CHEMISTRY LETTERS, pp. 167 - 170, 1984. © The Chemical Society of Japan 1984

A MODEL STUDY OF REGENERATION PROCESS IN RHODOPSIN VISUAL CYCLE

Minoru SAKURAI,* Osamu SHIROTA, Yoshio INOUE, and Riichirô CHÛJÔ
Department of Polymer Chemistry, Tokyo Institute of Technology,
12-1 Ookayama 2-chome, Meguro-ku, Tokyo 152

The regeneration process in rhodopsin was studied by an estimation of isomer population at photoequilibrium for the model compounds of N-retinylidene phosphatidylethanolimine. We suggested that the preferential formation of 11-cis isomer may be induced by the through-bond effect of oxygen atom of imine moiety of a Schiff base.

Light absorption by the chromophore, retinal, of rhodopsin induces its isomerization from the 11-cis to the all-trans form and this triggers a series of reactions leading to visual excitation. Reisomerization of the all-trans retinal thus formed to the 11-cis isomer, followed by resynthesis of rhodopsin, is an essential process for maintaining unimpaired vision. There are at least two possible modes in the step of retinal reisomerization: dark and light reactions. In the dark, all-trans retinal is converted to 13-cis and 9-cis retinals in the presence of catalysts, and not to 11-cis retinal, the least thermodynamically stable isomer.[1-3] From these facts, the presence of retinal isomerase in bovine retinas was suggested,[4] but not supported by later studies.[5,6] On the other hand, Shichi and Somers[6] found that 11-cis isomer is exclusively formed by irradiation of 460 nm light on a protonated all-trans N-retinylidene phosphatidylethanolimine(NRPE), which is located in the disc membrane of rod outer segment. This is the most attractive finding for the step of retinal reisomerization at present. MO calculation[7] indicated that the coulombic interaction between the negative charge on phosphate group and the positive charge along the polyene chain significantly contributes to the specific conversion to the 11-cis form in the photoisomerization of all-trans NRPE, but not followed by experimental data. In order to better understand the mechanism of regeneration process, further information would be required for the role of phosphatidylethanolamine(PE) moiety on the photoisomerization of NRPE.

We investigated the effect of the terminal polar group of Schiff bases on their isomer population at photoequilibrium. We used all-trans N-retinylidene ethanolimine(NRE) as a simplified analog of NRPE and the following compounds for a comparison; all-trans N-retinylidene butylimine(NRB), all-trans N-retinylidene propanolimine(NRPr), all-trans N-retinylidene pentanolimine(NRPe), and all-trans retinal. Retinal was synthesized by a procedure described elsewhere[8] and purified by TLC immediately before use. Each Schiff base was prepared by adding excess amine(100-fold excess) to a methanol solution of each retinal isomer in quartz cell and subsequently it was irradiated with a light of 15w fluorescence lamp at 10 °C. It has been reported that almost 100 percent of retinal is present as Schiff base at equilibrium, when it reacts with 40-fold excess of ethanolamine in ethanol.[9)] The above condition seems to be sufficient for full reaction of retinal. All the Schiff bases had the absorption maxima at 365 nm in methanol. UV absorption spectra were measured on a Beckman model 25 spectrometer.

Isomer population of each sample at photoequilibrium was determined according to the method of Kropf and Hubbard.[10)] We obtained the absorption spectra of photoisomerized mixture and analyzed under following assumptions: the mixture was composed of all-trans, 9-cis, 11-cis, and 13-cis isomers and the observed absorption spectra were fitted by the sum of the spectra of these isomers. It was confirmed that in the case of retinal, isomer population obtained from this method is more reliable than the one determined by spectrophotomeric assay of components eluted after TLC. We believe that the method of spectral fitting is the best available one to examine the isomer population of Schiff bases, considering that it is difficult to separate their isomers by HPLC.

In Fig.1 is shown the time dependence of isomer population starting from a pure all-trans retinal. The absorption spectra recorded in three minutes interval during irradiation. The successive spectra indicate that photoequilibrium is established after about 30 minutes.

