift in pigment absorbance, a shift that was readily reversed by DTT, which of itself produced neither spectral change, nor pigment destruction (Crescitelli, 1975). I proceed now to the additional point that chloride ions need to be considered in this PMB action and this is shown in Fig. 1. In this experiment a digitonin extract was divided into two portions in separate microcuvettes. One was retained, as prepared, in the chloride-deficient state; the second was bathochromically shifted by the addition of NaCl to a concentration of 2.48 x 10⁻¹ M, more than enough to produce the maximum "red" shift (curves 1 and 3, Fig. 1). PMB was then added to both aliquots which resulted in a significant "blue" shift in the pigment without added chloride (curve 2) while the one with chloride was unaffected (curve 4).