Tetrahedron Letters, Vol.31, No.34, pp 4841-4844, 1990
Printed in Great Britain

0040-4039/90 $3.00 + .00
Pergamon Press plc

β-Lactoglobulin Directed Photoisomerization of Retinal and Related Compounds#

Xiao-yuan Li, Alfred E. Asato, R. S. H. Liu*

Department of Chemistry, University of Hawaii, Honolulu, HI 96822 U. S. A.

Abstract. The 11-cis enriched photostationary state mixture of retinal trapped in BLG, a water soluble protein, is similar to that of retinal in heptanol. The results, along with those of the lower homologs, are consistent with a model involving specific protein interaction with the cyclohexenyl portion of the substrate and remote protonation of the aldehyde carbonyl group.

The direction of photoisomerization of a polyene, such as retinal and related compounds, is known to be highly sensitive to environmental effects.[1] Thus, by varying solvent polarity the visually important 11-cis isomer of retinal can either be completely absent from the product mixture or formed in excess of 25%.[2] And, when confined in a protein binding site, the retinyl chromophore can isomerize either specifically around the middle portion of the chromophore (the 11,12 or the 9,10 bond in visual pigments) or near the terminal portion of the chromophore (in bacteriorhodopsin).[3] For a better understanding of the role of the host protein in affecting direction of isomerization, we have initiated a study of photoisomerization of protein encapsulated polyenes. In this paper we wish to report results on photoisomerization of β-lactoglobulin (BLG)-bound retinal and related compounds for comparison with those in solution.

BLG is an abundant globular protein, the principal whey protein in the milk of mammals. It has a molecular weight of approx. 18,000; but, under physiological conditions or neutral pH, it exists as a dimer. Its three dimensional structure has been identified through recent determination of its crystal structure at a resolution of 2.5 - 2.8 A.[4] It revealed a β-barrel inner core similar to that in the plasma retinol-binding protein.[4]

Spectroscopic properties of the BLG complex with retinol have been studied in some detail. The initially formed weakly fluorescent complex rearranged to a compound possessing a highly structured fluorescent spectrum. It was suspected to be that of a dehydrated form of vitamin A. Both complexes were shown to be CD active, but of opposite sign.[5] A BLG complex with all-trans retinylidenepropyl amine is also known.[6]

We have now prepared purified BLG complexes of all-trans retinal, the C_{18}-ketone and the C_{15}-aldehyde,[7] and compared their photochemical behaviors to the same compounds solubilized in hexane and several alcohols. Since at the outset of this investigation we found that formation of the all-trans retinal and C_{15}-aldehyde complexes of BLG was accompanied by slow isomerization of the polyene to the terminal cis isomers, we decided that it would not be feasible to carry out accurate quantum yield or initial product measurements. Instead, we determined

photostationary compositions of these mixtures. Results of such an investigation are summarized in Tables 1 & 2.

Table 1. Photostationary State Compositions of Retinal in BLG in the Organic Solvents[a,b]

	Conc. M	13-cis	11-cis	9-cis	all-trans
BLG[c]	$3x10^{-5}$	23.3±0.4	16.8±0.3	7.3±0.6	52.5±0.3
Hexane (Hx)	$1.0x10^{-4}$	27.4±0.3	--	2.7±0.1	69.9±0.3
Ethanol	$5.7x10^{-3}$	25.3±0.4	15.2±0.5	7.0±0.1	52.5±0.2
	$1.0x10^{-3}$	23.6±0.2	18.5±0.2	6.5±0.2	51.3±0.6
	$5.7x10^{-4}$	23.8±0.4	19.5±0.2	5.8±0.1	50.8±0.6
	$1.0x10^{-4}$	23.3±0.3	21.4±0.4	5.6±0.2	49.7±0.5
1-Butanol	$5.7x10^{-4}$	27.5±0.5	17.0±0.5	5.0±0.2	50.5±0.2
1-Heptanol	$5.7x10^{-3}$	30.4±0.2	7.7±0.4	7.3±0.5	54.6±0.5
	$5.7x10^{-4}$	28.4±0.4	14.5±0.4	5.2±0.5	51.8±0.2
Hx + 1% Ethanol	$1.0x10^{-4}$	28.7±0.1	2.0±0.3	3.0±0.3	66.3±0.2
Hx + 5% Ethanol	$1.0x10^{-4}$	30.9±0.2	5.5±0.2	3.3±0.2	60.3±0.3

a. Irradiated with 366nm band using Corning 0-52 and 7-60 filter plates and a 200W Hanovia medium pressure Hg lamp until a constant composition was reached. Minor peaks (< 1%) are not included. b. HPLC analyses at 360nm. All data corrected for difference absorptivity of isomers at the wavelength of detection. c. Starting with a mixture of 24.6% 13-cis and 75.4% all-trans due to catalyzed isomerization by BLG. All others starting with the all-trans isomer.

