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INTERACTION OF PURPLE MEMBRANE WITH SOLVENTS

II. MODE OF INTERACTION

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Summary

Using the solubility parameter mapping technique (Eisenbach, M., Caplan, S.R. and Tanny, G. (1979) Biochim. Biophys. Acta 554, 269—280) we studied spectroscopically the mode of interaction between the purple membrane of *Halobacterium halobium* and pure organic solvents or solvent mixtures. Although the interacting solvents formed a well-defined closed region in the interaction maps, mapping the modes of interaction did not reveal a closed region for each spectrally classifiable type. A suggested interpretation for this is that interaction with the purple membrane chromophore requires that a solvent (or solvent mixture) possess apolar groups in order to obtain access to the chromophore, together with a polar character and hydrogen-bonding capacity. The mode of interaction, however, is dependent on the specificity of the reactive group of the solvent for retinal, and this has nothing to do with membrane properties.

We also examined the influence of the duration of the interaction and of illumination. Some solvents appeared to react more sluggishly than others, but no generalization in terms of the solubility parameter mapping was found, probably because the map describes thermodynamic rather than kinetic phenomena. The only effect of illumination was to enhance the reaction of some of these solvents. It did not change the solubility parameters of purple membrane.

Introduction

Several studies of the interaction between organic solvents and the purple membrane of *Halobacterium halobium* have been carried out in recent years

[1—5] (for review see Stoeckenius et al. [6]). Most of them were concerned mainly with the mode of retinal-protein or protein-protein interactions in bacteriorhodopsin, and therefore used relatively few mixtures of water with organic solvents. These mixtures usually bleached the chromophore.

In a preceding report [7] we showed how solubility parameter mapping could be used to quantitatively classify solvents according to chemical interaction and non-interaction with purple membrane. This was based on the solubility parameters of the solvents, δ_d, δ_p, and δ_h, which represent the contribution of dispersion forces, polar forces, and hydrogen bonding, respectively, to the cohesive energy of the solvent. Such classification of solvents revealed that most of them did not interact with bacteriorhodopsin, and those which did were within a well-defined area in these maps. These interactions were examined after 24 h incubation of purple membrane in the solvents. Whether other incubation periods yield different interacting areas in the maps or different interaction modes, and whether solvents located close to each other within the interaction area have the same mode of interaction, remained to be established. Illumination of purple membrane leads to configurational and conformational changes in bacteriorhodopsin (Refs. 2, 8—11; for review see Ref. 12); do these different conditions affect the interaction maps? The present report attempts to answer these questions by having a closer look at the interaction mode of purple membrane with solvents and by making use of the solubility parameter mapping technique.

Materials and Methods

H. halobium M-1 strain was grown as described by Danon and Stoeckenius [13], using a modified growth medium [14]. Purple membrane fragments were isolated according to Oesterhelt and Stoeckenius [15]. The fragments were frozen in liquid nitrogen and then dried under vacuum. Accurately weighed, dried samples of purple membrane (about 5 mg) were suspended (using a Teflon homogenizer) under illumination in 12 ml of the test solvent. Each suspension was divided under illumination into four equal portions, and treated as follows:

Portion I was illuminated for 30 min, then rehomogenized under illumination, followed by fast spectral measurement (without illumination).

Portion II was illuminated for 30 min, then centrifuged for 10 min in a clinical centrifuge (to precipitate membrane aggregates and to decrease or prevent light scattering), and a spectrum of the supernatant was quickly recorded (no illumination provided).

Portion III was illuminated for 30 min and then kept in the dark for an additional 24 h. At the end of this period it was rehomogenized in the dark and spectrally measured. The purpose of this treatment (which is an extension of the treatment given to portion I) was to examine reversible processes that might occur in the dark following illumination.

Portion IV was illuminated for 30 min and then kept in the dark for an additional 24 h. At the end of this period it was centrifuged for 10 min in a clinical centrifuge, and spectral measurement of the supernatant was taken (no illumination provided).

Another identical 5 mg sample of purple membrane was suspended and divided into four portions in the dark, and then treated in a way similar to that of the previous sample, with the exception that all the treatments were carried out in the dark and the purple membrane was never exposed to light after being suspended (we assume that the measuring beam of the spectrophotometer is too weak to interfere with the experiment).

