Price, M., Lemaistre, M., Pischetola, M., Di Lauro, R., & Duboule, D. (1991) *Nature 351*, 748-751.
Qian, Y. Q., Billeter, M., Otting, G., Muller, M., Gehring, W. J., & Wuthrich, K. (1989) *Cell 59*, 573-580.
Rayle, R. (1991) *Dev. Biol. 146*, 255-257.
Regulski, M., Dessain, S., McGinnis, N., & McGinnis, W. (1991) *Genes Dev. 5*, 278-286.
Robert, B., Sassoon, D., Jacq, B., Gehring, W. J., & Buckingham, M. (1989) *EMBO J. 8*, 91-100.
Rosenfeld, M. G. (1991) *Genes Dev. 5*, 897-907.
Ruberti, I., Sessa, G., Lucchetti, S., & Morelli, G. (1991) *EMBO J. 10*, 1787-1791.
Saiga, H., Mizokami, A., Makabe, K. W., Satoh, N., & Mita, T. (1991) *Development 111*, 821-828.
Sasaki, A. W., Doskow, J., MacLeod, C. L., Rogers, M. B., Gudas, L. J., & Wilkinson, M. F. (1991) *Mech. Dev. 34*, 155-164.
Sauer, R. T., Smith, D. L., & Johnson, A. D. (1988) *Genes Dev. 2*, 807-816.
Scott, M. P., & Weiner, A. J. (1984) *Proc. Natl. Acad. Sci. U.S.A. 81*, 4115-4119.
Scott, M. P., Tamkun, J. W., & Hartzell, G. W., III (1989) *Biochim. Biophys. Acta 989*, 25-48.
Schulz, B., Banuett, F., Dahl, M., Schlesinger, R., Schafer, W., Martin, T., Herskowitz, I., & Kahmann, R. (1990) *Cell 60*, 295-306.
Shepherd, J. C., McGinnis, W., Carrasco, A. E., DeRobertis, E. M., & Gehring, W. J. (1984) *Nature 310*, 70-71.
Stern, S. A., Tanaka, M., & Herr, W. (1989) *Nature 341*, 624-630.
Treisman, J., Conczy, P., Vashishtha, M., Harris, E., & Desplan, C. (1989) *Cell 59*, 553-562.
Treisman, J., Harris, E., & Desplan, C. (1991a) *Genes Dev. 5*, 594-604.
Treisman, J., Harris, E., Wilson, D., & Desplan, C. (1991b) *Bioessays* (in press).
Verrijzer, C. P., Kal, A. J., & van der Vliet, P. C. (1990) *Genes Dev. 4*, 1964-1974.
Vincent, J.-P., Kassis, J. A., & O'Farrell, P. H. (1990) *EMBO J. 9*, 2573-2578.
Volhlbrecht, E., Veit, B., Sinha, N., & Hake, S. (1991) *Nature 350*, 241-243.
Voss, J. W., Wilson, L., & Rosenfeld, M. G. (1990) *Genes Dev. 5*, 1309-1320.

Accelerated Publications

Design, Chemical Synthesis, and Expression of Genes for the Three Human Color Vision Pigments†

Daniel D. Oprian,* Ana B. Asenjo,‡ Ning Lee,‡ and Sandra L. Pelletier

Graduate Department of Biochemistry, Brandeis University, Waltham, Massachusetts 02254

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ABSTRACT: Color vision in humans is mediated by three pigments from retinal cone photoreceptor cells: blue, green, and red. We have designed and chemically synthesized genes for each of these three pigments. The genes were expressed in COS cells, reconstituted with 11-*cis*-retinal chromophore, and purified to homogeneity using an immunoaffinity procedure. To facilitate the immunoaffinity purification, each pigment was modified at the carboxy terminus to contain an additional eight amino acid epitope for a monoclonal antibody previously used to purify bovine rhodopsin. The spectra for the isolated pigments had maxima of 424, 530, and 560 nm, respectively, for the blue, green, and red pigments. These maxima are in excellent agreement with the maxima previously observed by microspectrophotometry of individual human cone cells. The spectra are the first to be obtained from isolated human color vision pigments. They confirm the original identification of the three color vision genes, which was based on genetic evidence [Nathans, J., Thomas, D., & Hogness, D. S. (1986) *Science 232*, 193].

Human color vision is mediated by three visual pigments present in retinal cone photoreceptor cells [cf. Boynton (1979)]. The spectra for these pigments have been determined by microspectrophotometry of individual human cone cells (Dartnall et al., 1983). The spectra show absorption maxima at 420, 530, and 560 nm for the blue, green, and red cone pigments, respectively. Despite their very different absorption maxima, these pigments all contain an identical 11-*cis*-retinal chromophore covalently attached to the protein by means of a Schiff base linkage to the ϵ-amino group of a conserved lysine residue. Thus, the different absorption maxima for the pigments arise from differences in the amino acid sequence of the individual proteins and in the interaction of these amino acids with the chromophore.

