Spectral Shifts of Protonated Schiff Bases and their Analogues in a Polyethylene Matrix

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Large shifts of the maxima in the absorption spectra of protonated retinal Schiff bases and their benzenoid analogues were observed on changing the counter ion from $CF_3CO_2^-$ to Cl^-.

Visual pigments contain 11-*cis*-retinal[1,2] bound to the ε-amino lysine residue of various lipoproteins through a protonated Schiff base linkage.[3,4] Although all these pigments possess the same chromophore they display absorption maxima which lie at different wavelengths, ranging from 400 to 600 nm.

Calculations have indicated that the position of the

Table 1. Absorption maxima of protonated salts of *N*-retinylidene-n-butylamine in MeOH, CH_2Cl_2, and in a polyethylene matrix.

	λ_{max}(nm)		
Anion	MeOH	CH_2Cl_2	Polyethylene
Cl^-	445	455	410
Br^-	445	465	420
ClO_4^-	445	475	428
$CF_3CO_2^-$	445	455	453

absorption maximum of a protonated retinal Schiff base is dependent on the distance between the positive nitrogen and its counter anion.[5a,c,6a] Accordingly, the isolated 'naked' cation *in vacuo* will absorb at *ca.* 600 nm.[7] The blue shift observed in solution has been explained by the effect of the counter ion, which stabilizes the ground state of the protonated Schiff base.

It was shown by Blatz *et al.*,[5a,c] that increasing the size of the counter ion shifts the λ_{max} of the retinal chromophore by up to 20 nm. This increase was attributed to the larger distance between the centres of the cationic and anionic charges. To study the effect of the distance between the positive nitrogen and the counter anion we have incorporated the protonated retinal Schiff base into a polyethylene film,† which acts as a rigid matrix and restricts the free movement of the ions.

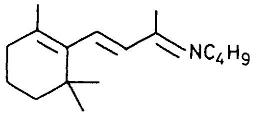
Retinal Schiff bases protonated with an excess of HCl, HBr, and $HClO_4$ absorbed in the matrix at 410, 420, and 428 nm, respectively (Table 1). These values show a similar dependence on the size of the counter anion to that in non-polar solvents but are considerably shifted to shorter wavelength. As such, they indicate small distances between the positive charges and the counter ions due to an association, probably in the form of tightly bound ion pairs, or as aggregates.

We have observed an apparent anomaly in the spectrum of retinal Schiff base protonated with CF_3CO_2H. The λ_{max} value in polyethylene was shifted to the red by 43 nm ($\Delta\nu$ = 2315 cm^{-1}) from the corresponding values of Schiff bases protonated with HCl in the same matrix, although in both polar and non-polar solvents the two salts absorbed at similar wavelengths (Table 1).

Exceptionally large red shifts of 50 and 40 nm in the spectra of retinal Schiff bases protonated with CF_3CO_2H and CCl_3CO_2H respectively have been observed by Kliger *et al.*,[8] and Blatz *et al.*,[5b] when the concentration of the acids was increased a 1000 fold in non-protic solvents. We have found, on the other hand, that a similar increase in the concentration of inorganic acids like H_2SO_4, shifts λ_{max} of this chromophore by less than 10 nm.

Concentration effects have previously been observed in the ^1H n.m.r. spectra of retinal Schiff bases protonated with CF_3CO_2H.[9] To explain this effect which occurs in CH_2Cl_2 but is absent in MeOH, Lauterwein[9] suggested homoconjugation[10] of $CF_3CO_2^-$ counter anion with a neutral CF_3CO_2H molecule. This suggestion can explain the spectral anomalies, since homoconjugation in non polar solvents as well as in the polyethylene film, will remove the $CF_3CO_2^-$ counter anion from the vicinity of the positive nitrogen atom and thereby causing a red shift in the absorption spectrum.

