Electron Donor-Acceptor Properties of 10(S)-Chlorophyll a

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Evidence for the self-aggregation of 10(S)-chlorophyll a (= chlorophyll a') in nonpolar solvents has been obtained by means of absorption spectroscopy in the visible and infrared regions. The visible absorption spectrum of chlorophyll a' exhibits a shoulder at 680 nm in aliphatic hydrocarbon solvents and the infrared spectrum measured in carbon tetrachloride contains an “aggregation peak” at 1650 cm⁻¹. IR spectroscopy shows, in addition, that the C-10 epimers of chlorophyll a are both present as monomers in tetrahydrofuran. However, in the presence of a small amount of aliphatic alcohol or water, the IR spectrum in tetrahydrofuran at lowered temperatures exhibits an intense, wide absorption band at ~1650 cm⁻¹ which was interpreted as arising from the hydrogen bonding of the hydroxyl group to the C-9 keto carbonyl of the chlorophyll.

Introduction

The electron donor-acceptor or coordination properties of chlorophyll (Chl) molecules are of particular interest in relation to the organization and function of these molecules in photosynthetic membranes [1–5]. According to the structural formula usually written for Chl a (I), the coordination number of the central magnesium atom is 4. Recent investigations [6–11] by IR and NMR spectroscopy have shown, however, that there is a strong tendency for one or both of the magnesium axial positions to be occupied by an electron donor ligand (nucleophile, Lewis base, polar solvent, amino acid sidechain of a protein). In monofunctional polar solvents (acetone, tetrahydrofuran, diethyl ether, pyridine), monomeric chlorophyll species, Chl • L₁ or Chl • L₂, are formed. In bifunctional polar solvents (dioxane, pyrazine, water) cross-linked chlorophyll species can be formed. Water is a particularly important bifunctional ligand, as it can form water-linked Chl dimers or adducts with exhibit spectroscopic properties similar to those of P 700 in the reaction center I of photosynthesis [1–4]. More significantly, the Chl-water adducts are also photoactive, i.e. they are photo-oxidized to the corresponding cation radicals in the presence of a suitable electron acceptor, e.g. tetranitromethane [1, 4]. In the absence of extraneous nucleophiles, the C-9 keto carbonyl of one Chl molecule can function as an electron donor to the central magnesium of another Chl molecule. This results in the formation of dimers and oligomers, (Chl)ₙ. On the basis of the similarities between the electronic absorption spectrum of the in vivo chlorophyll and that of the in vitro prepared oligomeric chlorophyll, it has been suggested that the “antenna chlorophyll” consists of chlorophyll oligomers [2–4].

While there has been considerable progress in attempting to relate the structure and properties of the Chl molecule to its function in photosynthesis, several open questions still remain. One of these is the relationship between the stereochemical and the electron donor-acceptor properties [2–4] in the Chl molecule. To answer this question, we have investigated the aggregation properties of 10(S)-chlorophyll a (= Chl a', 2) which is epimeric with the natural Chl a (1) at C-10. Previous work [12] had suggested that the unexpected properties of Chl a' are best interpreted in terms of the conformational alterations arising from the single stereochemical change at C-10. The unfavorable stereochemical properties of the prime derivative prevent it from forming precipitous chlorophyll-water adducts. It also seems likely that the C-9 keto carbonyl of Chl a' has reduced coordination tendency in comparison to Chl a, owing to the steric hindrance caused by the C-10 methoxy carbonyl group [12–14]. The first experimental evidence for the self-aggregation of Chl a' in nonpolar solvents has been provided by the 'H NMR spectra of Chl a' measured in benzene-d₆ (c_chl = 10⁻² M) [12]. This paper reports further evidence for the aggregation of Chl a' in nonpolar solvents. This evidence, which is based on visible and infrared absorp-
tion spectroscopy, is contradictory to the proposal [15] that Chl a' essentially lacks any acceptor properties at its central magnesium atom.

**Experimental**

*Absorption spectra*

The electronic absorption spectra were recorded on a Cary 219 spectrophotometer at 25 °C using 1.0 cm sample cells. The infrared absorption spectra were measured on a Beckman AccuLab 6 IR spectrophotometer.

*Chlorophyll a (1)*

The natural chlorophyll a with 10(R) configuration, was isolated from *Trifolium*-leaves as described previously [14, 16]. On the basis of spectroscopic and other purity criteria [17], the chlorophyll preparation was of a high purity.

