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A Study of the Binding Site Requirements of Rhodopsin Using Isomers of α-Retinal and 5-Substituted α-Retinal Analogs

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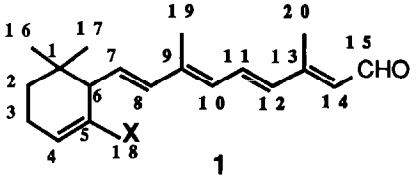
Results of the binding interaction of isomers of α-retinal and six substituted α-retinals (5-butyl, 5-phenyl, 5-isopropyl, 5-heptyl, 5-decyl, and 10-fluoro) with bovine opsin are reported. Their implications on the binding site requirements are discussed. © 1989 Academic Press, Inc.

INTRODUCTION

The basic stratagem of structurally modifying the retinal chromophore in rhodopsin in order to probe for specific protein/substrate interactions as well as to study photochemical properties has been widely applied with richly rewarding results. A number of comprehensive reviews on this broad subject are now available (*1*).

Because of our longstanding interest in the use of isomers of retinal and its analogs as models for the generation of a detailed topological map of the binding cavity of rhodopsin (*2*), we undertook the preparation of specifically modified retinal analogs that would address a previously neglected region of the binding site, namely the available space (if any) in the vicinity of the C-5 position of the cyclohexenyl ring. Due to synthetic expediency, we incorporated into our study the shifting of the ring double bond from the C-5 position (β-isomer, **2**) to the C-4 position (α-isomer, **1**). Hence the following α-retinal analogs (**1a–g**) were prepared and reactions of the isomers with bovine opsin examined. The study expands the sparse information available in the literature on the α-retinal system (*3*).



1

a. X = CH_3
b. X = n-C_4H_9
c. X = C_6H_5
d. X = i-Pr
e. X = n-C_7H_{15}
f. X = n-$C_{10}H_{21}$
g. X = CH_3, 10F

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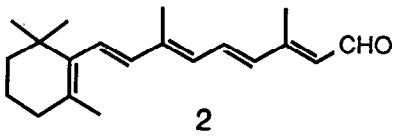

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The expected change of the ring/chain conformational relation between the α- and the β-isomer requires a closer examination of structural relation between the two retinals. We have, therefore, carried out in parallel a comparative structural study between the two forms of all-*trans*-retinal. This paper summarizes results of these investigations.

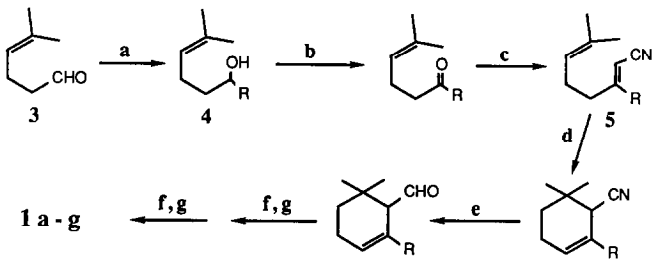
EXPERIMENTAL

Materials. Synthesis of α-retinal analogs. Well-established experimental methodologies for the preparation of normal β-homologs of retinal were directly adapted for the synthesis of the compounds employed in this study as outlined under Results. Commercially available starting materials and reagents were used throughout. Solvents were purified and dried in the usual manner. Structural and configurational assignments were based on both uv (hexane and/or ethanol) and NMR spectroscopy ($CDCl_3$ or acetone-d_6, TMS). Photoisomerization of the α-retinals was carried out according to established procedures involving irradiation in different solvents (*4*) or with a silica gel slurry (*5*).

Methods. X-ray crystal structural studies of α-retinal were conducted on an Enraf-Nonius CAD4 diffractometer using a crystal of 0.40 × 0.40 × 0.30 mm. A total of 2190 reflections was collected. Structural solution and refinement involved the use of direct methods conducted with the aid of a MicroVAX II computer and the SDPVAX (Enraf-Nonius & B. A. Frenz & Associates, Inc.) software. The coordinates of atoms in α-retinal obtained from its crystal structure (Table 1) were used for calculation of the minimized structure of all-*trans*-α-retinal by employing a molecular mechanics program modified for π-systems (MMP2-85) (*6*). A microGRAF V molecular modeling package (Chemical Design Co.) was employed for dihedral angle drive and structural matching studies between α- and β-retinal. The minimized structure of the latter obtained by the same method was reported recently (*7*). Procedures for opsin isolation and formation of rhodopsin analogs were essentially those reported before (*8*). The uv–vis spectra were recorded on a P.E. −λ5 spectrometer interfaced with a PE-2500 data station. NMR spectra were recorded on a NR-80 or a GE-QE300 spectrometer.