The spectral measurements were carried out with a Cary 15 spectrophotometer in the wavelength range 250—700 nm. Absorbances were corrected for light scattering as follows. A spectrum of non-absorbing (bleached) membrane fragments was recorded as a representative spectrum of light scattering. From this spectrum the relative value of scattering at wavelength λ in comparison to that at 680 nm was calculated (S_λ/S_{680}). In addition, the absorbance of the absorbing sample was also measured at 680 nm (A_{680}), as an indication of the light scattering of the measured sample at this wavelength (at which the absorbance of bacteriorhodopsin is practically zero). Thus, the corrected value of the absorbance at any wavelength A_λ (cor.) was calculated according to the formula, A_λ (cor.) = $A_\lambda - A_{680} \cdot S_\lambda/S_{680}$, where A_λ is the apparent absorbance (including the scattering contribution) at the given wavelength. The validity of this equation was experimentally verified by measuring the scattering of several suspensions with different concentrations of bleached membrane fragments, and plotting a calibration curve. This correction was only possible if the turbidity of the suspension was stable and no precipitate formed during the measurement. The samples were illuminated by a slide projector provided with an iodine quartz lamp (24 V, 150 W) through a Corning 3534 'cut on' filter (transferring light in the wavelength region of $\lambda \geqslant 530$ nm). Light intensity was measured by a YSI-Kettering radiometer (model 65A) and found to be 250 W/m^2.

The solvents in this study and their code numbers are listed in the preceding joint paper [7]. The values of the solubility parameters are taken from Hansen and Beerbower [16].

Results

Interaction modes

In the preceding report [7] we examined 73 individual solvents and six binary solvent mixtures for their interaction with purple membrane and classified them as 'interacting' and 'non-interacting' solvents. Table I lists all the interacting solvents (including solvent mixtures) from this representative group of 79 solvents. For close spectral examination of the interaction mode of these solvents, the spectra of both the suspension and the supernatant (after spinning down the purple membrane, see Materials and Methods) were recorded. The latter enabled us to identify products released to the medium. Table I shows a variety of interaction modes between purple membrane and the solvents, as judged from the many different absorption peaks. An attempt to identify these interaction modes, to classify them, and to evaluate their significance, is made in Discussion.

The effect of time

The results shown in Table I and in the preceding report were measured after

TABLE I

THE MODE OF INTERACTION OF SOLVENTS WITH PURPLE MEMBRANE IN THE DARK

Purple membrane fragments were suspended at room temperature in the dark for 24 h, treated as described in Materials and Methods, and then their spectra were recorded. Only solvents (individual or binary mixtures) which give absorption spectra different than 560 nm with purple membrane are included. The binary mixtures examined were 1 : 1 (by vol.). Unless light scattering did not perturb the measurement, ultraviolet spectra (250—350 nm) were recorded for the supernatants only.

Code No.	Solvent	Absorption peaks of the suspension (nm)	Absorption peaks of the supernatant after centrifugation (nm)
39.2	1,1,2,2-tetrachloroethane	390	395 300
51.2	tetrahydrofuran	360	375 280
66.2	methyl acetate	375 [a]	375
69.1	trimethyl phosphate	380	380
72.2	2-ethoxyethyl acetate	382 280	382 280
75.1	triethyl phosphate	370	375 283
89	ethanolamine	368 288	b
89.3	2-pyrrolidone	370	370 310
90	pyridine	425/400/380 (triplet) 305	b
93	aniline [a]	560 400	400 c
95.1	diethylene triamine	370	370
96	cyclohexylamine	355	355
96.1	quinoline	395	395 c
98	formamide [d]	520 370 285	520 370 285
99	dimethyl formamide	365	365 285
101	dimethyl sulfoxide	369 332	b
120	methanol	380	380
121	ethanol	382	382
121.1	ethylene cyanohydrine	390	390
122	1-propanol	380	380 270
123	2-propanol	375	372
123.1	3-chloropropanol	465 368 280	b
125	1-butanol	375	380
126	2-butanol [a]	560 380	375
126.2	benzylalcohol	395	b c
127	cyclohexanol [a]	560 400	400 290
128	1-pentanol [a]	570 395	380 255
129.1	diacetone alcohol	400	400 c
130	ethyl lactate	380	b
132.2	diethylene glycol monoethyl ether	370—400 280	b
140	formic acid	450	450 280
141	acetic acid	430	430 280
145	*m*-cresol	460	b c
150.1	propylene glycol	400	400 280
151	diethylene glycol	400	400 280
152	triethylene glycol [d]	395	395 280
153	hexylene glycol [a,d]	570 380	570 380
29 + 120	chloroform + methanol	390	390 280
36 + 89.3	carbon tetrachloride + 2-pyrrolidone	370	370 290
51.3 + 148	1,4-dioxane + water	380	385
55 + 148	acetone + water	385	385

[a] Purple aggregates were formed.

