Proc. Natl. Acad. Sci. USA
Vol. 73, No. 12, pp 4282–4286, December 1976
Chemistry

Covalently linked chlorophyll *a* dimer: A biomimetic model of special pair chlorophyll

(photosynthesis/photo-reactive chlorophyll/photo-oxidized chlorophyll/chlorophyll–ligand interactions/chlorophyll–protein interactions)

MICHAEL R. WASIELEWSKI, MARTIN H. STUDIER, AND JOSEPH J. KATZ

Chemistry Division, Argonne National Laboratory, Argonne, Illinois 60439

Contributed by Joseph J. Katz, October 4, 1976

ABSTRACT **The synthesis of a covalent dimer of chlorophyll *a* which possesses properties strikingly similar to those exhibited by P700 special pair chlorophyll *in vivo* is described. The covalent dimer is characterized by several spectroscopic techniques. Hydrogen bonding nucleophiles, such as water, primary alcohols, and primary thiols, are effective in generating a species from solutions of 10 μM covalent dimer in hydrophobic solvents which absorbs light near 700 nm. Formation of this *in vitro* special pair is a rapid, spontaneous process at room temperature. The range of nucleophiles which promote this process suggests that amino acid residues may function in a similar fashion to form P700 in chlorophyll–protein complexes. The photochemical properties of this *in vitro* special pair mimic those of *in vivo* P700 species. The 697 nm absorption of the *in vitro* special pair undergoes photo-bleaching rapidly in the presence of iodine that results in the production of a cation radical which exhibits an electron spin resonance signal similar to that of oxidized P700 observed in *Chlorella vulgaris*.**

Investigations into the molecular organization of chlorophyll in green plants strongly suggest that the chlorophyll molecules act cooperatively in the primary light absorption process of photosynthesis (1–3). The light energy is captured by an extended array of antenna chlorophyll molecules that absorb strongly at 678 nm (4). The electronic excitation produced by the light absorption is transmitted via the antenna chlorophyll to a special pair of chlorophyll molecules absorbing at about 700 nm, that functions as a low energy trap (5). The trapping of the excitation energy by the P700 special pair results in one electron oxidation of the pair to yield the special pair cation radical, and an electron which ultimately furnishes the reducing potential of photosystem I (6). There is good evidence from studies *in vivo* that the species designated P700 is actually a pair of chlorophyll *a* (Chl *a*) molecules.

In the visible spectra of systems *in vivo*, P700 undergoes bleaching upon illumination with red light to a greater extent than does P680 (7–10). The subsequent appearance of a photo-induced electron spin resonance signal of about 7 gauss linewidth with g = 2.0025 suggests the formation of $P700^{+}$ (11). A comparison between the signal of monomeric Chl^{+} and that of $P700^{+}$ reveals that the linewidth due to the *in vivo* species is narrower by $1/\sqrt{2}$ than that of *in vitro* Chl^{+}. This result agrees with theory regarding delocalization of one electron spin equally over two Chl *a* molecules. Further studies of *in vivo* systems by electron nuclear double resonance (ENDOR) spectroscopy (12) support the special pair proposal regarding photoreaction center chlorophyll.

Two distinct models have recently been proposed for the structure of special pair chlorophyll. The model of Shipman *et al.* (13) links two Chl *a* molecules by coordinating the magnesium atom of one Chl *a* to a nucleophile, e.g., ROH, which in turn hydrogen bonds to the keto carbonyl group of the second Chl *a*. Two such interactions result in a structure possessing C_2 symmetry with an approximate 8.9 Å Mg–Mg distance and 3.6 Å interplanar separation between the macrocycles. In this model, the carbomethoxy groups are pointed away from the center of the structure and the phytyl tails extend in similar directions from the macrocycles.

Spectroscopic evidence indicates that the keto carbonyl group of Chl *a* is the primary nucleophilic donor moiety and is of principal importance in Chl *a* aggregation (14–16). This evidence also supports a minor role for the carbomethoxy group as a donor. Nevertheless, Fong has proposed a model (17) for the structure of the special pair based on the coordination of a water molecule to the magnesium of one Chl *a* molecule, that in turn hydrogen bonds to the carbomethoxy group of a second Chl *a*. In this model, two such interactions result in a C_2 symmetric structure with the carbomethoxy groups pointing toward the interior of the structure. This results in a larger interplanar distance (about 5.6 Å) than the Shipman model with the phytyl tails situated at opposite ends of the molecule. The Fong model is based to a great extent on theoretical arguments.