Several years ago, Nathans et al. (1986a,b) cloned the genes for the blue, green, and red pigments using a homologous rhodopsin probe. The genes were identified by the following genetic criteria.

Blue Gene. Only one gene was localized to an autosome, chromosome 7. This gene was concluded to be that of the blue

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* To whom correspondence should be addressed.
‡ Biology Graduate Program, Brandeis University.

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pigment since blue color vision deficiencies are known to be inherited autosomally.

Green and Red Genes. Two of the three genes were localized to the X chromosome. These were identified as green and red because inherited green/red color vision deficiencies are known to be X-linked. Discrimination between the green and red genes was based on genomic Southern blots from individuals with known color vision deficiencies.

The identification of the color vision genes rests largely on indirect evidence. A demonstration that these three genes code for proteins with the expected spectral properties has not hitherto been provided. We present here the first direct demonstration that the blue, green, and red genes do in fact code for proteins that, when reconstituted with 11-*cis*-retinal, have absorption maxima of 424, 530, and 560 nm, respectively.

Experimental Procedures

Materials. 11-*cis*-Retinal was the generous gift of Peter Sorter and Hoffman-LaRoche (Nutley, NJ). CHAPS,[1] egg yolk PC (type XI-E), HEPES, Sepharose 4B, DTT, and PMSF were from Sigma (St. Louis, MO). Dodecyl β-D-maltoside was from Calbiochem (La Jolla, CA). Peptide I (Asp-Glu-Ala-Ser-Thr-Thr-Val-Ser-Lys-Thr-Glu-Thr-Ser-Gln-Val-Ala-Pro-Ala) was purchased from American Peptide Co., Inc. (Santa Clara, CA). The monoclonal antibody rho 1D4 (Molday & MacKenzie, 1983; MacKenzie et al., 1984) was purified from 20 L of hybridoma culture medium by $(NH_4)_2SO_4$ fractionation and DEAE-cellulose chromatography, according to standard protocols as have been described (Oprian et al., 1987). The rho 1D4 antibody was coupled to the Sepharose 4B solid support by the method of Cuatrecasas (1970).

Design and Chemical Synthesis of the Human Color Vision Genes. The principles that were used in the design of the genes were discussed in previous publications (Ferretti et al., 1986; Oprian et al., 1986). The overriding consideration was to introduce a large number of unique restriction sites into the gene sequence to facilitate later mutagenesis studies. This was accomplished without altering the amino acid sequence of the proteins by taking advantage of the degeneracy inherent in the genetic code.

The reagents and procedures used for chemical synthesis of the color vision genes were essentially as those used for synthesis of the genes for bovine rhodopsin (Ferretti et al., 1986; Oprian et al., 1986) and β_2-adrenergic receptor (McPhee and Oprian, manuscript in preparation).

Expression of the Color Vision Genes. The genes were expressed in COS cells following transfection with DEAE-dextran, virtually as has been described for expression of the bovine rhodopsin gene (Oprian et al., 1987; Franke et al., 1988; Zhukovsky & Oprian, 1989; Zhukovsky et al., 1991). Cells were harvested 72 h posttransfection for reconstitution and purification of the pigments. The level of expression is 2–3-fold less than that achieved with rhodopsin. This corresponds to about 1 μg/100-mm culture dish (a 100-mm culture dish contains about 1×10^7 cells and 2 mg of total protein).

Reconstitution and Purification of the Pigments. The following procedure was used for the green and red pigments. Unless specifically noted to the contrary, all procedures were performed at 4 °C or on ice. Typically, a single protein purification began with 10–20 culture dishes (100-mm diameter) of transfected COS cells. The cells were washed twice while still attached to the plates with 5 mL/plate of buffer P, which contained 50 mM HEPES, pH 6.6, 140 mM NaCl, and 1 mM DTT. The cells were harvested by adding 1 mL/plate of buffer P and then scraping with a rubber policeman, followed by collection in a clinical centrifuge (3 min at approximately 1000*g*). The cell pellet was washed by resuspending the cells and then pelleting in the clinical centrifuge two additional times with 1 mL/plate of buffer P. The cells were then incubated for 30 min with 20 μM 11-*cis*-retinal in buffer P (1 mL/plate). The incubation with retinal and all subsequent procedures were performed in the dark or under dim red illumination (15-W bulb with a Kodak No. 2 Safelight filter).