RESULTS

Retinal analogs. The preparation of retinal analogs **1a–g** was carried out according to the following straightforward nonselective reaction sequence.



a. RMgX; b. CrO_3–H_2SO_4, ether; c. $(EtO)_2\overset{\overset{O}{\|}}{P}CH_2CN$; d. CH_3SO_3H, CH_2Cl_2;
e. DIBAL; f. $(EtO)_2\overset{\overset{O}{\|}}{P}CH_2(CH_3)C = CHCN$, LDA, THF; g. DIBAL.

The introduction of the various substituents at 5-position was accomplished early in the sequence by the reaction of an appropriate organometallic reagent with

TABLE 1

Coordinates of Carbon and Oxygen Atoms of all-*trans*-α-Retinal from Its Crystal Structure and Their Estimated Standard Deviations[a]

Atom	*x*	*y*	*z*	B(Å^2)
C1	0.7985(4)	0.1427(3)	0.1047(2)	6.89(9)
C2	0.9537(5)	0.2191(3)	0.1138(2)	8.4(1)
C3	1.1160(5)	0.1681(3)	0.0993(2)	9.0(1)
C4	1.1400(4)	0.0630(3)	0.1296(2)	7.8(1)
C5	1.0231(4)	0.0090(2)	0.1547(2)	6.39(8)
C6	0.8478(4)	0.0523(2)	0.1591(2)	5.56(7)
C7	0.8476(4)	0.0796(2)	0.2391(2)	5.34(7)
C8	0.7830(4)	0.0196(2)	0.2856(2)	5.47(7)
C9	0.7882(3)	0.0387(2)	0.3644(2)	4.99(7)
C10	0.7375(4)	−0.0363(2)	0.4056(2)	5.48(7)
C11	0.7315(3)	−0.0333(2)	0.4831(2)	5.32(7)
C12	0.6821(4)	−0.1139(2)	0.5208(2)	5.78(8)
C13	0.6694(3)	−0.1137(2)	0.5981(2)	5.56(7)
C14	0.6184(4)	−0.2025(3)	0.6271(2)	6.65(8)
C15	0.5964(5)	−0.2166(3)	0.7034(2)	8.6(1)
C16	0.6415(5)	0.2001(4)	0.1211(2)	10.7(1)
C17	0.7583(6)	0.0986(4)	0.0249(2)	9.9(1)
C18	1.0560(5)	−0.1011(3)	0.1818(2)	8.9(1)
C19	0.8483(4)	0.1421(3)	0.3957(2)	6.21(8)
C20	0.7116(4)	−0.0171(3)	0.6420(2)	7.13(9)
O21	0.5524(3)	−0.2984(2)	0.7262(2)	11.80(8)

[a] Cell constants and space group: a = 7.919(2) Å, b = 12.972(2) Å, c = 18.357(2) Å, β = 101.12(2), $\bar{x}$ = 4. Radiation used: Cu$K\alpha$.

TABLE 2

Initial Product Distribution in Photoisomerization of all-*trans*-α-Retinal

Solvent[a]	Initio product ratio 13-*cis* : 11-*cis* : 9-*cis*[b]
Silica gel slurry	44 : 53 : 3
Methanol	47 : 50 : 3
Ethanol	51 : 46 : 3
CH_3CN	62 : 27 : 11
Hexane	73 : 3 : 24

[a] Deoxygenated solutions.

