Vol. 73, No. 1, 1976 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS

# THE EFFECTS OF CHLORIDE ION UPON CHICKEN VISUAL PIGMENTS

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Received August 26,1976

Summary: The absorbance maximum of a part of the visual pigment extracted from the retina of the chicken (Gallus domesticus) has been found to be dependent upon the chloride ion concentration of the preparation. This unusual finding shows that simple chemical treatment can cause a spectral shift of over 50 nm, and may mean that 'iodopsin' - the presumptive cone pigment previously reported in such extracts - may be a modified rod pigment.

## Introduction

There have been several attempts to extract the visual pigments of retinal cone receptors, the most notable being the extraction of the chicken retina by Wald et al. (1). Their extract contained two photosensitive pigments, a rhodopsin of $\lambda_{max}$ = 510 nm that apparently came from the rod receptors, and one of $\lambda_{max}$ = 562 nm that was thought to come from the cones and was named 'iodopsin'. The proportion of iodopsin varied from 29 to 50% of the pigment extracted.

For most of their preparations, Wald et al. used saline solutions to wash the receptors before extracting the visual pigments. This paper presents evidence that extracts prepared under ion-free conditions contain very little iodopsin, but a pigment of $\lambda_{max}$ c.560 nm can be generated in them simply by the addition of low concentrations of chloride ion. The visual pigment molecule is thought to be quite stable, and only one other case of displacement of the absorbance spectrum by such mild chemical treatment has been reported: Crescitelli (2) has shown that gecko receptors isolated in saline yield a pigment of $\lambda_{max}$ =

521 nm, while if prepared in water, their extract has a $\lambda_{max}$ of about 495 nm. Microspectrophotometer (MSP) measurements showed the pigment to be in the 521 nm form in the receptor, and so it was the removal of chloride that caused the formation of the anomalous pigment. The chicken data are less readily interpreted, but suggest that the opposite effect occurs, the addition of chloride generating an artifact from the 506 nm rod pigment which resembles the 569 nm pigment of the cone receptors.

## Experimental

Chickens were dark-adapted overnight and killed in dim white light. Their retinas were removed, the receptor outer segments isolated by flotation in 35% sucrose, and the residual screening pigments removed by one of two methods: (i) by drying and petroleum ether extraction, a method used by Wald *et al.*, and (ii) by high-speed centrifugation on a gradient of 25 to 45% sucrose (3). Both techniques gave essentially the same product, though the second method was more effective and much faster. The outer segments were then washed and extracted with 2% digitonin solution. Three different media were used for the washing and isolation of the receptors: (i) 0.9% saline - the principal method used by Wald *et al.*, (ii) 0.1M sodium phosphate buffer, and (iii) glass-distilled water alone. In each case, the receptor preparation was extracted overnight with 2% digitonin solution. A second extract was made from the residues of the first; this was generally found to contain about 30% of the pigment found in the first, but was otherwise identical. A third extraction gave a negligible amount of pigment. Extraction of whole eyes from fully dark-adapted chickens gave similar yields of pigment of identical composition. Experiments were also performed with isolated receptors suspended in 35% sucrose.

The extracts were analysed by partial bleaching, first exposing them to 'far-red' light ($\lambda > 650$ nm), then 'red' light ($\lambda > 580$ nm) and finally orange light ($\lambda > 500$ nm). The far-red bleach had a negligible effect on the rod pigment ($\lambda_{max} = 506$ nm).

## Results and Discussion

Only extracts prepared using saline contained the high proportion of iodopsin reported by Wald *et al.* With phosphate buffer, partial bleaching showed the extract to contain a pigment sensitive to the far-red light, but the $\lambda_{max}$ of this species was at 540 nm, not 560 nm, recalling the anomalous 544 nm pigment extracted by Bridges from the pigeon retina (4).

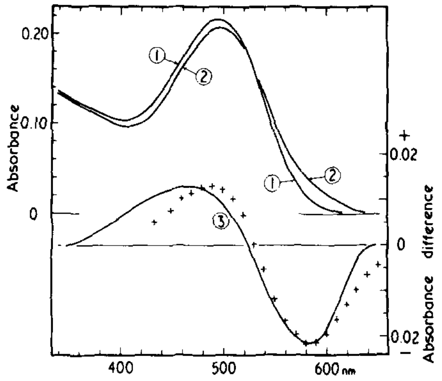

Fig. 1 Curve 1: Extract prepared from chloride-free chicken receptors in 2% digitonin (left-hand scale); Curve 2: 1 min after the addition of 50mM NaCl, corrected for dilution; Curve 3: Difference spectrum for the chloride effect (Curve 1 - Curve 2, right-hand scale). The crosses represent the difference spectrum for the conversion of the 506 nm rod pigment (Ref. 5) to iodopsin (Ref. 1).

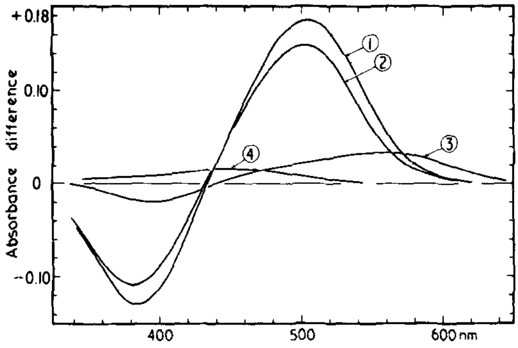

Fig. 2 Difference spectra for the partial bleaching of chicken visual pigment extracts. A. The extract was exposed to far-red light which caused little change, then red light which bleached the principal component (Curve 1), and finally exposed to orange light which gave little further change. B. After the addition of chloride (as in Fig. 1), the far-red bleach removed a pigment of $\lambda_{max}$ = 560 nm (Curve 3), the red bleach removed a reduced amount of the 506 nm pigment (Curve 2), and the final bleach removed a species of $\lambda_{max}$ c.450 nm (Curve 4).