[b] No centrifugation was carried out since a clear solution was formed.

[c] Ultraviolet spectrum could not be measured because of very high absorbance of the solvent in this region.

[d] Centrifugation did not eliminate the turbidity completely.

TABLE II

THE EFFECT OF TIME ON THE INTERACTIN OF SOLVENTS WITH PURPLE MEMBRANE IN THE DARK

The spectra of four portions of purple membrane in each of the listed solvents, treated in the dark as described under Materials and Methods, were recorded (for details see text). All the solvents (including mixtures) which were studied in the preceding joint paper [7] are examined here, but only those which exhibit spectral change between 30 min and 24 h are included in this table. A_{sus} and A_{sup} stand for the absorption peaks and absorbances (in brackets) of the suspension and the supernatant after spinning down the purple membrane aggregates, respectively. Unless light scattering did not perturb the measurement, ultraviolet spectra (250—350 nm) were recorded for the supernatants only.

Code No.	Solvent	30 min				24 h			
		A_{sus} (nm)		A_{sup} (nm)		A_{sus} (nm)		A_{sup} (nm)	
39.2	1,1,2,2-tetrachloroethane	465 (0.24)		465 (0.18)	285 (0.55)	390 (0.25)		395 (0.27)	300 (0.18)
51.2	tetrahydrofuran	560 (0.04) [a,b]		370 (0.02)	285 (0.11)	360 [a]		375 (0.22)	280 (0.37)
55	acetone	560 (0.16)		none		560 (0.16)		370 (0.05)	
66.2	methyl acetate	a,b		none		a,b	375 (0.11)	375 (0.13)	
72.2	2-ethoxyethyl acetate	440 (0.19)	280 (0.80)	440 (0.19)	280 (0.80)	382 (0.25)	280 (0.23)	382 (0.25)	280 (0.23)
75.1	triethyl phosphate	560 (0.07) [a]	370 [a]	375 (0.10)	285 (0.24)	370 (0.34)		375 (0.30)	283 (0.56)
96.1	quinoline	560 (0.15)	375 (0.28)	none	c	395 (0.19)		395 (0.19)	c
98	formamide [d]	520—540 (0.15)		525 (0.07)	285 (0.32)	520 (0.04)	370 (0.12) 285 (0.37)	520 (0.02)	370 (0.16) 285 (0.35)
121.1	ethylene cyanohydrine	560 (0.13)		none		390 (0.21)		390 (0.26)	
123	2-propanol	560 (0.06)		none		a,f		372 (0.08)	
125	1-butanol	560 (0.08)		none		375 [a]		380 (0.18)	
126	2-butanol	560 (0.14)		none		560 (0.08)	380 (0.03)	375 (0.05)	
126.2	benzyl alcohol	550—560 (0.19)	430—440 (0.17)	c,e		395 (0.25)		c,e	
130	ethyl lactate	435 (0.32)		e		380 (0.40)		e	
150.1	propylene glycol	460 (0.17)		460 (0.03)	280 (0.06)	400—420 (0.07) [a]		400 (0.09)	280 (0.09)
151	diethylene glycol	440 (0.10)		440 (0.10)	280 (0.60)	400 (0.05)		400 (0.05)	280 (0.26)
152	triethylene glycol [d]	430—460 (0.12)		430—460 (0.08)	280 (0.40)	390—400 (0.32)		390—400 (0.32)	280 (0.30)
153	hexylene glycol [d]	560 (0.22)		560 (0.22)		570 (0.03) [a]	380 (0.14) [a]	570 (0.03) [a]	380 (0.14) [a]

[a] The high turbidity of the suspension prevented accurate determination of the peak location and absorbance.
[b] The suspension was purple before the centrifugation.
[c] Ultraviolet spectrum could not be measured because of very high absorbance of the solvent in this region.
[d] Centrifugation did not eliminate the turbidity completely.
[e] No centrifugation was carried out since a clear solution was formed.
[f] The suspension was brown before centrifugation.