[b] Less than 10% conversion. Corrected for different extinction coefficients at 360 nm, the HPLC detecting wavelength.

aldehyde **3**. Oxidation of the resultant alcohol **4** by the method of Brown *et al.* (*9*) followed by an Emmons reaction with diethylphosphonoacetonitrile afforded dienonitrile **5** which was cyclized (methanesulfonic acid) and subsequently reduced (DIBAL) to the requisite cyclocitral analog. The synthetic route adopted for this study was regiospecific with respect to the introduction of the intended C_5-substituent. However, an isomeric mixture was obtained upon subsequent ring closure with the α-form predominating and minor amounts of the β- and γ-forms. The lack of regioselectivity in the construction of the α-retinal analog was in part offset by their separation and isolation in isomerically pure form by conventional normal phase HPLC.

The syntheses were completed in the usual manner by the application of two successive C_5-chain extension reactions (phosphonosenecionitrile and LDA in THF, FC purification followed by DIBAL reduction) (*4*). In this manner the final synthetic mixtures consisted of varying ratios of the all-*trans* 9-*cis*, 13-*cis*, and 9,13-di*cis* isomers in their α-, β-, and γ-forms. The all-*trans* form was first purified by preparative HPLC.

The 11-*cis* isomer necessary for binding interaction was formed by photoirradiation of the all-*trans* isomer. Unlike the β-series, irradiation in a silica gel slurry (*5*), instead of acetonitrile, was found to give the highest amount of 11-*cis*-α-retinal in the photostationary state mixture (53%). The initial ratios of isomers formed are listed in Table 2.

Individual isomers were separated and purified by preparative HPLC prior to regeneration experiments. Their spectral data are listed in Tables 3 and 4. The uv–vis absorption spectra for isomers of α-retinal are shown in Fig. 1. They are typical of α-retinals: all exhibiting fine structure, but less for the nonplanar 11-*cis*.

Binding interaction with bovine opsin. Artificial pigment analogs were prepared by standard methods (*8*) involving incubation of the requisite retinal analog with digitonin-solubilized opsin at ambient temperature for 0.5–2 h. The absorption properties for the pigment analogs are listed in Table 4 together with their relative

TABLE 3

^{1}H NMR Data of Isomers of α-Retinal and 5-Substituted α-Retinals[a]