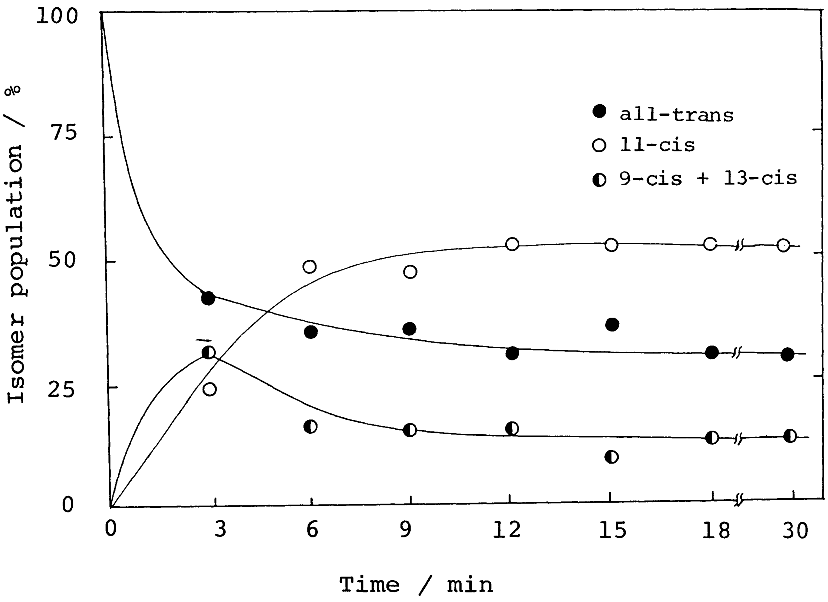

Fig. 1. The time dependence of isomer population starting from pure all-trans retinal in methanol.

Table 1 shows the isomer population of NRE, NRB and retinal at photoequilibrium in methanol. It is noted that 11-cis isomer is exclusively formed upon irradiation of all-trans NRE, although in the case of NRB and retinal, 9-cis and 13-cis isomers are also formed as major products. It has been recognized that the composition of photoisomerized products depends on the polarity of solvents in the direct irradiation of all-trans retinal: the 11-cis isomer is formed in addition to 9-cis and 13-cis isomers in alcoholic or polar solvents, but not in nonpolar solvents.[10),11)] This has been explained by the change of the relative ordering of n-π^* and π-π^* states depending on whether the solvent is polar or nonpolar.[11)] The isomer population of NRB is rather similar to that of retinal. The polarity of solvents may be also responsible for the preferential formation of

11-cis isomer in NRB. However, the absence of 9-cis and 13-cis isomers in NRE clearly indicates that there is another factor in the selective photoisomerization of NRE. NRE is different from NRB in the structure of terminal imine. It is quite natural to suppose that an alcoholic group of imine moiety may play an important role of retinyl moiety of a Schiff base.

Table 1. Isomer population of NRE, NRB and retinal at photoequilibrium in methanol.

Compound	Percentage composition		
	all-trans	11-cis	9-cis and 13-cis
NRE	48.3	51.7	0.0
NRB	23.8	37.8	38.4
Retinal	30.0	48.4	21.6

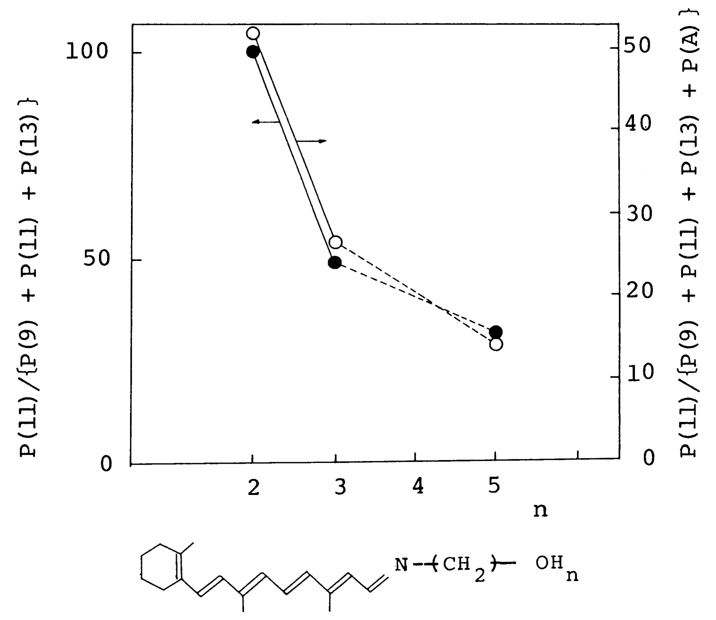

Fig. 2. The dependence of the yield of 11-cis isomer on the terminal structure of Schiff bases in their trans-cis photoisomerization.