TABLE III

THE EFFECT OF ILLUMINATION ON THE INTERACTION OF SOLVENTS WITH PURPLE MEMBRANE

Eight portions of purple membrane in each of the listed solvents or solvent mixtures were spectrally compared as described under Materials and Methods. A_{sus} and A_{sup} stand for the absorbance peaks and absorbances (in brackets) of the suspension and the supernatant *after spinning down* the purple membrane aggregates, respectively. Unless light scattering did not perturb the measurement, ultraviolet spectra (250—350 nm) were recorded for the supernatants only.

Code No.	Solvent	Illuminated sample (nm)		Sample kept in the dark (nm)	
		A_{sus}	A_{sup}	A_{sus}	A_{sup}
Incubation for 30 min					
39.2	1,1,2,2-tetrachloroethane	460 (0.24)	460 (0.24) 285 (0.28)	465 (0.24)	465 (0.18) 285 (0.55)
51.2	tetrahydrofuran	370 (approx. 0.02) [a,b]	370 (0.05) 285 (0.11)	560 (approx. 0.04) [a,c]	370 (0.02) 285 (0.11)
51.3	1,4-dioxane	570 (0.13)	none	560 (0.09)	none
55	acetone	570 (0.15)	none	560 (0.16)	none
67.1	propylene carbonate [d]	565 (0.25)	565 (0.07) 290 (0.55)	560 (0.14)	560 (0.04) 290 (0.14)
69.1	trimethyl phosphate [d]	370 (0.06) [a]	370 (0.08) 285 (0.40)	a	370 (0.10) 285 (0.40)
75.1	triethyl phosphate	570 (0.05) [a] 375 [a]	375 (0.16) 285 (0.40)	560 (0.07) [a] 370 [a]	375 (0.10) 285 (0.24)
93	aniline	565 (0.10)	e	560 (0.14)	e
96	cyclohexylamine	365 (0.28)	365 (0.28) e	365 (0.35)	365 (0.35) e
98	formamide	365 (0.15)	365 (0.13) 285 (0.34)	520—540 (0.15)	525 (0.07) [d] 285 (0.32) [d]
121.1	ethylene cyanohydrine	380 (0.12)	380 (0.15)	560 (0.13)	none
123	2-propanol	a, c	none	560 (0.06)	none
125	1-butanol	570 [a] 360 [a]	375 (0.03)	560 (0.08)	none
126	2-butanol	570 (0.06)	none	560 (0.14)	none
126.2	benzyl alcohol	430—440 (0.17)	e,g	550—560 (0.19) 430—440 (0.17)	e,g
149	ethylene glycol	460—540 (0.04) [a,h]	285 (0.30)	560 (0.11) [c]	560 (0.02) 285 (0.08)
150.1	propylene glycol	450 (0.11) [a]	275 (0.10)	460 (0.17)	460 (0.03) 280 (0.06)
150.2	1,3-butanediol [d]	570 (0.12)	570 (0.02) 295 (0.08)	560 (0.16)	560 (0.03) 295 (0.06)
51.3 + 148	1,4-dioxane + water	380 [a]	385 (0.27)	382 [a]	385 (0.27)
148 + 150.1	water + propylene glycol	570 (0.46)	570 (0.08) 285 (0.07)	563 (0.35)	560 (0.03) 285 (0.04)

Dark incubation for 24 h (following the 30 min period)