Compound	H_4	H_7	H_8	H_{10}	H_{11}	H_{12}	H_{14}	H_{15}	$J_{7,8}$	$J_{10,11}$	$J_{11,12}$
all-*trans*-**1a**	5.45	5.72	6.15	6.18	7.12	6.38	5.99	10.11	15.4	11.3	15.1
13-*cis*-**1a**	5.51	5.72	6.17	6.21	7.02	7.29	5.86	10.22	15.6	11.0	14.9
11-*cis*-**1a**	5.45	5.70	6.15	6.53	6.68	5.93	6.11	10.12	15.5	12.6	11.5
9-*cis*-**1a**	5.47	5.71	6.63	6.06	7.26	6.31	5.99	10.14	15.3	11.5	15.1
9,13-di*cis*-**1a**	5.47	5.72	6.63	6.09	7.20	7.18	5.87	10.22	15.3	10.0	11.8
9,11-di*cis*-**1a**	5.46	5.72	6.64	6.38	6.81	5.89	6.09	10.11	15.3	12.3	11.8
11,13-di*cis*-**1a**	5.43	5.64	6.05	6.17	6.74	6.09	5.99	9.71	15.4	11.8	10.7
all-*trans*-**1b**	5.43	5.72	6.14	6.20	7.13	6.38	5.99	10.13	15.3	10.4	15.1
13-*cis*-**1b**	5.44	5.72	6.15	6.21	7.02	7.29	5.86	10.23	15.4	11.3	14.8
11-*cis*-**1b**	5.43	5.70	6.13	6.52	6.67	5.93	6.11	10.12	15.7	12.2	11.7
9-*cis*-**1b**	5.46	5.72	6.62	6.05	7.24	6.31	5.99	10.14	15.2	11.6	15.0
9,13-di*cis*-**1b**	5.46	5.73	6.61	6.09	[c]	[c]	5.86	10.23	15.1	10.5	[c]
all-*trans*-**1c**	5.42	5.71	6.13	6.18	7.13	6.38	5.99	10.13	15.3	10.7	15.1
9-*cis*-**1c**	5.45	5.72	6.62	6.05	7.25	6.31	5.99	10.14	15.3	11.6	15.1
9,13-di*cis*-**1c**	5.45	5.72	6.61	6.09	[c]	[c]	5.87	10.23	15.3	10.5	[c]
all-*trans*-**1d**	5.41	5.70	6.12	6.16	7.11	6.36	5.70	10.10	15.4	11.6	15.1
13-*cis*-**1d**	5.42	5.70	6.13	6.19	7.00	7.27	5.84	10.20	15.4	11.4	14.7
11-*cis*-**1d**	5.41	5.68	6.11	6.50	6.66	5.91	6.10	10.09	15.4	12.3	12.0
9-*cis*-**1d**	5.44	5.70	6.60	6.03	7.23	6.29	5.97	10.10	15.3	11.6	15.0
all-*trans*-**1e**	5.43	5.71	6.13	6.18	7.13	6.38	5.98	10.12	15.2	10.6	15.1
13-*cis*-**1e**	5.43	5.72	6.15	6.21	7.02	7.29	5.86	10.23	15.6	11.4	14.6
11-*cis*-**1e**	5.43	5.70	6.13	6.52	6.67	5.93	6.11	10.12	15.7	12.3	11.5
9-*cis*-**1e**	5.45	5.72	6.62	6.05	7.25	6.31	5.99	10.14	15.3	11.6	15.1
9,13-di*cis*-**1e**	5.45	5.72	6.60	6.09	7.20	7.17	5.89	10.23	15.3	10.4	[c]
all-*trans*-**1f**	5.40	5.65	6.07	6.11	7.04	6.33	5.84	10.00	15.4	11.5	15.0
13-*cis*-**1f**	5.41	5.66	6.09	6.16	6.95	7.72	5.73	10.10	15.5	11.2	14.9
9-*cis*-**1f**	5.47	5.74	6.59	6.03	7.23	6.29	5.97	10.10	15.3	11.5	15.0
all-*trans*-**1g**[b]	5.46	5.71	6.67	−124.43	6.87	6.67	6.08	10.15	15.4	26.4	15.4
13-*cis*-**1g**	5.46	5.72	6.68	−124.18	6.77	7.55	5.93	10.28	14.7	26.7	15.2
11-*cis*-**1g**	5.44	5.66	6.58	−122.44	6.30	6.05	6.00	10.09	15.6	29.9	12.9
9-*cis*-**1g**	5.47	5.69	6.37	−120.38	6.99	6.62	6.09	10.16	15.2	26.8	15.4
9,13-di*cis*-**1g**	5.47	5.70	6.35	−120.20	6.89	7.50	5.94	10.27	15.2	26.7	15.2

[a] 300 MHz spectra; in $CDCl_3$; TMS internal standard.
[b] F–NMR recorded on a NR-80 spectrometer. $CFCl_3$ as internal standard.
[c] Overlapping or poorly resolved signals.

binding propensities. In general, the structured absorption spectra of the free chromophores gave way to broad, featureless absorption maxima for the protein-bound species. Rates of pigment formation are lower for the α-isomer than those of the corresponding β-isomer (Fig. 2). Spectral data for 5-butyl-α-rhodopsin **(1b)** are shown in Fig. 3 as a representative example. The pigment is shown to be stable toward hydroxylamine and 11-*cis*-retinal.

The dicis pigment analog. Because of recent findings that the di*cis* rhodopsin analogs undergo extensive isomerization during binding interaction (*10*), we have carried out chromophore extraction studies with 9-*cis*,11-*cis*-α-rhodopsin in the

TABLE 4

Ultraviolet Absorption Data of Isomers and Analogs of α-Retinal and 5-Substituted α-Retinals