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The absorbance spectrum for the ion-free extract is shown in Fig. 1 (Curve 1). When analysed by partial bleaching, the far-red light caused little bleaching; red light bleached a considerable amount of a pigment of $\lambda_{max}$ = 506 nm (Fig. 2, Curve 1), which is identical with the pigment found in situ in individual rod outer segments by MSP measurement (5).

On adding sodium chloride to the extract to give a final concentration of 50mM, an immediate change took place, the absorbance falling at 506 nm and rising at 560 nm (Fig. 1, Curve 2). The difference between these is plotted as Curve 3, and appears to be due to the conversion of a pigment of $\lambda_{max}$ c.500 nm to a product of $\lambda_{max}$ c.560 nm. The calculated difference spectrum for the conversion of the 506 nm rod pigment to iodopsin - assuming their peak extinction coefficients to be the same - is plotted as crosses for comparison.

A partial bleach of the chloride-treated pigment showed the presence of three species (Fig. 2), far-red light bleaching a considerable amount of a pigment of $\lambda_{max}$ = 560 nm (Curve 3), red light bleaching a 506 nm pigment, apparently identical to the original pigment but reduced in amount, and the final orange bleach removing a pigment of $\lambda_{max}$ c.450 nm. Addition of 11-cis-retinal to the fully bleached preparation caused the immediate regeneration of the 560 nm species, while the 506 nm pigment regenerated more slowly. Partial bleaching showed the regenerated material to be composed of the same two pigments as before. The long-wave pigment was slowly destroyed on the addition of hydroxylamine in the dark.

Thus the original extract contained little of the 560 nm pigment, but this could be generated merely by the addition of small amounts of chloride ion, and appeared identical in every way to the iodopsin described by Wald et al. The same effect was seen when sodium chloride was added to a sucrose suspension of chloride-free receptors, high chloride concentrations converting more than half of the total pigment to the 560 nm species. The composition was determined by partial bleaching as before, and the amounts of pigment calculated on the basis that the peak extinction coefficients for the pigments were the same.

Matsumoto *et al.* (6) report a displacement of the $\lambda_{max}$ of iodopsin on the addition of 9-*cis*-retinal which they ascribe to the exchange of the chromophoric group. Migration of the chromophoric group cannot explain the present observations as the chloride effect is still seen after addition of 25mM hydroxylamine to an ion-free extract. This rapidly reacts with free retinal, which suggests that the chloride effect must be intramolecular.

These experiments show that the $\lambda_{max}$ of the chicken pigments measured in extracts or receptor suspensions is not necessarily the same as those measured in the intact receptor cell. The question remains whether the species of $\lambda_{max}$ c.560 nm seen in chloride-treated extracts is the same as the 569 nm pigment observed in the cones by MSP (5). The chicken retina is considered to have a high proportion of cones, a recent count showing them to comprise 67 to 90% of the receptor population in different areas of the retina (7). Of these, about 80% contain the 569 nm (5). The peak absorbance per unit length of the cones is about 80% of that of the rods (5), and the pigment concentration is therefore probably proportionately lower. The mean diameters of the cone outer segments are about one-third of those of the rods (Bowmaker and Knowles, unpublished; Dr V. B. Morris, personal communication), and so the 569 nm cone pigment comprises between 11 and 33% of the total pigment in the retina, while the rod pigment is between 86 and 60% of the total. Thus despite the high proportion of cones, their small size means that their total pigment content is insufficient to explain the amount of 560 nm pigment seen in chloride-treated extracts and receptor suspensions. Quantitative extractions of completely dark-adapted whole eyes indicate that all of the pigment was removed in the normal extraction procedure. This was confirmed by absorbance measurements on isolated retinas mounted in a perfusion chamber. Partial bleaching of the retina gave similar results to the ion-free extracts and suspensions, confirming that the absorbance of the 569 nm cone pigment is less than 10% of that of the rod pigment.

I therefore conclude that extracts and suspensions prepared under chloride-free conditions truly represent the relative amounts of the pigments in the chicken retina, and that the 560 nm species generated by the addition of chloride results from a modification of the rod pigment. The similarity between its spectrum and that of the 569 nm pigment seen in the cones by MSP may reflect some basic similarity in the molecular structure of the rod and cone pigments.

The change of $\lambda_{max}$ may result from the chloride ion forming a charged site in the pigment protein, or could arise from a minor change in the protein conformation. In either case, the spectral shift would seem to be the result of a change in the electronic interaction between protein and chromophoric group.

## Acknowledgments

I wish to acknowledge the kindness of Mr H. J. S. Jones and Mr M. Dennett of the Plumpton Agricultural College in providing dark-adapted chicken heads, Dr J. K. Bowmaker for the MSP data and many helpful discussions, Dr V. B. Morris for allowing the use of her unpublished observations, and the invaluable assistance of Mrs Anne Priestley.

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