39.2	1,1,2,2-tetrachloroethane	385 (0,20)		390 (0.20)	300 (0.22)	390 (0.25)		395 (0.27)	300 (0.18)
51.2	tetrahydrofuran	370 [a]		372 (0.22)	270 (0.08)	360 [a]		375 (0.22)	280 (0.37)
51.3	1,4-dioxane	560 (0.16)		none		560 (0.11)		none	
55	acetone	560 (0.12)		370 (0.05)		560 (0.16)		370 (0.05)	
67.1	propylene carbonate [d]	560 (0.15)		560 (0.12)	290 (0.50)	560 (0.15)		560 (0.03)	290 (0.10)
69.1	trimethyl phosphate [d]	370 (0.12)		370 (0.12)	285 (0.45)	370 (0.19)		370 (0.19)	285 (0.50)
75.1	triethyl phosphate	372 (0.37)		373 (0.33)	283 (0.66)	370 (0.34)		375 (0.30)	283 (0.56)
93	aniline	560 (0.09)		400 (0.17)	e	560 (0.08)		400 (0.19)	e
96	cyclohexylamine	365 (0.38)		365 (0.38)	e	365 (0.38)		365 (0.38)	e
98	formamide	370 (0.10)	285 (0.39)	370 (0.14)	285 (0.42)	520 (0.04)	370 (0.12)	520 (0.02) [d]	370 (0.16) [d]
						285 (0.37)		285 (0.35) [d]	
121.1	ethylene cyanohydrine	380 (0.12)		390 (0.17)		390 (0.21)		390 (0.26)	
123	2-propanol	370 (0.09) [f]		372 (0.13)		a,f		372 (0.08)	
125	1-butanol	375 [a]		380 (0.21)		375 [a]		380 (0.18)	
126	2-butanol	560 (0.06)		380 (0.06)		560 (0.08)		375 (0.05)	
126.2	benzyl alcohol	390 (0.19)		e,g		395 (0.25)		e,g	
149	ethylene glycol	540—550	380 [a]	540—550	380 (0.07)	530—560		530—560	285 (0.12)
		(0.03) [a,h]		(0.02)	285 (0.32)	(0.12) [c]		(0.02)	
150.1	propylene glycol	400 (0.10)		380—400	285 (0.03)	400—420		400 (0.09)	280 (0.09)
				(0.08)		(0.07) [a]			
150.2	1,3-butanediol	560 (0.12)		295 (0.06)		560 (0.07)		295 (0.03)	
51.3 + 148	1,4-dioxane + water	380 [a]		385 (0.27)		380 [a]		385 (0.27)	
148 + 150.1	water + propylene glycol	563 (0.40)		560 (0.06)	285 (0.07)	563 (0.29)		560 (0.03)	285 (0.03)

[a] The high turbidity of the suspension prevented accurate determination of the peak location and absorbance.
[b] The suspension was brown-yellow before centrifugation.
[c] The suspension was purple before centrifugation.
[d] Centrifugation did not eliminate the turbidity completely.
[e] Ultraviolet spectrum could not be measured because of very high absorbance of the solvent in this region.
[f] The suspension was brown before centrifugation.
[g] No centrifugation was carried out since a clear solution was formed.
[h] The color of the suspension was orange-pink before centrifugation.

24 h of purple membrane suspension in the solvents. Extension of the incubation period beyond 24 h, in the dark, neither changed the number of interacting solvents nor altered the interaction mode (not shown). This was not the case when the incubation period was shortened: several solvents then showed different spectra. Table II contains a comparison of the spectral peaks of these solvents between two periods of incubation: 30 min and 24 h. As a matter of fact, all the 73 solvents and six solvent mixtures [7] were examined, but only those listed in the table behaved significantly differently at these time periods. None of the mixtures yielded any spectral difference between 30 min and 24 h of incubation with purple membrane. Two classes of solvents may be distinguished in Table II: those whose interaction with purple membrane is spectrally observable only after periods of incubation longer than 30 min (e.g. the alcohols, No. 123, 125, 126, 126.2); and those which interact during the first 30 min of incubation, but this interaction continues with time and yields other products (e.g. tetracholoroethane, No. 39.2).

The effect of illumination

Making use of the interaction maps previously described [7], we studied the effect of illumination on representative solvents from each region of these maps. This was done by comparing an illuminated sample with an identical sample kept in the dark, both being incubated in the solvents for 30 min. To determine if the observed effect of illumination is reversible (the light-adapted form of bacteriorhodopsin reverses to the dark-adapted form when not illuminated [8,17]), a fraction of the illuminated sample was kept for 24 h in the dark following the 30 min illumination period. The latter was compared with an identically treated sample which had not been exposed to light. Table III summarizes the results of this comparison. One distinct conclusion from the table is that generally after 24 h in the dark there was no difference between samples which had been previously exposed to light and samples which were kept all the time in the dark. (Ethylene glycol (No. 149) seems to be an exception: a peak at 380 nm appeared only when the sample had been previously illuminated.) However, a difference was observed when the samples were measured at the end of the 30 min of illumination. Here the solvents can be divided into three categories:

(I) Solvents which interacted with purple membrane under illumination, but not in the dark (e.g. ethylene cyanohydrine, No. 121.1). All these belong to the group of solvents which slowly interact with purple membrane (cf. Table II). Since there was no difference after 24 h between these solvents and others which had not been previously exposed to light, we may conclude that the role of illumination is only to stimulate this interaction. However, not all the solvents included in Table II show this behavior. In addition, no reversibility in the dark was observed among the solvents whose interaction was stimulated by illumination.