Further insight into the structure of the special pair may be obtained through a biomimetic approach based on carefully chosen *in vitro* structures. The examination of well-defined *in vitro* structures has proven exceedingly useful (3) in developing an understanding of photosynthetic processes *in vivo*.

Recent results from this laboratory (13) showed that concentrated solutions of Chl *a* in toluene containing a 1.5 molar excess of ethanol, when cooled to dry ice temperatures, form predominantly a species which absorbs light at 700 nm. In addition, these solutions exhibit an additional infrared absorption band in the carbonyl region due to hydrogen bonding of the keto carbonyl group. Fong and Koester have also observed 700 nm absorption by Chl *a* solutions in hydrocarbons containing water at low temperatures (18).

The entropy requirements for the formation of P700 chlorophyll in hydrocarbon solvents are severe as noted by the need to use high chlorophyll concentrations and low temperatures. This problem may be overcome by chemically linking two intact Chl *a* macrocycles. This linkage should bring the two Chl *a* macrocycles to within approximately the same distance that they might occur within a P700 protein, a probable location for the *in vivo* special pair. Due to the synthetic difficulties involved in preparing a Chl *a* dimer, a recent communication (19) outlined the properties of a covalent pyrochlorophyll *a* dimer. Although this molecule is capable of forming a P700 species, it cannot reflect the rather stringent steric requirements imposed by the carbomethoxy group. It is well known that the keto carbonyl group of pyrochlorophyll *a* functions as a stronger donor moiety than does that of Chl *a* (3). This may be due to steric restrictions imposed by the carbomethoxy group. In addition, the interplanar distance between the macrocycles in the

Abbreviation: Chl, chlorophyll.

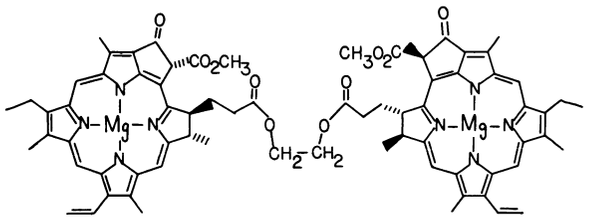

FIG. 1. The structural formula of bis(chlorophyllide *a*) ethylene glycol diester.

special pair models is a strong function of the stereochemistry at C-10. To better evaluate the proposed structures of the special pair, we synthesized a biomimetic model of the special pair by covalently linking two Chl *a* macrocycles.

SYNTHETIC ASPECTS OF THE COVALENT Chl *a* DIMER

The synthesis of a covalently linked Chl *a* dimer is difficult because of the intrinsic reactivity of the Chl *a* macrocycle. Chlorophyll *a* is rapidly degraded by a variety of conditions normally encountered in synthetic work. For example, treatment of Chl *a* with acid at pH < 3 results in expulsion of its magnesium atom (20). On the other hand, strong bases and nucleophiles react with the ketone at C-9 and cleave ring V (21–22). Chlorophyll *a* is also readily oxidized at C-10 (23). Finally, heating Chl *a* in the presence of even weakly basic solvents results in decarboxylation at C-10 to yield pyrochlorophyll *a* (24). Thus, Chl *a* reactivity results primarily from the presence of the β keto ester group in moderately strained ring V.

Although removal of the 10-carbomethoxy group alleviates many of these problems, various structural models of the P700 species utilize the carbomethoxy group as a point of hydrogen bonding interactions (5, 17). In addition, the stereochemistry about C-10 is an important consideration in any structural proposal. Consequently, the synthesis of an unaltered dimer of Chl *a* is most desirable.

The original work of Willstätter and Stöll (25), later amplified by Fischer and Lambrecht (26), disclosed that the phytyl ester of Chl *a* can be selectively hydrolyzed by the enzyme chlorophyllase with retention of the magnesium atom or by strong aqueous acid to yield the free acids, chlorophyllide *a* and pheophorbide *a*, respectively. Similarly, in the presence of an excess of simple alcohols, e.g., methanol, the same reactions yield the corresponding transesterified alkyl chlorophyllide *a* (26) or alkyl pheophorbide *a* (27). Thus, the propionic acid side chain of the macrocycle may be used as a starting point to construct a linked dimer in the form of a bis(chlorophyllide *a*) diester of an alkanediol, e.g., ethylene glycol, without altering the functionality in ring V. The structure of this molecule is presented in Fig. 1.