The proteins were solubilized from cell membranes essentially as has been described by Okano et al. (1989) for the resolution and purification of chicken visual pigments. Cells were collected by centrifugation, and the pellet was resuspended in 1 mL/plate of buffer S (buffer P containing 0.75% CHAPS, 0.8 mg/mL PC, 1 mM $MnCl_2$, 1 mM $CaCl_2$, and 1 mM PMSF). Membranes were allowed to solubilize for 1 h. The suspension was then spun in a clinical centrifuge to remove nuclei and incubated with rho 1D4–Sepharose 4B as has been described for rhodopsin (Oprian et al., 1987) except that buffer S was used throughout the purification. The pigments were eluted from the column with 370 μL of 50 μM peptide I in buffer S.

The procedure used for the blue pigment was essentially identical to that described for the green and red except that dodecyl maltoside was used instead of CHAPS/PC for solubilization and purification of the protein. The initial solubilization was in 1% dodecyl maltoside; subsequent washing and elution of the immunoaffinity matrix was in 0.1% dodecyl maltoside.

Absorption Spectroscopy. UV/visible absorption spectra were recorded on samples eluted from the rho 1D4–Sepharose 4B immunoaffinity matrix using an Hitachi Model U-3210 spectrophotometer that was specifically modified by the manufacturer for use in a dark room. Data were acquired with the aid of an Everex System 1700 microcomputer using Spectra Calc software from Galactic Industries Corp. (Salem, NH). All spectra were recorded on samples of 1.0-cm path length in thermostated cell holders with the temperature maintained at 4 °C. Difference spectra were determined from samples before and after bleaching in the presence of 100 mM hydroxylamine (Wald, 1968; Wald et al., 1955).

Results

Design and Chemical Synthesis of the Genes. Genes for each of the three color vision pigments were designed such that they coded for the naturally occurring amino acid sequence of the proteins inferred from cDNA clones (Nathans et al., 1986a). The genes for each pigment contained, in addition to the wild-type sequence, 8 amino acids at the carboxy terminus derived from the sequence of rhodopsin. These eight additional amino acids, Glu-Thr-Ser-Gln-Val-Ala-Pro-Ala, correspond exactly to the carboxy terminus of rhodopsin and are known to be the epitope for the monoclonal antibody rho 1D4 (MacKenzie et al., 1984) used previously for purification of rhodopsin from transfected COS cells. We knew from previous studies that this epitope could be placed on heterologous proteins and used for their purification with the rho 1D4 antibody (McPhee and Oprian, manuscript in preparation). Therefore, we placed the 8 amino acid epitope at the carboxy terminus of each pigment to allow their purification from COS cells. The spectra reported in this paper were obtained from pigments containing the rho 1D4 epitope.

[1] Abbreviations: CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate; PC, L-α-phosphatidylcholine; HEPES, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid; DTT, DL-dithiothreitol; PMSF, phenylmethanesulfonyl fluoride.

