Resonance Raman Spectra of Red-Shifted Retinal Schiff's Base

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Synopsis. We have found that the visible absorption maximum (λ_{max}) of retinal Schiff's base in fluoro alcohols is considerably red-shifted. The C=N stretching Raman line exhibits a frequency shift in deuterated fluoro alcohols, indicating that the Schiff's base is protonated. Frequencies of the in-phase C=C stretching Raman lines ($\nu_{C=C}$) changed linearly with λ_{max}, and the $\nu_{C=C}$ vs. λ_{max} correlation coincides with that observed for retinoid proteins.

In attempts to elucidate the structure-function relationship of retinoid proteins, resonance Raman (RR) spectroscopy has been extensively used to explore the molecular events which befall the chromophore of visual pigments[1-7] and bacteriorhodopsin[8-13] upon light irradiation. One of the remaining problems to be clarified is the origin of the opsin shift, that is, the large red shift of the absorption maximum of a retinal Schiff's base (RSB) in proteins compared with that in organic solvents. Recently we found that RSB in fluoro alcohols such as 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) and 2,2,2-trifluoroethanol (TFE) (Abbreviations for the chemical substances are summarized in the footnote of Table 1) gives an absorption band which is strongly red-shifted at the expense of the ordinary band in the blue region.[14] The amount of the red shift is larger than that usually observed for protonation of a Schiff's base by a strong acid, and the relative intensity of the red-shifted band to the ordinary band depends on the solvent composition and temperature; dilution of the fluoro alcohol solution with an inert solvent such as chloroform or rise of temperature decreases the relative intensity of the red-shifted band to the blue band. We are curious to know what occurs on the chromophore which exhibits such a large red shift of λ_{max}. In this note we report on RR spectra of all-*trans*-RSB of Ile-Ala-Phe-OBzl (RetIAF) and of butylamine (RetBu) in the fluoro alcohols, pointing out that the interaction between the Schiff's base nitrogen and alcoholic hydroxyl group alters the π delocalization of retinal and changes $\nu_{C=C}$ in linear correlation with λ_{max}.

Experimental

RetIAF was synthesized by mixing all-*trans*-retinal with Ile-Ala-Phe-OBzl[15] in ethyl acetate. 40 mg of HCl Ile-Ala-Phe-OBzl was dissolved in 5 ml of water and the solution was adjusted to pH 9 by 5% $NaHCO_3$. The solution became a gel. After extraction with 15 ml of ethyl acetate the organic layer was dried over Na_2SO_4 and filtered. Molecular sieve (3A) was added to the filtrate and then 100 mg of all-*trans*-retinal was incorporated into the solution, which was left to stand at 0 °C for 24 h. After removal of the molecular sieve, the solution was evaporated to give a yellow oil. The slightly yellow precipitate, which was formed on addition of hexane, was collected, washed with hexane, and dried in vacuum; the yield was 24 mg. The purity of the compound was confirmed by ^1H and ^{13}C NMR. RetBu was prepared according to the literature.[16] Deuterated HFIP or TFE was obtained by distillation of a mixture of D_2O and the fluoro alcohol (4:1). Other solvents were distilled immediately before use. The Schiff's base solution at a concentration of ca. 0.1 mM (1 M=1 mol dm^{-3}) was used for the Raman measurements. Raman spectra were measured with a JEOL-400D Raman spectrometer and an Ar laser (Spectra Physics, model 164) or a He/Cd laser (Kinmon Electrics, CDR80SG). The laser power at the sample point was 2—24 mW. About 50 µl of the sample solution in a cylindrical cell kept at 4 °C was used for the measurement.

Results and Discussion

The RR spectra of RetIAF and RetBu in the 1400—1700 cm^{-1} region are shown in Figs. 1(A) and (B), respectively. It is seen that the frequencies of the Raman lines are sensitive to the solvent and this solvent effect is most pronounced for the C=C and C=N stretching modes. The in-phase C=C stretching modes ($\nu_{C=C}$) of RetIAF and RetBu appear at 1576 and