There are numerous mild esterification procedures available in the literature (refs. 28–30 are representative). The majority of these methods are adaptations of known peptide linkage procedures. All involve the preparation of an activated ester of the carboxylic acid with subsequent alcoholysis to form the desired ester. However, many of these procedures require conditions which destroy the integrity of ring V. Thus, the available methods are somewhat limited. We have found that the preparation of pheophorbide *a* esters proceeds smoothly, in high yield at room temperature, when the acid, dissolved in dry tetrahydrofuran containing an excess of pyridine, is activated with methyl chloroformate (31). The resulting mixed

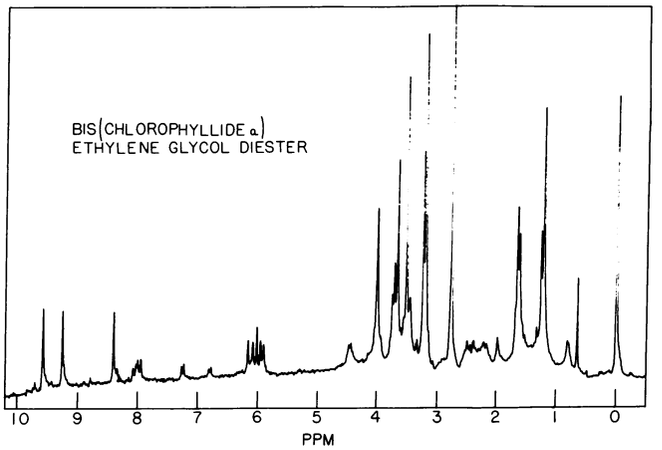

FIG. 2. The 220 MHz proton nuclear magnetic resonance spectrum of bis(chlorophyllide *a*) ethylene glycol diester in acetone-d_6 with hexamethyldisiloxane as an internal reference (0.00 ppm).

anhydride is treated *in situ* with an excess of the esterifying alcohol. In this fashion, pheophorbide *a* ethylene glycol monoester is produced rapidly in high yield (>90%).

The reaction of pheophorbide *a* ethylene glycol monoester with a molecule of pheophorbide *a* activated by methyl chloroformate is slow, relative to hydrolysis of the carbonic anhydride intermediate by traces of water in the reaction mixture. Consequently, no coupling occurs. Thus, coupling requires that the activated ester survives long enough to couple with the glycol, and that no other nucleophiles are present to react with the activated ester. Very few methods comply with these restrictions at the same time preserving the chemical integrity of ring V.

Coupling is achieved by reacting a concentrated (~0.1 M) solution of a 2:1 molar ratio of pheophorbide *a* ethylene glycol monoester to pheophorbide *a* in pyridine at 0° with a slow stream of phosgene. The phosgene is a mild acylating agent which activates the carboxylic acid (presumably by formation of the acid chloride) and does not attack ring V. Aqueous workup and then chromatography on silica gel results in a 17% yield of dimer.

The magnesium atoms are inserted into the bis(pheophorbide *a*) ethylene glycol diester by the recently published method of Eschenmoser (32). All magnesium complexing procedures applicable to simpler phorbins fail. Further synthetic details concerning this preparation will appear elsewhere.

SPECTROSCOPIC CHARACTERIZATION OF THE COVALENT Chl *a* DIMER

The structure of bis(chlorophyllide *a*) ethylene glycol diester was established from data obtained by various spectroscopic techniques. The 220 MHz proton nuclear magnetic resonance spectrum (^{1}H NMR) of the dimer in acetone-d_6 is illustrated in Fig. 2. The striking feature of the spectrum is the close correspondence between the proton chemical shifts of the dimer with those of monomeric Chl *a* species (14). Thus, the two macrocycles under these solvation conditions are both independent and equivalent, and result in complete overlap of their respective resonances. The unique resonance at 4.00 parts per million (ppm) is assigned to the four ethylene glycol protons of the link. This sharp singlet indicates that not only are both macrocycles equivalent, but rotation about the C–C bond of the link is rapid on the nuclear magnetic resonance time scale. If these conditions were not fulfilled, then the glycol resonance would appear as either an A_2B_2 or ABCD multiplet.