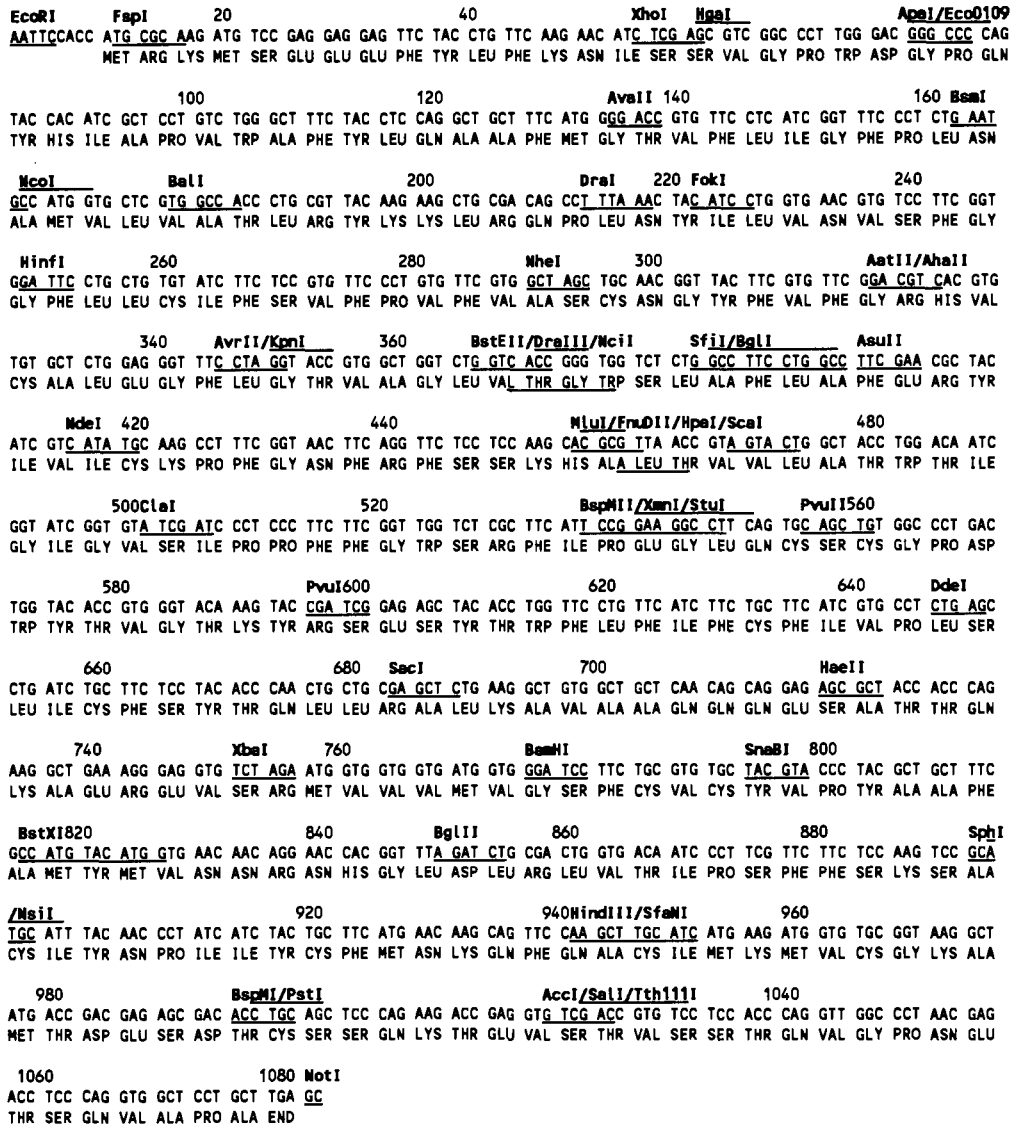

FIGURE 1: Nucleotide and corresponding amino acid sequence for the synthetic blue gene. The DNA sequence shown is for the coding strand. The corresponding amino acid sequence is also indicated. Numbers refer to the nucleotide sequence beginning with the first A of the 5′-flanking *Eco*RI site. The gene was designed as an *Eco*RI–*Not*I restriction fragment containing 51 unique restriction sites within the coding sequence. The restriction sites are indicated by bold type above the sequence and by horizontal lines demarcating the recognition sequence. The gene contains the complete coding sequence for the wild-type pigment plus an additional eight codons at the carboxy terminus corresponding to the rho 1D4 epitope.

The sequences for the blue, green, and red genes are shown in Figures 1 and 2. The salient features of each are summarized as follows.

Blue Gene. The sequence for the blue gene is shown in Figure 1. It was constructed as an *Eco*RI–*Not*I restriction fragment. The gene is 1080 bp in length, including codons for the rho 1D4 epitope. The protein encoded by this gene is 356 amino acids in length. There are 51 unique restriction sites within the coding sequence that define restriction fragments for mutagenesis. The gene was assembled from a total of 38 oligonucleotides.

Green and Red Genes. The green and red pigments are highly homologous, differing by only 15 amino acids out of a total of 364. The sequences for the green and red genes are shown in Figure 2. They were constructed as *Eco*RI–*Not*I restriction fragments. The genes are 1130 bp in length, including codons for the rho 1D4 epitope. The proteins encoded by these genes are 372 amino acids in length. There are a total of 45 unique restriction sites in each sequence that define restriction fragments for mutagenesis. The green and red sequences are very similar and contain with one exception the same complement of unique restriction sites. The one position different in the two genes is at about nucleotide 900, where the green gene has a *Kpn*I site and the red gene has an *Nsi*I site. Each gene was assembled from a total of 44 oligonucleotides.

The DNA sequence of all three genes was confirmed by sequence analysis of both strands using the dideoxy method (Sanger et al., 1977).

Absorption Spectra of the Isolated Color Vision Pigments. The synthetic genes were inserted into a modified version of the expression vector pMT-2 (Franke et al., 1988) and then transfected into COS cells for production of the proteins. After reconstitution with 11-*cis*-retinal and purification using the rho 1D4 immunoaffinity matrix, the pigments were placed in a spectrophotometer for a determination of their absorption spectra. The spectrum recorded for the blue pigment is shown in Figure 3A, and the spectra for the green and red pigments are shown in Figure 3B. Due to interference from light scattering at shorter wavelengths, the absorption maximum for the blue pigment was determined from a difference spectrum (Figure 3A, inset). This was not necessary for the green and red pigments. As is shown, the maxima are 424, 530, and 560 nm for the blue, green, and red pigments, respectively.