Compound	λ_{max} (nm)	(ε)	Analogs (yield)[a]	PSB (nm)[b]
all-*trans*-**1a**	351	(5.2×10^4)	—	420
13-*cis*-**1a**	346	(4.4×10^4)	—	420
11-*cis*-**1a**	354	(2.5×10^4)	468 (++), 467[c]	430
9-*cis*-**1a**	348	(4.7×10^4)	462 (++)	420
9,11-di*cis*-**1a**	348	(2.2×10^4)	478 (++)	420
9,13-di*cis*-**1a**	342	(3.3×10^4)	—	419
all-*trans*-**1b**	351	(4.8×10^4)	—	
13-*cis*-**1b**	347		—	
11-*cis*-**1b**	354	(2.9×10^4)	462 (++)	
9-*cis*-**1b**	350		460 (+)	
all-*trans*-**1c**	352	(5.8×10^4)	—	
9-*cis*-**1c**	349	(4.9×10^4)	460 (+++)	
9,13-di*cis*-**1c**	344	(4.2×10^4)	460 (+)	
all-*trans*-**1d**	354		—	
11-*cis*-**1d**	353		470 (+++)	
9-*cis*-**1d**	349		462 (++)	
all-*trans*-**1e**	352	(3.9×10^4)	—	
13-*cis*-**1e**	348	(3.4×10^4)	—	
11-*cis*-**1e**	350	(2.5×10^4)	— (−)	
9-*cis*-**1e**	348	(3.7×10^4)	460 (+)	
all-*trans*-**1f**	352	(2.2×10^4)	—	
9-*cis*-**1f**	349	(1.6×10^4)	—	
all-*trans*-**1g**	354	(4.2×10^4)	—	420
13-*cis*-**1g**	349	(2.7×10^4)	—	415
11-*cis*-**1g**	334	(1.4×10^4)	463 (++)	410
9-*cis*-**1g**	350	(3.3×10^4)	460 (+++)	420
9,13-di*cis*-**1g**	344		458 (++)	

[a] +++ ≡ >70%; ++ ≡ 30–70%; + ≡ 3–30%; − ≡ <3%. Calculated based on an assumed extinction coefficient of α-pigment being 90% of that of rhodopsin (i.e., ~38,000).

[b] Protonated *n*-butyl Schiff base with camphorsulfonic acid in ethanol.

[c] Data listed in Ref. (*3b*).

form of oximes. The procedure used in this study is essentially the same as those reported for rhodopsins. The HPLC chromatogram of the oximes of authentic samples of isomeric α-retinal oximes is shown in Fig. 4 (top). That of extracted oximes from the pigment(s) derived from 9-*cis*,11-*cis*-α-retinal is shown in Fig. 4 (bottom). Clearly, a substantial amount of isomerization to the 11-*cis* isomer has taken place. Lacking information on extinction coefficients of the oximes, we made no attempts to quantify the extent of isomerization and to calculate the absorption maximum for the di*cis* analog. But it is clear that the latter should

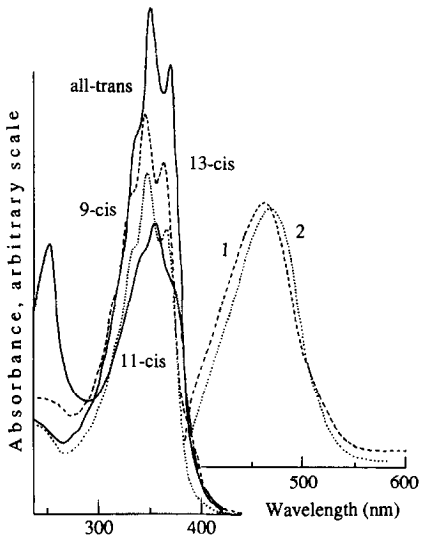


FIG. 1. Left. The uv–vis absorption spectra of isomers of α-retinal taken in hexane; arbitrary intensity scale. Extinction coefficients listed in Table 4. Right: difference absorption spectra of α-rhodopsin (curve 2) and 9-*cis*-α-rhodopsin (curve 1) in digitonin.

have, interestingly, a red shifted absorption maximum from those of the mono-*cis* isomers.

Structural analysis. The coordinates of the carbon and oxygen atoms in all-*trans*-α-retinal obtained from the X-ray crystal structure are listed in Table 1. These position parameters were used as initial input for calculation of the minimized structure of α-retinal using the molecular mechanics program modified for π-systems (MMP2-85) (*6*). The calculated torsional bond angles are listed together with those from the crystal structure in Table 5. These data are used for comparison with those of the minimized all-*trans*-retinal (β-form) (*7*).