(II) Solvents which interact with purple membrane either in the dark or under illumination (e.g. 1,1,2,2-tetrachloroethane, No. 39.2). The interaction modes of these solvents are identical under illumination and in the dark.

(III) Solvents which do not interact with purple membrane under illumination (e.g. 1,4-dioxane). All these solvents do not interact in the dark either, and

thus exhibit a 565—570 nm absorbance peak under illumination (the light-adapted form of bacteriorhodopsin [2]) and 560 nm in the dark (the dark-adapted form).

Discussion

The interaction of purple membrane with solvents

In this discussion we shall attempt to interpret the findings in this report in the light of those reported previously [7]. Based on the interaction maps of purple membrane [7] the nature of the interacting solvents may be interpreted (though somewhat speculatively) as follows. The high hydrophobicity of the purple membrane [7] requires the interacting solvent to possess a relatively high value of δ_d, thus giving access to the hydrophobic site [3,18] of the retinal. On the other hand, in order to interact with the chromophore or to release the retinal, the solvent should also possess a polar nature and/or a hydrogen-bonding capability (as reflected in the values of δ_p and δ_h, respectively). As an example we may use the case of acetone (No. 55) and water (No. 148), neither of which interacted with purple membrane, unlike their 1 : 1 mixture which did interact [7]. Acetone does not interact with purple membrane on account of its lack of hydrogen bonds ($\delta_h = 2.5$ only). At the other extreme, water cannot reach the hydrophobic site of the retinal, being highly hydrogen bonded ($\delta_h = 20.7$) and polar, and consequently does not interact with the purple membrane. However, a 1 : 1 mixture of acetone and water is well within the interaction area, evidently because the acetone carries the water into the hydrophobic retinal site, where the water can interact with the retinal.

It seems to be generally accepted that the retinal is covalently bound to the opsin by a Schiff's base linkage to a lysine residue [19]. The question may be raised, therefore, as to how the dissociation of such a covalent bond can be explained by a solubility phenomenon. The answer probably lies in the lability of the Schiff's base linkage [20]. The reaction of Schiff's base formation is reversible and reaches equilibrium noticeably short of completion [20]. Thus, we may assume that any solvent which (a) is capable of reaching the hydrophobic site of the retinal (according to its δ_d value), and (b) according to its δ_p and δ_h values may interact with the carbonyl group of the free retinal fraction or with the ϵ-amino group of the lysine residue (according to its δ_p and δ_h values), will probably drag the equilibrium towards partial or full release of the retinal. This shift in equilibrium requires water in stoichiometric amounts. Such a small quantity may be included in the undried solvent [7] or even in the freeze-dried purple membrane.

Oesterhelt and collaborators [1—3,21] studied the interaction mode of purple membrane with mixtures of water and organic solvents, and identified the observed spectral peaks with specific species. We are aware that spectral studies are not enough to elucidate the interaction mode of the solvent with purple membrane, but nevertheless a rough estimation may be obtained. By applying Oesterhelt's identification to Table I, one concludes that most of the interacting solvents release free retinal into the solution (a 370—380 nm peak is observed even after spinning down the membrane fragments). Other (but less