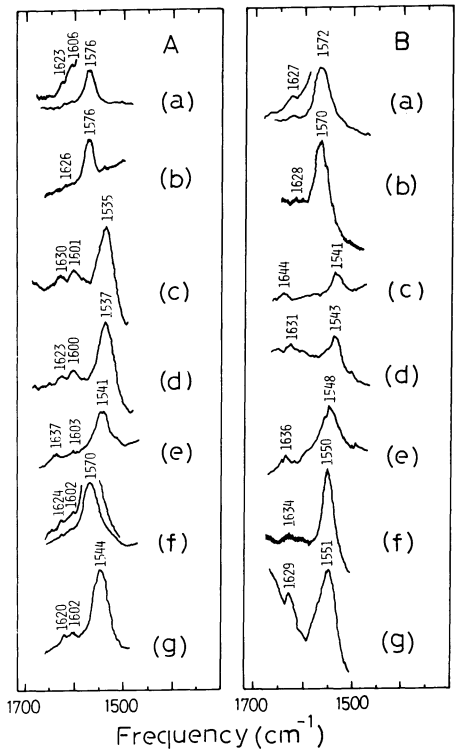

Fig. 1. The resonance Raman spectra of RetIAF (A) and RetBu (B) in the 1400—1700 cm^{-1} region. Excitation wavelengths and solvents are as follows: a): 441.6 nm, chloroform, b): 514.5 nm, chloroform, c): 514.5 nm, HFIP, d): 514.5 nm, HFIP (deuterated), e): 514.5 nm, TFE, f): 441.6 nm, TFE, g): 514.5 nm, TFE (deuterated).

1572 cm^{-1}, respectively, in chloroform (a) but they are shifted to 1535 and 1541 cm^{-1} in HFIP (c) and to 1541 and 1548 cm^{-1} in TFE (e). The Schiff's base C=N stretching modes ($\nu_{C=N}$) of RetIAF and RetBu appear at 1623 and 1627 cm^{-1}, respectively, in chloroform (a) but they are shifted to 1630 and 1644 cm^{-1} in HFIP (c) and to 1637 and 1636 cm^{-1} in TFE (e). Thus, the C=C and C=N stretching modes shift in opposite directions and their magnitudes are not correlated.

The visible absorption spectrum of RetIAF in TFE exhibits two absorption maxima at 368 and 494 nm, and the RR spectrum excited at 441.6 nm (f) is distinctly different from that excited at 514.5 nm (e). Therefore, it is deduced that there exist two molecular species in equilibrium in the TFE solution, each giving rise to an absorption band at either 368 or 494 nm. These are tentatively called the U and P species, respectively, in this paper. The bands in the 514.5 nm- and 441.6 nm-excited RR spectra are considered to arise predominantly from the P and U species, respectively. The U species gives the $\nu_{C=C}$ and $\nu_{C=N}$ lines at 1570 and 1624 cm^{-1} (f), respectively, while the P species gives the lines at 1541 and 1637 cm^{-1} (e). The RR spectrum of the TFE solution excited at 441.6 nm (f) is very close to that of the chloroform solution (a). The $\nu_{C=N}$ line of the P species exhibits frequency shift in deuterated fluoro alcohols [(d) and (g) in Figs. 1A and B], but that of the U species does not. Therefore, the P and U species are inferred to have protonated and unprotonated Schiff's bases, respectively.

On the other hand, protonation of RetIAF and RetBu in chloroform by a strong acid such as CF_3COOH caused frequency shifts of the $\nu_{C=C}$ mode by -32 and -15 cm^{-1}, respectively, and the $\nu_{C=N}$ mode by $+17$ and $+23$ cm^{-1}, respectively. The magnitude of the shift is smaller than the frequency difference between the chloroform and fluoro alcohol solutions for $\nu_{C=C}$, but it is larger for $\nu_{C=N}$. Table 1 summarizes the values of λ_{max}, $\nu_{C=C}$, and $\nu_{C=N}$ of RetIAF and RetBu in various solvents. When the values of λ_{max} are plotted against the values of $\nu_{C=C}$, it gives a straight line as displayed in Fig. 2. The linear correlation between λ_{max} and $\nu_{C=C}$ was previously reported for retinoid proteins,[5,9] but it is noteworthy here that the values of λ_{max} and $\nu_{C=C}$ of the present compounds in various solvents fall on the identical line with that of the retinoid proteins. This means that the solvent effect on the chromophore found in the present study is qualitatively the same as the role of surrounding proteins regarding the spectral property. Since the strength of the C=C bond in the conjugated linear molecules generally becomes weaker and thus the $\nu_{C=C}$ frequency becomes lower as delocalization of π electrons increases, the linear correlation between λ_{max} and $\nu_{C=C}$ suggests that the red-shift of λ_{max} of RetIAF and RetBu in fluoro alcohols as well as of retinylidene chromophore in proteins can be attributed to increas-

