NEWS AND VIEWS

Going in the Red

RHODOPSIN, the visual pigment of retinal rods, is a molecule that has caused many a theoretician to shed tears of bitter frustration. Its chemistry, thanks largely to the work of Wald and his colleagues, is understood in its essential details, but its absorption spectrum is all but unexplained. When the carotenoid prosthetic group, 11-*cis* retinal, which is pale yellow, is bound to the protein, opsin, an enormous spectral shift ensues, with the appearance of a red, or in the case of the cone pigment, iodopsin, a purple colour. To account for such a formidable wavelength shift—200 nm or even more—has taxed even the fertile imagination of the spectroscopists. There is good evidence that the terminal aldehyde group of the retinal is bound by way of a Schiff base linkage to the amino group of a lysine side chain in the protein, and an increase in the length of the π-electron system, with a consequent redshift in the absorption band, would result if a protonated aldimine group were added to the end of the carotenoid system.

Now the electronic spectra of the carotenoids are as well understood as anything in molecular spectroscopy, and certain it is that the extension of the π-system by this much can explain only a small part of the observed shift, as indeed is also experimentally obvious from the spectra of model compounds. Explanations of the spectra of the visual pigments, which directly determine the wavelength-dependence of the visual response, have therefore tended to invoke perturbations, unknown to science in any other context, involving special dispositions of charged groups around the chromophore, massive induced dipoles, and so forth. After all these years of whistling in the dark, an altogether more convincing hypothesis has at last appeared. This is propounded by Mendelsohn, writing in this issue of *Nature* (page 22), and with hindsight, it seems perhaps slightly surprising that this perception has not come sooner.

Mendelsohn has not actually worked with a retina pigment, but rather with the closely related material from the purple membrane of the halophilic bacterium, *Halobacterium halobium*. The chromophore, as in rhodopsin and iodopsin, is 11-*cis* retinal; it has been shown to be attached to the protein through a Schiff base with a lysine amino group, and its absorption spectrum is in essence that of a visual pigment, for which it may therefore be regarded as an analogue. The advantage of this material is that it is much more stable to light, and is not in fact photochemically bleached. This makes it a promising object for study by the resonance Raman technique, which, it has been lately pointed out, offers certain unique advantages in regard to chromophoric biological systems. The principle is that when the exciting line falls in the electronic absorption band, and there are no complications from fluorescence or photochemical lability, Raman-active frequencies of the chromophore are greatly enhanced in intensity, and the spectrum can therefore be examined at very low concentration, and without interference from the contributions of other species, the intensities of which are by comparison infinitesimal.

Thus laser excitation within the absorption band of the purple membrane leads at very low total pigment concentration to a Raman spectrum containing some fifteen peaks, which vanish if the pigment is first bleached by denaturation with cationic detergent. The most prominent band lies at 1,531 cm^{-1} and its relative intensity increases as the excitation wavelength approaches the visible absorption maximum. A second intense band at 1,568 cm^{-1}, on the other hand, becomes more intense when the excitation wavelength shifts towards the blue. It is inferred that the two vibrations are coupled respectively to the longest-wavelength electronic absorption band, and to a transition at shorter wavelength. All other vibrations behave like the 1,531 cm^{-1} band. With the aid of published assignments for the Raman spectra of retinal derivatives, the principal features in the resonance Raman spectrum can be identified, in particular those in the region containing C=C and C=N stretching modes. Comparison of the observed frequencies and their dependence on deuteration, with those of model compounds, makes it clear in the first instance that the chromophore contains an unprotonated Schiff base, which at once annihilates a widely held hypothesis. The most striking observation, however, is that the dominant 1,531 cm^{-1} band has no counterpart in the model compounds, and is surmised therefore to arise from perturbation of the π-electron system with some other element of the protein. Moreover the system is highly sensitive to solvent: addition of chloroform induces a reversible shift of 60 nm in the electronic absorption band, with a corresponding displacement of the intense Raman line.

All this is diagnostic of a charge-transfer interaction, and the best candidate for an electron donor in the protein is, as Szent-Györgyi perceived many years ago, the indole chromophore of tryptophan. Retinal and tryptophan when mixed in solution do indeed give rise to a new long-wavelength absorption band, indicative of the formation of a charge-transfer complex. The conclusion that the absorption band of the visual pigment arises in this way has yet, of course, to be proved, but its attractions and the several hitherto perplexing features of the system for which it at once provides an explanation render it a compellingly attractive proposition. It may moreover give pleasure to those spectroscopists who rallied fifteen years ago to Szent-Györgyi's banner, and set off in pursuit of Submolecular Biology, for it is not often that a charge-transfer complex turns up in nature, without a sprinkling of chloranil or iodine from the hand of the Master.

From our Molecular Biology Correspondent