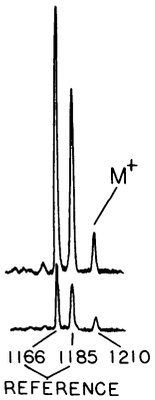

FIG. 3. A portion of the time-of-flight mass spectrum of bis-(pheophorbide *a*) ethylene glycol diester showing the parent ion (M^+) and the mass reference peaks labeled with their recorded mass.

Each resonance in the spectrum with the exception of the glycol resonance shows either broadening or the appearance of small satellite resonances. These effects are due to the presence of an equilibrium amount (~15%) of the *a'* diastereomer at C-10 in each macrocycle (33). This equilibrium is rapidly established when all chlorophyll molecules with β-keto-ester functions intact are dissolved in polar solvents exhibiting even weak basicity.

Because the molecule is dimeric, the stereoisomerism at C-10 results in a distribution which consists of about 73% *a*–*a* dimer, 25% *a*–*a'*, dimer, and 2% *a'*–*a'* dimer. As will be seen below, this distribution may be utilized to obtain information regarding the structure of special pair Chl *a*.

The mass spectroscopic determination of the molecular weight of the covalent dimer is essential to a secure characterization of the compound. However, it is very difficult to observe the parent ion of an organic compound with a molecular weight greater than 1000. Thermal decomposition of the molecule is significant at temperatures required to volatilize the compound. Because the volatility of pheophytin derivatives is greater than that of the corresponding chlorophylls, the magnesium-free dimer was examined. Most of the experimental difficulties were circumvented by the use of time-of-flight mass spectrometry. By using a thermal ionization source, we recorded the mass of the parent ion of bis(pheophorbide *a*) ethylene glycol diester at mass 1210 ± 1. This mass corresponds exactly to that calculated for the dimer. The mass spectrum obtained by this technique is illustrated in Fig. 3.

The visible absorption spectrum of bis(chlorophyllide *a*) ethylene glycol diester in ether is indistinguishable from the spectrum of Chl *a* recorded under identical conditions (34). Thus, the two chlorophyll *a* chromophores are electronically independent in highly polar media. In the presence of solvents in which monomeric Chl *a* is known to exist, the properties of the covalent dimer as indicated by all available spectral data are not unique. In solvent systems which promote Chl *a* aggregation, however, the singular properties of the covalent dimer become apparent.

BIOMIMETIC PROPERTIES OF THE COVALENT Chl *a* DIMER

The long wavelength portion of the visible absorption spectrum of 10 μM covalent dimer in dry CCl_4 is illustrated in Fig. 4A. The intense red absorption occurs at 677 nm. By comparison, the spectrum of Chl *a* recorded under the same conditions exhibits a broad absorption centered at 674 nm (35). Chlorophyll

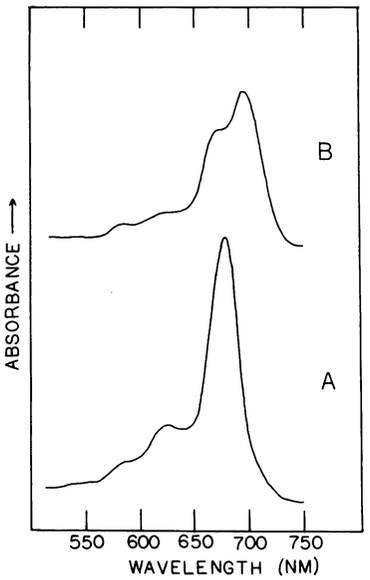

FIG. 4. The visible absorption spectrum of 10 μM bis(chlorophyllide *a*) ethylene glycol diester in (A) dry carbon tetrachloride; (B) water-saturated carbon tetrachloride.

a, at concentrations of 10 mM–10 μM in dry CCl_4, is known to form dimers (35). The keto carbonyl oxygen atom of one Chl *a* is coordinated to the Mg of the other Chl *a*; thus, one Chl *a* functions as a donor molecule while the other is an acceptor. We suspect that the covalent dimer aggregates intramolecularly at 10 μM. An examination of space-filling models of the covalent dimer reveals that the 10 atom chain linking the two macrocycles is sufficiently long to easily permit the keto carbonyl oxygen of one macrocycle to coordinate with the Mg of the other macrocycle. Further work is needed for an assignment of the electronic transitions comprising the red absorption band of the self-aggregated covalent dimer.