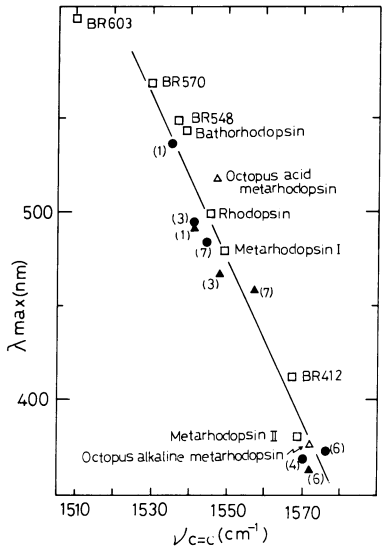

Fig. 2. Correlation between λ_{max} and $\nu_{C=C}$ for the present compounds and retinoid proteins. Data are obtained from rhodopsin,[3] bathorhodopsin,[1] metarhodopsin I and II,[5] bacteriorhodopsin,[12] octopus metarhodopsin,[7] and Table 1 (●: RetIAF and ▲: RetBu). Numbers besides the points represent the compounds specified in Table 1.

Table 1. Raman Frequencies and Absorption Maxima of RetIAF and RetBu in Various Solvents

Solvent	Ret IAF[a]			Ret Bu[a]		
	$\nu_{C=N}$ / cm^{-1}	$\nu_{C=C}$ / cm^{-1}	λ_{max} / nm	$\nu_{C=N}$ / cm^{-1}	$\nu_{C=C}$ / cm^{-1}	λ_{max} / nm
(1) HFIP[b]	1630	1535	536	1644	1541	494
(2) HFIP[b] (deuterated)	1623	1537		1631	1543	
(3) TFE[b]	1637	1541	494	1636	1548	467
(4) TFE[c]	1624	1570	368			
(5) TFE[b] (deuterated)	1620	1544		1629	1551	
(6) Chloroform[c]	1623	1576	372	1627	1572	363
(7) Chloroform + TFA[b]	1640	1544	484	1650	1557	458

a) Abbreviations used are; RSB, retinal Schiff's base; RetIAF, RSB with Ile-Ala-Phe-OBzl; RetBu, RSB with butylamine; HFIP, 1,1,1,3,3,3-hexafluoro-2-propanol; TFE, 2,2,2-trifluoroethanol. b) Raman frequencies are obtained from the spectrum excited at 514.5 nm. c) Raman frequencies are obtained from the spectrum excited at 441.6 nm.

ed π delocalization. It is important to note that such π delocalization is controlled by the interaction at the Schiff's base.

An interesting feature of this study is that the $\nu_{C=N}$ modes of the red-shifted RetIAF and RetBu shift to lower frequencies in deuterated fluoro alcohols. Upon deuteration of HFIP, the $\nu_{C=N}$ modes of RetIAF (1630 cm^{-1}) and RetBu (1644 cm^{-1}) exhibit the frequency shift of −7 and −13 cm^{-1}, respectively, while the deuteration of TFE gives corresponding shifts of −17 and −7 cm^{-1}, respectively. This type of frequency shift is usually seen upon deuteration of proteins having protonated RSB.[3] Accordingly, it is likely that the alcoholic hydroxyl group acts as a Lewis acid and a proton is shared between the Schiff's base nitrogen and the alcoholic oxygen. If this were simply a hydrogen-bonding interaction in which the Schiff base nitrogen serves as a proton acceptor, the $\nu_{C=N}$ line would not exhibit such a large frequency shift upon deuteration of the alcohols. Therefore, the interaction between the N and H atoms is rather of a weak covalent type but it would not be categorized as a hydrogen bond. Thus, RetIAF and RetBu in fluoro alcohols behave as if they were protonated RSB. The solvent-dependent difference in frequency shifts upon deuteration reflects that the degree of coupling between the C=N stretching and the N-H in-plane bending modes varies depending on the strength of the quasi-covalent N-H bond. It is due to this vibrational coupling that the frequency of $\nu_{C=N}$ is not correlated with the frequency of $\nu_{C=C}$ or the values of λ_{max} despite the fact that the C=N bond is also affected by the π delocalization. We would stress finally that fluoro alcohol is more effective than a strong acid in shifting λ_{max} to red and on lowering $\nu_{C=C}$.

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