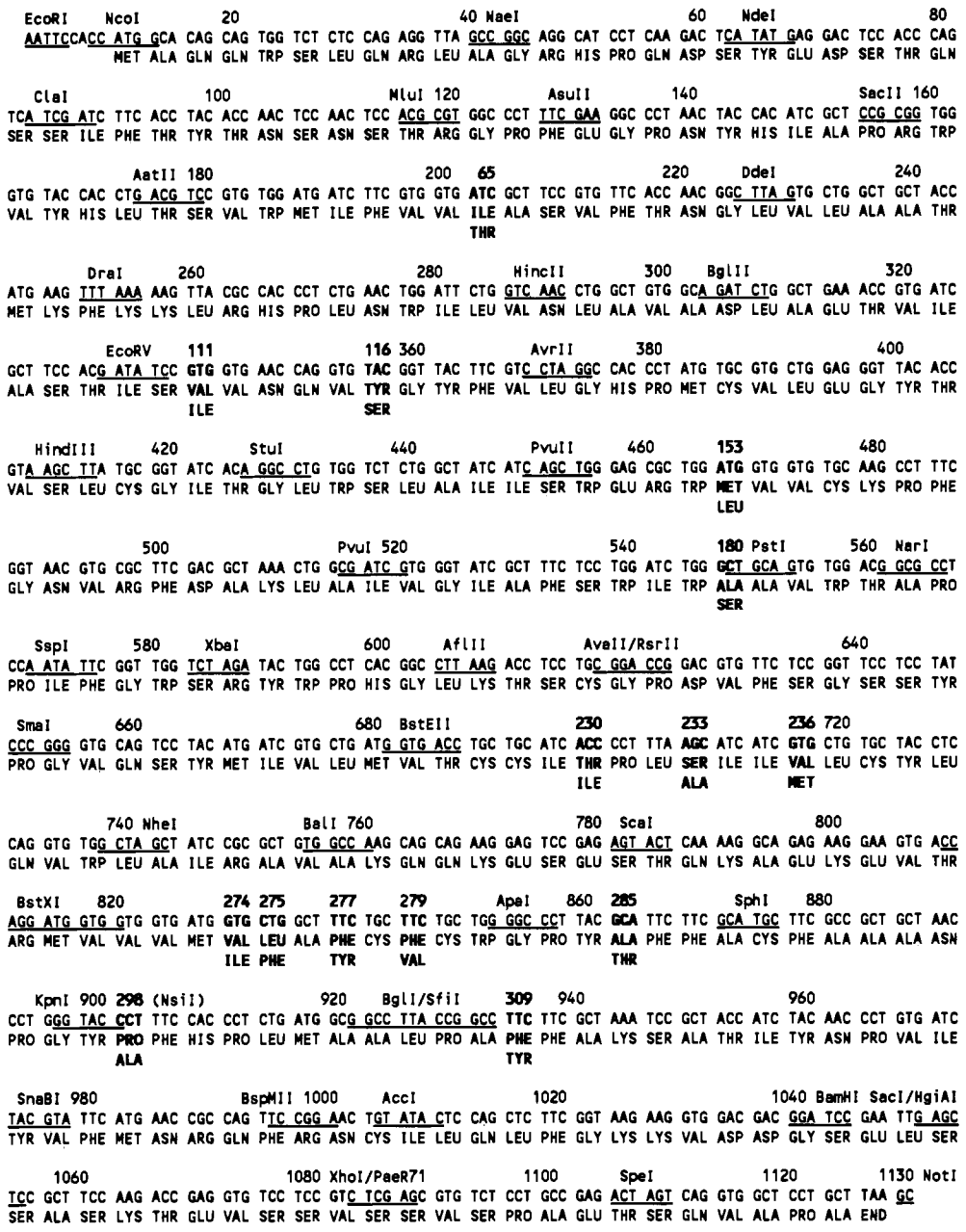

FIGURE 2: Nucleotide and corresponding amino acid sequence for the synthetic green and red genes. The DNA sequence shown is for the coding strand of the green gene. The corresponding amino acid sequence is also indicated. The number, codon, and amino acid for each of the 15 positions that are different between the two proteins are highlighted by bold type, with the amino acid of the red pigment indicated below that of the green pigment. The codons used in the red gene for the 15 different amino acids are as follows: Thr^{65}, ACA; Ile^{111}, ATC; Ser^{116}, AGC; Leu^{153}, CTG; Ser^{180}, TCT; Ile^{230}, ATC; Ala^{233}, GCT; Met^{236}, ATG; Ile^{274}, ATC; Phe^{275}, TTC; Tyr^{277}, TAC; Val^{279}, GTG; Thr^{285}, ACC; Ala^{298}, GCA; Tyr^{309}, TAC. The numbers shown that are not in bold type refer to the nucleotide sequence beginning with the first A of the 5′-flanking *Eco*RI site. The genes were designed as *Eco*RI–*Not*I restriction fragments containing 45 unique restriction sites within the coding sequence. The restriction sites are as indicated. The *Kpn*I site at position 900 in the green gene is not present in the red; instead, the red gene contains an *Nsi*I site (shown in parentheses). The genes contain the complete coding sequence for the wild-type pigments plus an additional eight codons at the carboxy terminus corresponding to the rho 1D4 epitope.