If a 10 μM solution of the covalent dimer in dry CCl_4 is bubbled with wet nitrogen, then the visible absorption spectrum changes dramatically. The intensity of the band at 677 nm diminishes and is simultaneously replaced with a band at 697 nm. These changes result in the spectrum illustrated in Fig. 4B. The 20 nm red shift of the principal absorption band can be attributed to the *in vitro* formation of a species similar to the P700 photo-reaction center chlorophyll *a* dimer observed *in vivo*.

Previous work on preparing photoactive Chl *a* species, which absorb red light at 700 nm, involved cooling hydrocarbon solutions of Chl *a* containing various amounts of ethanol (13) or water (18) to low temperatures (about −100°). These low temperatures indicate that the loss in free energy of the system due to structural folding is primarily in the $T\Delta S$ term. The covalent linkage of two Chl *a* macrocycles reduces the entropy of the system, thus allowing hydrogen bonding interactions between the macrocycles and water to fold the covalent dimer into the red-shifted species readily at room temperature.

Water is not the only hydrogen bonding nucleophile capable of interacting with the covalent dimer to form an *in vitro* special pair. Primary alcohols such as ethanol and methanol are as effective as water in promoting formation of the species absorbing at 697 nm. In addition, preliminary results indicate that primary alkanethiols are also effective in this regard. A 10 μM solution of covalent dimer in CCl_4 containing 10 mM ethanol or methanol exhibits a visible absorption spectrum identical with that in Fig. 4B. An increase in the alcohol concentration is expected to disaggregate the covalent dimer through coor-

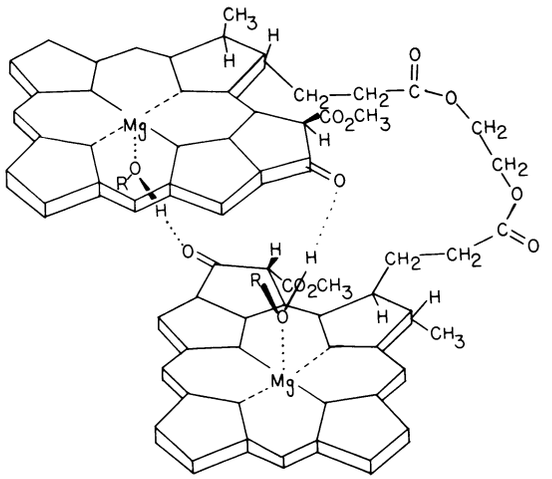

FIG. 5. The proposed structure of bis(chlorophyllide *a*) ethylene glycol diester in a hydrophobic solvent containing an excess of the hydrogen bonding nucleophile ROH.

dinative saturation of the magnesium atom in each macrocycle (5). The stability of the folded covalent dimer structure is illustrated by the need to employ an alcohol concentration in excess of 0.1 M to fully disrupt the long wavelength species.

The ability of non-aqueous hydrogen bonding nucleophiles to induce the formation of the *in vitro* special pair suggests the intriguing possibility that the nucleophiles commonly found in several amino acid residues such as serine, tyrosine, and cysteine may in fact be responsible for *in vivo* P700 formation in chlorophyll–protein complexes. The isolation of several active P700 chlorophyll–protein complexes point towards this possibility (for a comprehensive review of this area see ref. 36).

It is important to note that in addition to CCl_4, we have found that other non-polar solvents, such as benzene and toluene, serve as excellent solvents for the formation of the *in vitro* P700 species. This suggests the need for a hydrophobic environment surrounding the special pair.