Table 2. Photostationary state compositions of C_{15}-aldehyde[a] and C_{18}-ketone[b]

	Isomer Composition (%)[c]				
Solvent	11-cis[d]	9-cis	7-cis	7,9-dicis	all-trans
C_{18}-ketone					
BLG	33.3±.4	18.7±.2	3.9±.2	--	44.1±.1
Hexane	8.4±.1	7.8±.2	3.5±.1	--	80.3±.3
Ethanol	20.1±.4	18.0±.3	8.0±.5	--	53.8±.4
C_{15}-aldehyde					
BLG	--	11.9±.2	15.3±.1	4.4±.3	68.4±.4
Hexane	--	12.2±.5	22.6±.1	5.8±.3	59.4±.5
Ethanol	--	9.2±.2	19.4±.5	3.7±.4	67.7±.4

a. Irradiated with 313nm light from a LPS 251 Hg-Xenon lamp connected in sequence with a monochromator. b. Irradiated with 366nm light (see above). c. Analysis beam: 340nm for C_{18}-ketone and 290nm for C_{15}-aldehyde. Corrected for different absorbance of isomers (ref. 8). d. Overlapping with dicis isomers containing the 11-cis geometry.

Data in Table 1 show the presence of substantial amounts of the 11-cis isomer in the photostationary states of retinal trapped in BLG. Given the generally accepted hydrophobic characteristics of the binding site of a water soluble protein such as BLG and the noted solvent dependent photochemical properties of retinal, this result is somewhat surprising. For comparison, we have also re-determined the compositions of retinal[8] in several alcohols, at different concentrations and also in hexane and binary mixtures of hexane and ethanol. That the compositions are concentration dependent (Table 1) is in agreement with the early observations of Brown and Wald[9] and the more recently reported quantum chain process.[10] Cases of low retinal concentrations approximate the isolated chromophore trapped within a binding site. Data obtained in different solvents show that the amount of the 11-cis isomer in BLG is closer to those of the alcohols than that of hexane (closest to that in dilute heptanol). We have further demonstrated that small amounts of ethanol (1 and 5%) added to hexane can exert a significant effect on the amount of the 11-cis isomer in the photostationary composition.

The BLG-retinol complex is known to be CD active.[5] We have now demonstrated that the BLG complexes with all three substrates used in this study also exhibit induced circular dichroic behavior (Figure 1). On the basis of current understanding of the CD properties of the visual pigment analogs, it appears reasonable to suggest that the CD activity is associated with induced