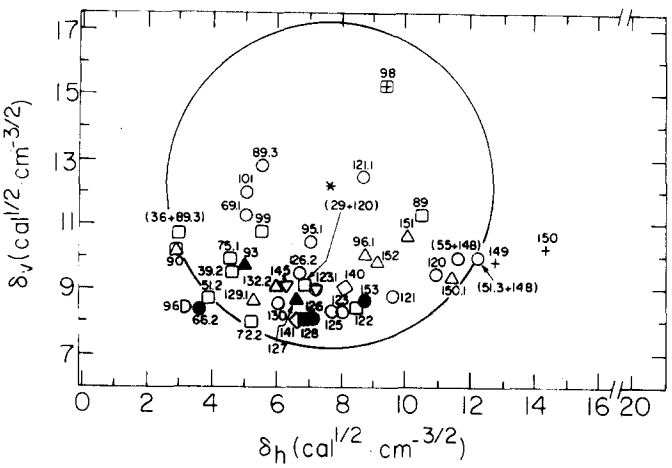

Fig. 1. Solubility parameter mapping (δ_v vs. δ_h) of the interaction mode of purple membrane with solvents. The solvents and solvent mixtures (with their corresponding code numbers) are those appearing in Table I. The data were taken from Table I. The circle was accurately redrawn from Fig. 1 in Ref. 7; *, the center of the circle. ○, solvents exhibiting an absorption peak at 370—380 nm; △, approx. 400 nm; ▽, approx. 460 nm; □, 380 and 280 nm; ◇, 440 and 280 nm; D, 355 nm; +, 520—530 nm. Full symbols mean that an absorption peak at 560 nm was observed in addition to the symbolized peak.

common) interactions may involve a non-protonated retinylidene protein (peaks at 380 nm and 280 nm *, e.g. cellosolve acetate, No. 72.2), a protonated retinylidene protein (peaks around 440 nm and 280 nm, e.g. formic acid, No. 140), a 400 nm chromophore [3] (e.g. cyclohexanol, No. 127), and a 460 nm chromophore [2,21] (e.g. *m*-cresol, No. 145). This identification of products is by no means certain, however, especially in the light of possible absorption peak shifts in different solvents.

If the above interpretation is correct, the solubility parameters can only determine which of the solvents will interact with purple membrane, but they cannot determine the mode of interaction. We therefore expect to see in the interaction maps something like random distribution of the interaction mode. In Fig. 1 the solvents and solvent mixtures of Table I were drawn according to their mode of interaction on a δ_v/δ_h map [7], and this seems to confirm the expectation. (The other modes of interaction maps discussed in Ref. 7 also yielded a random distribution.) Furthermore, Table I is in accord with this hypothesis, showing that those solvents which possess functional groups capable of reacting with either the retinal or the lysine indeed release retinal. Those solvents which lack this capability, show the other modes of interaction.

The effects of time and illumination

The theory behind solubility parameter mapping is based on thermodynamic principles (see Theory in Ref. 7). Hence, to find a correlation between the kinetics of the solvent-purple membrane interaction and location of the solvent on the interaction map is not to be expected. Modifying either of the maps in

* Peaks at 380 nm and 280 nm may also result from a separate release of retinal and protein to the medium.

Ref. 7 according to Table II, i.e. drawing interaction maps for incubation periods of 30 min, does not reveal such a well-defined interaction area as is found after an incubation period of 24 h. This is because the solvents which demonstrate different spectra at 30 min are scattered all over the interaction region. From the thermodynamic point of view, the 'correct' time for comparison of the solvents is when further incubation after the original incubation period does not alter the results. The period of 24 h fits this criterion. The present results do not supply an explanation for either the sluggish interaction of some of the solvents, or the classification of solvents based on Table II (cf. Results).

Studying the effect of illumination seemed more promising, since one might have expected that light-induced configurational and conformational changes would change the δ_i values of purple membrane, and that this would lead to a shifted interaction area in the maps. However, this was not the case. Table III demonstrated that the only clear general effect of illumination was to enhance some of the sluggish interactions. But in no case (except for ethylene glycol) did illumination cause a different behavior than that observed in the dark after 24 h, and among the 'fast' interacting solvents the interaction was identical under illumination or darkness. Furthermore, even those solvents which behaved differently after 30 min (category I in the Results) are scattered all over the interaction area of the maps. The latter observation indicates that the only effect of illumination is on the kinetics. Since the kinetic effect is not understood at present, neither is the effect of illumination. The effect of illumination can be caused either by light-induced configurational or conformational changes, or by faster interaction of one of the photointermediates of bacteriorhodopsin with the solvent (in relation to the ground state) [6,12,19]. The enhanced effect may be either higher accessibility to the solvent or increased lability of the chromophore. The present data do not allow us to distinguish between these alternatives.

In conclusion, we have demonstrated here how solubility parameter mapping can be used to reduce the number of solvents examined while nevertheless providing fuller, systematic information about the influence of external factors on the interaction between solvents and purple membrane. In principle, this technique could be applied to any membrane in which the reacting group is not exposed to the medium; in such a case a quantitative estimation of the hydrophobic and polar nature of the group's site as well as the degree of hydrogen bonding can be obtained.

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