These values are in very good agreement with the maxima expected on the basis of spectra determined by microspectrophotometry of individual human cone cells (Dartnall et al., 1983). These are the first spectra reported for isolated human pigments and provide a direct demonstration that the genes previously identified for the blue, green, and red pigments do in fact code for the blue, green, and red pigments.

Discussion

Rhodopsin, the visual pigment from rod photoreceptor cells, is a stable protein that is easily isolated in abundance from bovine retina. Consequently, a great deal is known about the biochemical and spectral properties of rhodopsin (Wald, 1968; Dratz & Hargrave, 1983). In contrast, much less is known about cone cell pigments. Iodopsin, the red pigment from chicken cone cells, was originally isolated in soluble form by Wald (Wald et al., 1955). This protein has a similar absorption maximum to the human red pigment and shares about 80% homology in amino acid sequence with the human protein (Kuwata et al., 1990; Tokunaga et al., 1990). Iodopsin has been resolved from the other chicken cone pigments (Fager & Fager, 1980; Yen & Fager, 1984) and purified to homogeneity (Okano et al., 1989). With the exception of iodopsin, very little is known of other cone cell pigments. The human cone cell pigments (color vision pigments) have never before been isolated. We have provided here the first spectra recorded

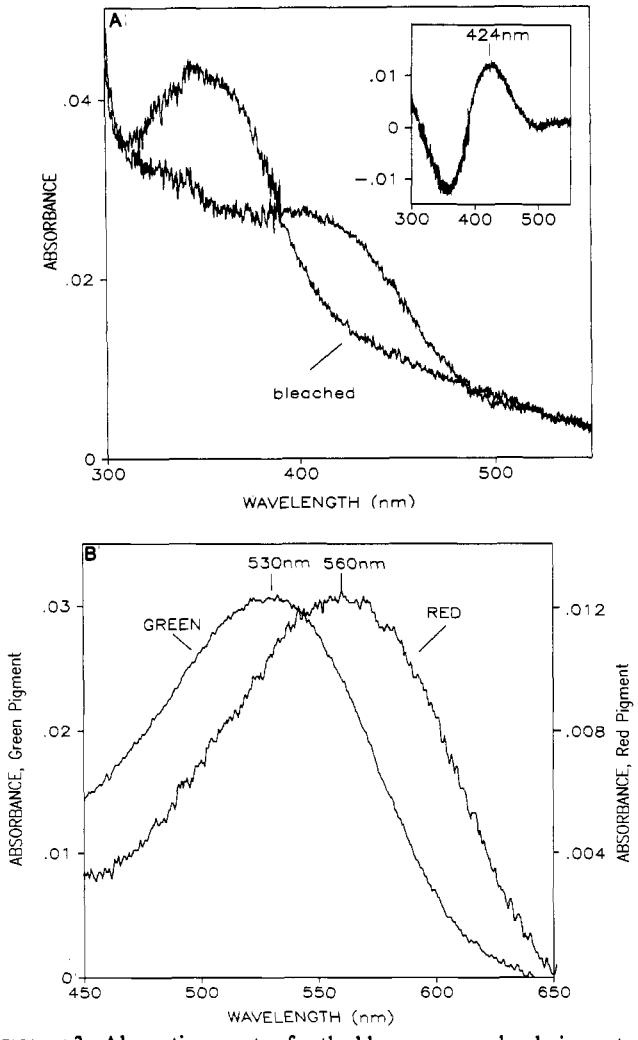

FIGURE 3: Absorption spectra for the blue, green, and red pigments. (A) Absorption spectrum for the blue pigment. The figure shows the spectrum obtained from a sample of the blue pigment that had been purified from 20 plates of transfected COS cells. Due to the short-wavelength maximum of this pigment, the spectrum is much more affected by light scattering than are those of the green and red pigments. For this reason, the absorption maximum of the blue pigment was determined from a difference spectrum. After the absorption spectrum for the isolated blue pigment was recorded, the sample was bleached in the presence of 100 mM hydroxylamine and the bleached spectrum was recorded. Inset: difference spectrum obtained by subtracting the bleached spectrum from the one obtained prior to bleaching. The absorption maximum is 424 nm. (B) Absorption spectra for the green and red pigments. These spectra were obtained from samples of the pigments that were purified from 20 plates of transfected COS cells. The absorption maximum of the green pigment is 530 nm, while that of the red pigment is 560 nm.

from isolated human color vision pigments. These spectra confirm the original identification of the genes by Nathans et al. (1986a,b). The absorption maxima are in excellent agreement with those determined by Dartnall et al. (1983) using microspectrophotometry of individual human cone cells. This was reassuring since it was certainly conceivable that the extra eight amino acids of the rho 1D4 epitope on the carboxy terminus of the pigments may have affected their spectral properties.