P(X) denotes the population of X-cis isomer and all-trans isomer, where X is 9, 11, and 13 for cis isomer, and A for all-trans isomer.
Schiff bases with n=2,3, and 5 correspond to NRE, NRPr, NRPe, respectively.

Figure 2 shows the dependence of the yield of the 11-cis isomer on the terminal structure of Schiff bases in their trans-cis photoisomerization. The selective formation of 11-cis isomer was observed only in NRE. This means that the ethanol-like structure of imine moiety is essentially required for the specific isomerization. From a consideration of the molecular structure of NRE using CPK molecular model, we could not find any special intramolecular interaction which stabilizes the 11-cis isomer of NRE. The single bond of ethanol moiety would freely rotate in solution. Furthermore, considering that the solute molecule is surrounded by solvent molecules, methanol, it is reasonable to be assumed that there is no specific interaction through space between the ethanolamine OH group and polyene moiety.

We conclude that the ethanolamine OH group influences mainly on the electronic states of polyene moiety by through-bond effect, that is, by the σ-inductive effect. This effect would be enhanced by the formation of hydrogen bond between the imine OH group and methanol. The above data suggested that two-bond separation between the oxygen atom and the Schiff base nitrogen atom is optimal for the formation of 11-cis isomer. In PE there is a similar type of oxygen in polar head group, so that the specific formation of 11-cis isomer may be expected for an unprotonated NRPE.

Recently, Becker et al. found that in the case of polar solvents, a protonation exert no influence on the type of isomerization already existing for the unprotonated NRB and little or no influence on the quantum yield.13) This indicated that the through-bond effect described above is also important for the photoisomerization of protonated form of NRE and NRPE. Therefore, the mechanism for retinal reisomerization process may not be understood without a consideration of the through-bond effect induced by oxygen atom of PE moiety. Our suggestion is essentially different from that previously reported by Rabinovich who has taken account of the through-space effect of the negative charge of phosphate group on the electronic states of polyene moiety, but not the through-bond effec owing to the crude approximation in MO calculation.7)

There has been no report on the relationship between the structure of imine moiety of a Schiff base and its photoproducts. In the present report, it is clearly demonstrated that the direction of photoconversion of a Schiff base is sensitively dependent on the structure of its imine moiety. We are now studying this with more extended model system of NRPE.

References

1) S. Futterman and M. H. Rollins, J. Biol. Chem., 248, 7773(1973).
2) G. W. T. Groenendijk, C. W. M. Jacobs, S. L. Bonting, and F. J. M. Daemen, Eur. J. Biochem., 106, 119(1980).
3) R. R. Rando and A. Chang, J. Am. Chem. Soc., 105, 2879(1983).
4) R. Hubbard, J. Gen. Physiol., 39, 935(1956).
5) E. O. Plante and B. Rabinovich, Biochem. Biophys. Res. Commun., 46, 725(1972).
6) H. Shichi and R. L. Somers, J. Biol. Chem., 249, 6570(1974).
7) B. Rabinovich, Photochem. Photobiol. 29, 567(1979).
8) Y. Inoue, Y. Tokito, R. Chûjô, and Y. Miyoshi, J. Am. Chem. Soc., 99, 5592(1977).
9) P. A. Plack and D. J. Pritchard, Biochem. J. 115, 927(1969).
10) A. Kropf and R. Hubbard, Photochem. Photobiol., 12, 249(1970).
11) W. H. Waddell and D. L. Hopkins, J. Am. Chem. Soc., 99, 6457(1977).
12) V. Ramamurthy, M. Denny, and R. S. Liu, Tetrahedron lett., 22, 2463(1981).
13) R. S. Becker, K. Freedman, and G. Causey, J. Am. Chem. Soc., 104, 5797(1982).

(Received October 6, 1983)