The effect of the counter anion on the spectrum in the polyethylene matrix decreases in retinal homologues with a shortening of their polyene chain (Table 2). Schiff base salts of the shortest member of this series, β-ionone, protonated with CF_3CO_2H or HCl exhibit the same λ_{max} in polar and non-polar solvents and in the polyethylene film. In addition, increasing the concentration of CF_3CO_2H has little effect on the λ_{max} of the $CF_3CO_2^-$ salt (shift only *ca.* 10 nm).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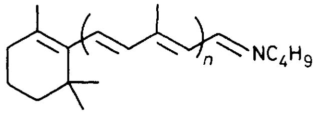
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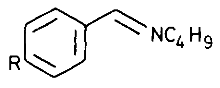
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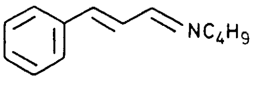
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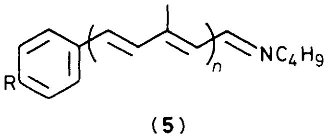
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Table 2. Absorption maxima of Cl^- and $CF_3CO_2^-$ salts of Schiff bases; values of $CF_3CO_2^-$ salts in parentheses when different from those of Cl^- salts.

		λ_{max} (nm)		
Compounds		MeOH[a]	CH_2Cl_2[a]	Polyethylene[b]
(1)		336	339	334
(2)	$n = 1$	382	390	360(393)
	$n = 2$	445	455	410(453)
(3)	R = H	274	276	276
	R = OMe	320	321	314(325)
(4)		324	324	317(323)
(5)	R = H; $n = 1$	371	375	363(375)
	R = H; $n = 2$	442	443	440(475)
	R = Me; $n = 2$	459	467	431(484)

[a] The salt was prepared by protonation of the respective Schiff base with one equivalent of the acid. [b] An excess of acid was used for the protonation.

Conversely, very large spectral shifts were observed for the Cl^- and $CF_3CO_2^-$ salts of Schiff bases of an analogue of retinal in which the cyclohexane ring was replaced by a *p*-tolyl ring. The Cl^- salt absorbs in the polyethylene film at λ_{max} 431 nm and the $CF_3CO_2^-$ salt at 484 nm ($\Delta\nu$ = 2540 cm^{-1}) (Table 2). A progressive decrease in the shift was observed when the methyl group of the aromatic ring was removed and when the polyene chain was shortened. Changing the counter anion did not change the λ_{max} of the shortest compound in this series, *N*-benzylidene-n-butylamine, in the polyethylene matrix (Table 2).

The spectral shifts of the protonated Schiff bases derived from long conjugated polyenes are the consequence of comparatively smaller delocalization of their positive charges in the ground than in the excited states. Thus, the proximity of the Cl^- counter ion to the positive nitrogen stabilizes the ground state relative to the excited state by electrostatic interaction. On the other hand, the homoconjugation of $CF_3CO_2^-$ with CF_3CO_2H removes this counter anion from the vicinity of the nitrogen and it has less influence on the chromophore transition.

It is plausible that in the Schiff bases derived from the shorter polyenes a smaller fraction of the positive charge is delocalized into the chain upon excitation and the stabilization by the Cl^- counter ion will not be much larger in the ground

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† A polyethylene film of 0.2 mm thickness was left immersed in a $CHCl_3$ solution of the Schiff base for about 24 h at room temp., washed and dried, and then dipped for a few min in a hexane solution of the respective acid.

than in the excited state and the spectral shift will be small. Conversely, the introduction of electron donating substituents into the benzene ring in the aromatic Schiff base salts tends to increase the charge delocalization in the excited states, and enhance the effect of the Cl^- on the absorption of the chromophore. Thus, Cl^- and $CF_3CO_2^-$ salts of *N*-benzylidene-n-butylamine absorb at the same wavelengths, while the Cl^- salts of the substituted derivatives are blue shifted by *ca.* 11—13 nm in polyethylene from the $CF_3CO_2^-$ salts.

We have shown that changing the counter anion in the protonated retinal Schiff bases, under specific conditions, may result in a shift of their absorption maxima by as much as *ca.* 3800 cm^{-1}. This result supports the previous suggestion[4,6] that the position of the long absorption maxima of the first intermediate in the bacteriorhodopsin photocycle, as well as in rhodopsin, is caused by the comparatively large distance between the positively charged nitrogen and the counter ion in the pigment.

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