The use of this epitope for purification of heterologous proteins may be generally applicable. In addition to the color pigments, we have used it to purify a synthetic β_2-adrenergic receptor from transfected COS cells (McPhee and Oprian, manuscript in preparation). The modified receptor has ligand binding properties that are indistinguishable from the wild type, and it activates adenylate cyclase in transfected CHO cell lines as would be expected of the wild-type protein. We note, however, that the purification is not as efficient as it is for rhodopsin. Typically, we lose about 50% of the protein because of inefficient binding to the immunoaffinity column (not shown), whereas with rhodopsin we recover almost 100% of the expressed protein (Oprian et al., 1987). Presumably, this reflects a slightly lower affinity of the antibody for the epitope when it is placed in a foreign context. A longer sequence of amino acids from the carboxy terminus of rhodopsin does not improve the results. We have tried adding 12 amino acids, instead of 8, to the green and red proteins, but we found no difference in yield of purified protein (not shown). The longer epitope was also without effect on the position of absorption maxima. The loss resulting from the lower binding affinity might be alleviated by preparing a more concentrated extract from the COS cells.

Our future efforts will be directed toward understanding the mechanism of wavelength regulation in the color vision pigments. The three pigments contain an identical 11-*cis*-retinal chromophore, and yet they absorb light with very different maxima. The different spectral properties are brought about by specific interactions of the chromophore with the proteins (Neitz et al., 1991). Site-directed mutagenesis is a promising approach to the analysis of these interactions and their relative roles in wavelength modulation. Previous mutagenesis studies using rhodopsin as a model for the color vision pigments have met with little success in terms of identifying the amino acids in cone pigments that are involved in wavelength modulation (Zhukovsky & Oprian, 1989; Nathans, 1990). The availability of the three synthetic genes and the ability to express them in functional form will greatly facilitate the progress of this work. This experimental system will also greatly facilitate studies of red/green color vision deficiencies, which are thought to arise from hybrid pigments resulting from unequal homologous recombination of the red and green genes (Nathans et al., 1986b; Piantanida, 1988; Neitz et al., 1989).

REFERENCES

Boynton, R. M. (1979) *Human Color Vision*, Holt, Rinehart and Winston, New York.

Cuatrecasas, P. (1970) *J. Biol. Chem. 245*, 3059–3065.

Dartnall, H. J. A., Bowmaker, J. K., & Mollon, J. D. (1983) *Proc. R. Soc. London, B 220*, 115–130.

Dratz, E. A., & Hargrave, P. A. (1983) *Trends Biochem. Sci. 8*, 128–131.

Fager, L. Y., & Fager, R. S. (1980) *Vision Res. 21*, 581–586.

Ferretti, L., Karnik, S. S., Khorana, H. G., Nassal, M., & Oprian, D. D. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 599–603.

Franke, R. R., Sakmar, T. P., Oprian, D. D., & Khorana, H. G. (1988) *J. Biol. Chem. 263*, 2119–2122.

Kuwata, O., Imamoto, Y., Okano, T., Kokame, K., Kojima, D., Matsumoto, H., Morodome, A., Fukada, Y., Shichida, Y., Yasuda, K., Shimura, Y., & Yoshizawa, T. (1990) *FEBS Lett. 272*, 128–132.

MacKenzie, D., Arendt, A., Hargrave, P., McDowell, J. H., & Molday, R. S. (1984) *Biochemistry 23*, 6544–6549.

Molday, R. S., & MacKenzie, D. (1983) *Biochemistry 22*, 653–660.

Nathans, J. (1990) *Biochemistry 29*, 937–942.

Nathans, J., Thomas, D., & Hogness, D. S. (1986a) *Science 232*, 193–202.

Nathans, J., Piantanida, T. P., Eddy, R. L., Shows, T. B., & Hogness, D. S. (1986b) *Science 232*, 203–210.

Neitz, J., Neitz, M., & Jacobs, G. H. (1989) *Nature 342*, 679–682.