*Chlorophyll a' (2)*

The prime chlorophyll with 10(S) configuration, was prepared from 1 by the method developed recently [17]. UV/Vis and 1H NMR spectra of the preparation were identical with those previously reported [17], and TLC on sucrose [18] revealed the presence of only one component.

*Purity of solvents*

The organic solvents (light petroleum, b.p. 60–80 °C, 1-propanol, carbon tetrachloride and tetrahydrofuran) were of reagent grade purity (Merck, Darmstadt). They were used as provided except for drying.

**Results and Discussion**

On washing a ~ 10^{-4} M solution (100 ml) of Chl a' in light petroleum containing 0.5% 1-propanol with
cold water, the colour of the solution changed from blue-green to green. No chlorophyll-water adducts precipitated, however, as a result of this treatment, and the visible absorption spectrum measured from this solution (Fig. 1, solid line) showed that the chlorophyll was still predominantly in a monomeric state. After permitting the washed solution to stand overnight at −30 °C, the visible absorption spectrum (Fig. 2, broken line) was quite similar to that described for Chl a dimers [19]. When the solution was allowed to stand at −30 °C for several days, crystal-like black micelles were formed. This shows that also Chl a′ can form oligomeric molecular species, (Chl)ₙ. The 'H NMR spectrum measured from the precipitated Chl a′ in benzene-d₆ was similar to that reported for the aggregated Chl a in nonpolar solvents [6]. The addition of a small amount of acetone-d₆ to the benzene solution caused disaggregation, and a proton spectrum similar to that reported for monomeric Chl a′ [12], was obtained.

Fig. 2 presents a comparison of the carbonyl stretch regions in the IR spectra of Chl a (A) and Chl a′ (B), both measured in dry carbon tetrachloride. The similarity of these IR spectra is obvious. Both spectra show an absorption band at 1740 cm⁻¹ which arises from the C-10a and C-7a ester carbonyl vibrations, and another band at 1695 cm⁻¹ caused by the C-9 keto carbonyl. The band at ~1650 cm⁻¹ is the “aggregation peak” resulting from the 9-C = O···Mg interaction [6, 9]. The fact that this band is of lower intensity in spectrum B as compared to spectrum A, supports the view that
the C-9 keto carbonyl of Chl a' is a somewhat weaker electron donor than that of Chl a [12, 13].

When solid Chl a' was dissolved in dry tetrahydrofuran (THF), the IR spectrum shown in Fig. 2C was obtained. This spectrum is closely similar to that reported for Chl a in THF [6]. However, if a $\approx 10^{-4}$ M solution of Chl a' in light petroleum containing 0.5% 1-propanol, was directly evaporated to near "dryness" at reduced pressure and temperature and the residue dissolved in cold (0 °C) THF, the IR spectrum shown in Fig. 2D was obtained. This spectrum exhibits an intense, broad band at $\sim 1650$ cm$^{-1}$ which probably arises from the hydrogen bonding of the hydroxyl group of propanol or water to the C-9 keto carbonyl. A confirmation to this conclusion has recently been obtained by $^1$H NMR spectroscopy [20].

It has been postulated [15] that the central magnesium atom of Chl a' essentially lacks any electron acceptor properties. This postulation is, however, in discrepancy with the present results which show that also Chl a' self-aggregates and probably with the same mechanism as Chl a, i.e. the central magnesium atom of one Chl molecule accepts the lone-pair electrons of the C-9 keto carbonyl of another Chl molecule. Further evidence against the postulation [15] is provided by the comparison of the electronic absorption spectra of Chl a and Chl a' in THF [21]. Both spectra show the $Q_{0-0}$ band located principally at 626 nm, which has been interpreted as indicating the formation of hexacoordinated Chl species [22, 23].

In summary, the present results indicate that Chl a' can form self-aggregates comparable to those formed by Chl a. Nevertheless, the aggregation tendency of Chl a' appears to be somewhat weaker than that of Chl a. This effect can be interpreted in terms of the steric hindrance of the C-9 keto carbonyl by the C-10 methoxycarbonyl in Chl a'. The electron acceptor properties of the central magnesium atom of Chl a' appear to be quite similar to the corresponding properties of Chl a.

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