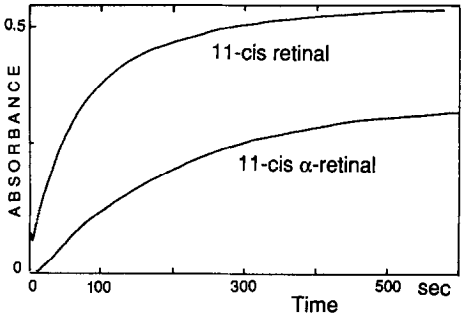


FIG. 2. Comparison of rates of pigment formation of rhodopsin and α-rhodopsin followed by increase of absorbance at 490 nm. Concentration of opsin = 3×10^{-5} M; concentration of retinal = 1×10^{-4} M for both isomers. The calculated ratio of pseudo unimolecular rate constants is 3.9.

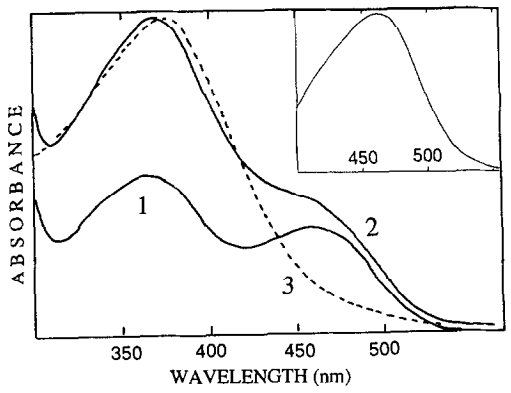


FIG. 3. 5-Butyl-α-rhodopsin. Curve 1, pigment absorption from incubation of 11-*cis*-α-5-butylretinal (3.3×10^{-5} M) in a digitonin solution of opsin (2.4×10^{-5} M). Curve 2, above after addition of 11-*cis*-retinal to a final concentration of 2.4×10^{-5} M. Curve 3, difference absorption spectrum from subtracting curve 2 from curve 1 showing no rhodopsin formed. Insert: difference absorption spectrum of the pigment from before and after photobleaching in the presence of hydroxylamine.

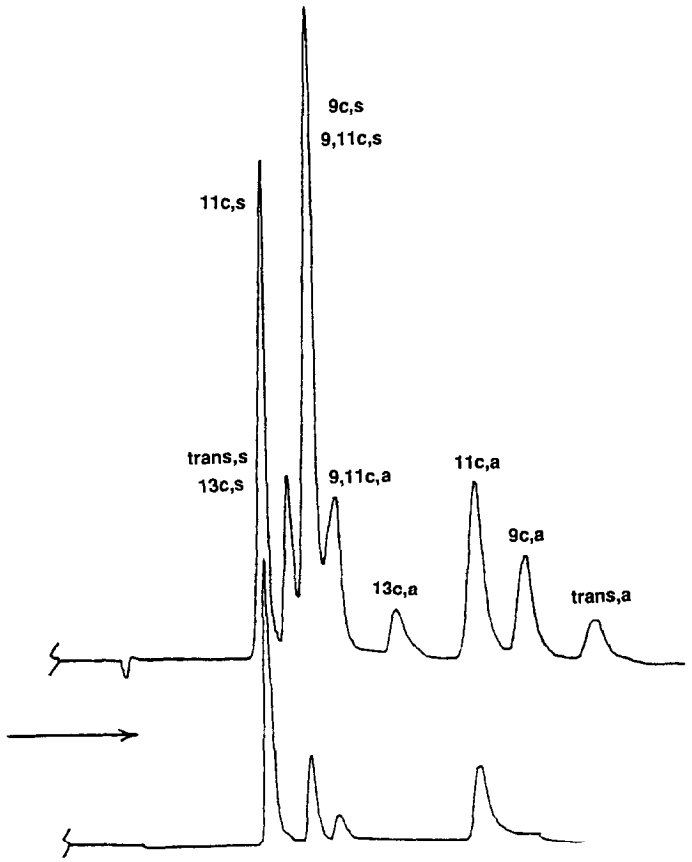


FIG. 4. Upper. HPLC chromatogram of authentic samples of isomeric α-retinal oximes. Solvent, 10% ether, 0.5% ethanol in hexane on a Microsorb column; detecting beam, 360 nm (c = *cis*, s = syn, a = anti). Retention time for *trans*,anti = 24.7 m. Lower. HPLC chromatogram of the oximes extracted from the pigment(s) derived from 9-*cis*,11-*cis*-α-retinal.