Neitz, M., Neitz, J., & Jacobs, G. H. (1991) *Science 252*, 971-974.
Okano, T., Fukada, Y., Artamonov, I. D., & Yoshizawa, T. (1989) *Biochemistry 28*, 8848-8856.
Oprian, D. D., Nassal, M., Ferretti, L., Karnik, S. S., & Khorana, H. G. (1986) in *Protein Engineering: Applications in Science, Medicine, and Industry* (Inouye, M., & Sarma, R., Eds.) pp 111-123, Academic Press, Inc., New York.
Oprian, D. D., Molday, R. S., Kaufman, R. J., & Khorana, H. G. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 8874-8878.
Piantanida, T. (1988) *Trends Genet. 4*, 319-323.
Sanger, F., Nicklen, S., & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 5463-5467.
Tokunaga, F., Iwasa, T., Miyagishi, M., & Kayada, S. (1990) *Biochem. Biophys. Res. Commun. 173*, 1212-1217.
Vollrath, D., Nathans, J., & Davis, R. W. (1988) *Science 240*, 1669-1672.
Wald, G. (1968) *Science 162*, 230-239.
Wald, G., Brown, P. K., & Smith, P. H. (1955) *J. Gen. Physiol. 38*, 623-681.
Yen, L., & Fager, R. S. (1984) *Vision Res. 24*, 1555-1562.
Zhukovsky, E. A., & Oprian, D. D. (1989) *Science 246*, 928-930.
Zhukovsky, E. A., Robinson, P. R., & Oprian, D. D. (1991) *Science 251*, 558-560.

Photolysis Intermediates of Human Rhodopsin†

J. W. Lewis,‡ F. J. G. M. van Kuijk,§ T. E. Thorgeirsson,‡ and D. S. Kliger*,‡

Department of Chemistry and Biochemistry, University of California, Santa Cruz, California 95064, and Department of Chemistry and Biochemistry, Montana State University, Bozeman, Montana 59717

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ABSTRACT: Photochemical studies were conducted on human rhodopsin at 20 °C to characterize the intermediates which precede the formation of metarhodopsin II, the trigger for the enzyme cascade mechanism of visual transduction. Human rhodopsin was prepared from eyes which had previously been used for corneal donations. Time resolved absorption spectra collected from 10^{-8} to 10^{-6} s after photolysis of human rhodopsin in detergent suspensions displayed biexponential decay kinetics. The apparent lifetimes obtained from the data are 65 ± 20 and 292 ± 25 ns, almost a factor of 2 slower than the corresponding rates in bovine rhodopsin. The spectra can be fit well using a model in which human bathorhodopsin decays toward equilibrium with a blue-shifted intermediate (BSI) which then decays to lumirhodopsin. Spectra and kinetic rate constants were determined for all these intermediates using a global analysis which showed that the spectra of the human intermediates are remarkably similar to bovine intermediates. Microscopic rate constants derived from this model are 7.4×10^6 s^{-1} for bathorhodopsin decay and 7.5×10^6 s^{-1} and 4.6×10^6 s^{-1} for the forward and reverse reactions of BSI, respectively. Decay of lumirhodopsin to later intermediates was studied from 10^{-6} to 10^{-1} s after photolysis of rhodopsin in human disk membrane suspensions. The human metarhodopsin I ⇄ metarhodopsin II equilibrium appears to be more forward shifted than in comparable bovine studies.

Rhodopsin, the sensory pigment of scotopic vision, is the most successfully characterized receptor protein, particularly in its bovine form. Extending this level of understanding to human rhodopsin is important because mutations affecting the rhodopsin amino acid sequence have recently been shown to cause human disease. It thus becomes vital to characterize human rhodopsin so that the defects in health-related variants can be understood mechanistically. It is also interesting to compare rhodopsins with different sequences because this photoreceptor protein has emerged as a model for other more elusive membrane receptors, strongly posing the question of how amino acid sequence affects membrane protein function. While bovine rhodopsin has been extensively characterized, previous studies of human rhodopsin have been confined to the slower processes occurring after the signal initiated by light absorption has been transmitted to other proteins in the rod outer segment (ROS).[1] Here we characterize the more rapid stages which lead to the activated form of human rhodopsin which triggers the enzyme cascade in the human eye.

Dramatic spectral changes which follow exposure of rhodopsin to light attracted attention as early as the 19th century (Kuhne, 1878). Modern time-resolved spectral studies have expanded our knowledge of these changes and have shown that photolysis leads to a number of discrete intermediate states of the protein (Ottolenghi & Sheves, 1989; Birge, 1990). The importance of understanding the mechanism of rhodopsin function has gained even wider significance in recent years, first because at least one form of retinitis pigmentosa has been shown to be caused by a variety of point defects in the rhodopsin gene (Dryja et al., 1990, 1991; Heckenlively et al., 1991; Ingelhearn et al., 1991; Sung et al., 1991) and second because

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* Author to whom correspondence should be addressed.
‡ University of California.
§ Montana State University.

[1] Abbreviations: BSI, blue-shifted intermediate; EDTA, ethylenediaminetetraacetic acid; ROS, rod outer segments; TBS, Tris-buffered saline.

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