Strong Circular Dichroism of Chlorophyll a in 50:50 Ethanol–Water Solution

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Summary Strong circular dichroic absorption bands have been found in solutions of chlorophyll a in the concentration range 10^{-5}—10^{-4}M in 50 : 50 ethanol–water mixtures, whereas in 40 : 60 and 60 : 40 mixtures of ethanol and water such absorption is very weak

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ALTHOUGH chlorophyll a (Chl a) contains three asymmetric carbon atoms,[1] solutions in ethanol show only weak circular dichroic bands When water is added to such a solution in a controlled manner, strong bands appear (Figure and Table) when the ratio of ethanol to water is in the region of 50 50

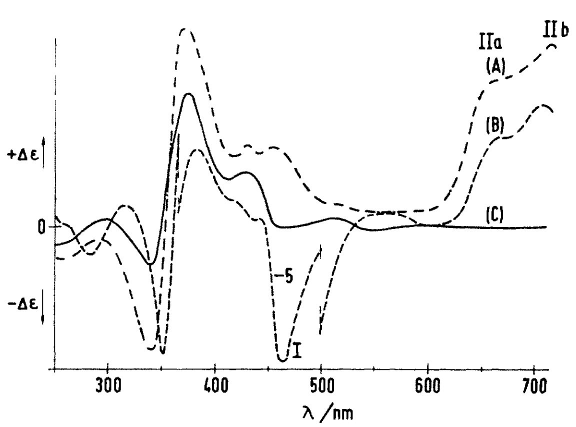

FIGURE C d spectrum of chlorophyll a in aqueous ethanol (A), 5×10^{-5} M in 50:50 EtOH–H_2O, (B), 1 25 $\times$ 10^{-4} M in 50.50 EtOH–H_2O, (C), 1 25 $\times$ 10^{-4} M in 45 55 EtOH–H_2O (For clarity, the curves are not drawn exactly to scale Exact data are given in the Table)

Further addition of water causes the intensity of the bands to fall again so that at an ethanol to water ratio of 40 : 60, the c d signal essentially disappears again A similar appearance of c d bands in ethanol–water mixtures containing >50% water has been reported by Lematre *et al* [2] for xanthophyll, they did not, however, observe any disappearance of the bands in solutions containing excess of water and they interpreted the c d bands as being due to dimer formation The xanthophyll signal was destroyed upon addition of the nonionic detergent Triton X100 Addition of Triton X100 had no effect upon the Chl a c d spectrum Buffering the solution at pH 7 also had no effect

TABLE Δε values for the curves in the Figure

λ/nm	Curve (A)	(B)	(C)
340	−161	−251	−79
372	+279	+742	+248
430	+109	+91	+94
457	+109	−1303	—
660	+197	+170	—

It was found that (a) band I was only present at concentrations $\geqslant 10^{-4}$M (*cf* curve B), (b) bands IIa and IIb decreased in intensity as the concentration of Chl a was increased above 10^{-4}M, (c) the intensities of the bands in the 250—450 nm region were not very reproducible between duplicate experiments, but the bands were less sensitive than I and IIa,b to solvent composition (compare curves C with A and B) The u v –visible absorption spectra of these solutions showed no anomalies All spectra were recorded at room temperature (*ca* 20 °C)

In ethanol Chl a forms dimers only at much higher concentrations [3] However, Chl a is insoluble in water so that it is not unreasonable to suppose that as the proportion of water is increased, dimer formation occurs at much lower concentrations When solutions of $> 5 \times 10^{-5}$M Chl a in 50% ethanol were kept overnight in a refrigerator, precipitation occurred, showing that the presence of this amount of water lowers the solubility of Chl a dramatically Assuming that the c d absorption is due to dimer formation, the disappearance of the signal upon addition of water in excess of 50% would be due to the formation of optically inactive oligomers It is not feasible to conduct meaningful c d measurements of Chl a in 100% ethanol at concentrations where dimers are formed because of the high optical densities encountered

It has been shown that Chl a in CCl_4 solution forms a high proportion of Chl a dimers [4] We found no c d absorption for Chl a in CCl_4 solution However, in CCl_4, the dimer has the simple structure $(\text{Chl a})_2$, whereas in hydrogen-bonding solvents, it has been proposed that in the dimer, Chl a is bound by solvent bridges H-bonded to the ketone functions [5,6] Other structures for Chl a aggregates have also been proposed [6]

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