TABLE 5

Experimental and Calculated Torsion Angle of all-*trans*-α-Retinal in Degrees

Torsion angle				Crystal data[a]	Calculated data[b]
C5	C6	C1	C2	−48.25 (0.33)	−45.9
C5	C6	C1	C16	−167.81 (0.28)	−166.5
C5	C6	C1	C17	71.61 (0.34)	75.1
C7	C6	C1	C2	74.50 (0.32)	76.3
C7	C6	C1	C16	−45.07 (0.37)	−44.3
C7	C6	C1	C17	−165.65 (0.28)	−162.7
C1	C6	C5	C4	22.82 (0.41)	18.7
C1	C6	C5	C18	−157.56 (0.28)	−162.8
C7	C6	C5	C4	−103.10 (0.34)	−106.7
C7	C6	C5	C18	76.53 (0.33)	71.7
C1	C6	C7	C8	137.13 (0.31)	122.2
C5	C6	C7	C8	−98.23 (0.34)	−112.5
C6	C1	C2	C3	58.49 (0.35)	58.8
C16	C1	C2	C3	179.34 (0.29)	−179.7
C17	C1	C2	C3	−59.90 (0.38)	−61.9
C1	C2	C3	C4	−39.68 (0.40)	−42.0
C2	C3	C4	C5	11.92 (0.51)	13.4
C3	C4	C5	C6	−3.34 (0.53)	−1.7
C3	C4	C5	C18	177.05 (0.33)	179.9
C6	C7	C8	C9	175.98 (0.27)	178.4
C7	C8	C9	C10	−171.11 (0.30)	−175.5
C7	C8	C9	C19	9.65 (0.45)	3.2
C8	C9	C10	C11	−179.55 (0.27)	177.3
C19	C9	C10	C11	−0.34 (0.48)	−1.4
C9	C10	C11	C12	−178.38 (0.29)	−177.8
C10	C11	C12	C13	−178.18 (0.28)	178.3
C11	C12	C13	C14	−179.48 (0.29)	−175.6
C11	C12	C13	C20	0.74 (0.45)	4.0
C12	C13	C14	C15	−179.24 (0.29)	179.0
C20	C13	C14	C15	0.53 (0.49)	−0.6
C13	C14	C15	O1	−179.10 (0.33)	−179.9

[a] Estimated standard deviation in parentheses.
[b] From minimized structure by MMP2(85) calculations.

DISCUSSION

α- and β-Isomers. The crystal structures of all-*trans*-retinal and all-*trans*-α-retinal have been used for comparing structural similarities between the two isomers. Because of the difference in ring/chain conformation between the two isomers, the two rings are nearly orthogonal to each other. The overlap improves substantially (Fig. 5) after rotation of the 6,7-bond in the α-retinal from −113° for the relaxed α-retinal to −34° to that of the relaxed β-retinal (this raised the energy of the α-isomer by 0.7 kcal/mol). The flexible hydrophobic pocket of the binding site, as demonstrated in the early work of adamantyl analogs (*11*), apparently can

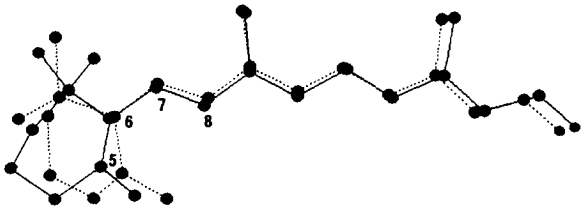


FIG. 5. Matching all-*trans*-α- and β-retinals. A minimized structure of all-*trans*-α-retinal generated by molecular mechanics calculations (MMP2-85) was converted to one with the same ring/chain torsional angle as in the β-isomer by rotating the 6,7-bond from −113° to −34°, the latter reported for the minimized structure of the β-isomer (Ref. (*7*)). The structures shown correspond to the best match of the thus generated α-retinal (dotted line) with the minimized β-retinal (solid line). The matching routine in the CHEMX program was used.

readily accommodate this small difference in spatial requirement. Hence it is not surprising that isomers of α-retinal exhibit the same selectivity as those in the β-series in their interaction with bovine opsin: namely giving a moderate to high yield of pigments with the 9-*cis* and 11-*cis* isomer and none with the 13-*cis* and all-*trans*.