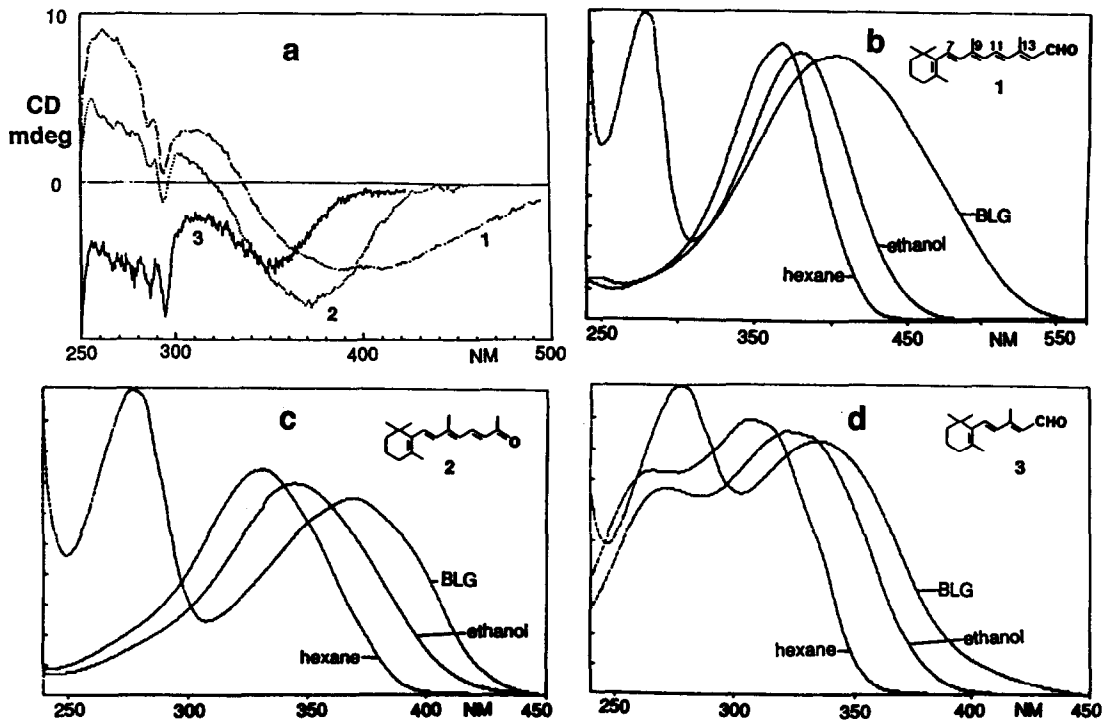

Figure 1. (a) CD curves of all-trans isomer of retinal (1). C_{18}-ketone (2) and C_{15}-aldehyde (3) in BLG-A. The concentrations of all BLG-complexes were 0.1% in 0.1M of phosphate buffer, pH 7.5. The spectra are averages of 4 scans, path length is 10mm. Baselines were corrected with 0.1M of phosphate buffer, pH 7.5. UV spectra of all-trans isomer of (b) retinal, (c) C_{18}-ketone and (d) C_{15}-aldehyde in hexane, ethanol and BLG-A.

ring-chain chirality as a result of specific protein-substrate interactions. Therefore, we suggest that the observed photochemical properties of retinal in BLG result from specific hydrophobic interaction of the binding site with the trimethylcyclohexenyl ring in conjunction with protonation of the carbonyl group by a remote proton source from the protein. The latter effect could give rise to the observed chemistry equivalent to that in a polar medium. In agreement with this interpretation is the observed red shift of the absorption spectra of the bound substrates (Figure 1b-d) which are reminiscent of that reported for protonated retinal obtained in the presence of phenol as a proton donor.[11] However, at this time, it is difficult to pin-point the specific amino acid residue that is likely to serve as the proton donor.

The minor variation of relative amounts of the 9-cis and 13-cis isomers of retinal in BLG from those obtained in solution probably reflects local steric constraints within the protein binding site. The variations of isomer distribution in the C_{18}-ketone and the C_{15}-aldehyde (Table 2) are less dramatic as demonstrated earlier in solution studies.[8] The major notable trends for the C_{18}-ketone are the smaller amount of the 7-cis isomer and the larger amount of the 11-cis isomer with the BLG sample. The former is consistent with the notion of a tight, specific hydrophobic pocket for the cyclohexenyl ring and the latter could be due to the shape of the cavity near the C13-C14 bond.[12]

References:

#Photochemistry of polyenes 26. For 25, see: P. Arjunan & R. S. H. Liu, Tetrahedron Lett., 29, 853-856 (1988).

1. R. S. H. Liu & A. E. Asato, Tetrahedron, 40, 1931-1986 (1984).
2. R. Hubbard & A. Kropf, Photochem. Photobiol., 12, 249-260 (1970).
3. H. Shichi in "Biochemistry of Vision", Academic Press, New York, 1983, Chapters 6 amd 11.
4. (a). M. Z. Papiz, L. Sawyer, E. E. Elipoulos, A. C. T. North, J. B. C. Findlay, R. Sivaprasadarao, T. A. Jones, M. E. Newcomer & P. J. Kraulis, Nature, 324, 383-385 (1986). (b). H. L. Monaco, G. Zanotti, P. Spadon, M. Bolognesi, L. Sawyer & E. E. Elipoulos, J. Mol. Biol., 197, 695-706 (1987).
5. R. D. Fugaste & P. Song, Biochim & Biophys. Acta, 625, 28-42 (1980).
6. J. Horwitz & J. Heller, J. Biol. Chem., 249, 4712-4719 (1974).
7. The following is a typical procedure for preparation of a BLG-polyene complex. To a 50ml solution of 0.5% BLG in phosphote buffer, pH=7.5 was added 2ml of a 9 x 10^{-3}M ethanol solution of retinal (or its analog). The solution was allowed to stand overnight at room temperature. The complex was then purified on a Sephodex G-75 column (3.5 x 90 cm) with 0.1M phosphate buffer, pH=7.5 as eluant. The collected fractions were analyzed by uv-vis spectroscopy.

   For analysis, 10ml of the 0.2% BLG complex were denatured by mixing with 2ml of methylene chloride. The aqueous layer was separated from the gel and an equal volume of methylene chloride as the remaining gel was added. Upon mixing on a Tissumizer, a clear solution resulted. The methylene chloride layer was filtered on a short silica gel column. After concentrating the solution, the mixture was analyzed by hplc.
8. V. Ramamurthy, M. Denny & R. S. H. Liu, Tetrahedron Lett., 22, 2463-2466 (1981).
9. P. K. Brown & G. Wald, J. Biol. Chem., 222, 865-877 (1956).
10. S. Ganapathy, A. Trehan & R. S. H. Liu, Chem. Comm., 2, 199-200 (1990).
11. T. Takemura, P. K. Das, G. Hug & R. Becker, J. Am. Chem. Soc., 100, 2626-2630 (1978).
12. This work was supported by a grant from the National Science Foundation (CHE-16500).

(Received in USA 24 May 1990)