While the yields of pigment are not substantially different between the two series (Table 4 and Ref. (*2a*)), rates of pigment formation are different with pigments in the β-series forming at least three times more rapidly (Fig. 2). The latter could be due to the cumulative effect of lower amounts of the reactive conformer present in the α-series and the difference in orientation of the rings for the two isomers. An exceptional case is the 9,11-di*cis* isomer which isomerized to the 11-*cis* isomer instead of the 9-*cis* isomer as in the case of 9,11-di*cis* rhodopsin (*12*).

Effect of 5-substituent. The general appearance of all the maxima of 5-substituted analogs at 465 (±8) nm is similar to that of the parent α-rhodopsin and other tetraene analogs (*13*). They suggest relatively unencumbered chromophores, thus in general free of any substantial interaction of the protein with the C-5 pendant. However, in a very qualitative sense, it was observed that the relative rates of artificial pigment formation as well as the approximate yields were roughly inversely proportional to the size of the C-5 substituent. These data could be interpreted to imply some protein-based discrimination toward the retinal analog.

The size-exclusion limit for the C-5 substituent in terms of chain length appears to be between 7 and 10 carbons. Thus, whereas the 9-*cis* along **1a** formed a pigment analog, 18-nonylretinal (5-decyl, **1f**) failed to form an artificial rhodopsin. Unfortunately, the scope of our study did not allow for the ascertainment of C-5 side chain conformation, nor for that matter, its relationship to the polyene side chain of the analogs. However, it is reasonable to conclude that there exists substantial latitude for the eventual introduction of bulkier substituents in this particular region of the binding site of rhodopsin. We might add that based on part of the preliminary results of the current study, it was noted that the binding site constructed from binding isomeric retinals should be enlarged near the 5-methyl region (*7*).

The failure of 5-phenyl retinal to form a pigment analog while 18,18-dimeth-

ylretinal (5-isopropyl, **1d**) interacts normally with the apoprotein deserves special mention. Although their steric demands are at first glance quite similar, the former compound's lack of reactivity can be ascribed to significant nonbonded interaction of the phenyl group, constrained to coplanarity with the C-4 double bond, and with the polyene side chain which introduces an unacceptable perturbation in the optimal ring–chain dihedral angle. The high yield of pigments derived from isomers of 10-fluoro-α-retinal provides another set of pigment analogs for examination of specific protein/substrate interaction as shown in the 10-fluororhodopsin series (*14*). Such a photochemical study will be carried out in conjunction with other halogenated analogs.

General comments. Finally, analog studies such as this current investigation invite comparison with results of other studies featuring the systematic variations of pendant groups on the retinal chromophore. Artificial visual pigments have been prepared from a variety of retinal analogs with differing substituents at the C-9 position of the side chain (C-9: R = H (*15*), Me, Et (*1c,d*), *i*-Pr, Bu, pentyl (*16*), *n*-Pr (*17*), and diazoacetoxymethylene (*18*)). The major exception was the inability of 9-phenylretinal (*16*) to regenerate with opsin, presumably for the same reason as in the 5-phenylretinal case; i.e., coplanarity induced by conjugative stabilization imparting unusual and unacceptable ring–chain conformational twists in the potential chromophore.

In contrast to the above examples, other systematic studies have led to the general conclusion that there is rather limited space available for modification on the retinal side chain. Thus, at C-14, R = H, F (*19*), Cl (*16*), and Me (*20*) are acceptable whereas R = Et (*16*) is too large to be accommodated in the protein cavity. Variation at C-13 of R = H, Me, Et, and Pr (*21*) produce pigment analogs, but R = butyl (*16*) is unacceptable. At C-12, even less room is available since for R = Me or Cl, pigment yield is greatly reduced in comparison to R = H or F (*2c*). This also is true for variation at the C-10 position. Here, R = F is acceptable (*19*), but R = Me or Et significantly reduces the yield of rhodopsin analog (*2b*) and R = hexyl (*16*) forms no pigment at all.

The enhanced sensitivity of the chromoprotein to modification in the vicinity of the aldehydic terminus is consistent with stricter spatial demands on the chromophore, in terms of both attached pendant functionalities and side chain conformational changes brought on by the introduction of these substituents.

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