Further examination of the visible absorption spectrum in Fig. 4B reveals that a small amount of the species absorbing at 677 nm remains in the water saturated CCl_4 solution. The intensity of the 697 nm band relative to that at 677 nm is about 3:1. By recalling that the covalent dimer is a mixture of diastereomers about the C-10 position in each macrocycle, we find it necessary to examine the stereochemistry of the carbomethoxy groups in relation to the ability of the dimer to fold into a structure capable of exhibiting a 700 nm visible absorption. The *a*–*a* and *a'*–*a'* dimers are both able to fold into the closely spaced, C_2 symmetric, stacked structure proposed by Shipman *et al.* (13). In this structure, illustrated in Fig. 5, both carbomethoxy groups point away from the interior of the structure. However, space-filling models predict that the *a*–*a'* isomer, comprising about 23% of the equilibrium mixture, should have difficulty folding into a similar structure due to steric compression of one carbomethoxy group between the macrocycles. This suggests the possibility that the residual absorption at 677 nm is due to the *a*–*a'* covalent dimer. Preliminary results of nuclear magnetic resonance studies indicate that the C_2 symmetric structure of Shipman *et al.* (13) is correct. The analysis is complicated by the presence of an isomeric mixture of covalent dimers, as noted above.

An identification of the covalent dimer *in vitro* as the biomimetic analog of the P700 species *in vivo* demands that the covalent dimer exhibit the photochemical properties observed for P700 *in vivo* (11, 37). When a 10 μM solution of covalent dimer in water-saturated CCl_4 was treated with an equimolar amount of I_2 (E_0 = 0.54 V), bleaching of only the 697 nm absorption occurred in the dark over a period of 10 min. In a similar experiment, the sample was illuminated by red light (light from a 200 W incandescent lamp was filtered through 2 inches of H_2O and a Corning 2030 filter, $\lambda > 648$ nm) that resulted in complete bleaching of the 697 nm absorption within 30 sec. These experiments indicate that the oxidation potential of the *in vitro* special pair must be close to 0.5 V. The oxidation potential of P700 from spinach chloroplasts has been estimated as 0.43 V by Kok (37). Thus, the biomimetic special pair exhibits redox behavior quite similar to the *in vivo* system despite the environmental differences between the two systems. In another experiment, a 1 mM solution of covalent dimer in water-saturated CCl_4 containing an equimolar amount of I_2 was irradiated with red light (300 W Eimac Xenon arc lamp, Corning 2030 filter, $\lambda > 648$ nm) in the microwave cavity of an electron spin resonance spectrometer. An intense Gaussian signal with a linewidth of 7.54 gauss was observed. This linewidth corresponds well to that predicted by theory for an electron delocalized over two chlorophyll *a* macrocycles (11). The linewidth of the signal from the oxidized *in vitro* special pair compares favorably with the linewidth of the electron spin resonance signal obtained via photooxidation of P700 in *Chlorella vulgaris* (11). Further work is in progress to determine the reversibility of the photooxidation of the *in vitro* special pair.

CONCLUSIONS

We have synthesized a covalent dimer of Chl *a* which possesses properties strikingly similar to those exhibited by P700 special pair Chl *a* *in vivo*. We have shown that several hydrogen bonding nucleophiles are effective in generating a species from a solution of the covalent dimer in hydrophobic solvents which absorbs light near 700 nm. Once the entropy of bringing two Chl *a* molecules close together has been overcome, *in vitro* special pair formation is a rapid, spontaneous process at room temperature. The range of nucleophiles which promote this process suggests that amino acid residues might function in a similar fashion to form P700 in chlorophyll–protein complexes.

The photochemical properties of the covalent dimer are truly biomimetic. The *in vitro* special pair undergoes photobleaching rapidly in the presence of iodine that results in the production of a cation radical which exhibits an electron spin resonance signal which is narrowed compared to that of monomeric Chl^+ due to delocalization of the odd electron over two Chl *a* macrocycles. These properties are exactly analogous to *in vivo* P700 special pair Chl *a*.

These and other biomimetic properties of the covalent dimer are currently under further investigation. The *in vitro* special pair is an extremely useful model in exploring new concepts concerning photoreaction centers in photosynthesis.

We thank Messrs. Walter Svec and Ben Cope for preparing the pheophorbide *a* used in this study. We also thank Dr. Walter Oettmeier for assistance in obtaining the electron spin resonance data. This work was performed under the auspices of the U.S. Energy Research and Development Administration.

1. Emerson, R. & Arnold, W. (1931–1932) *J. Gen. Physiol.* **15**, 391–420.
2. Emerson, R. & Arnold, W. (1932–1933) *J. Gen. Physiol.* **16**, 191–205.
3. Katz, J. J., Oettmeier, W. & Norris, J. R. (1976) *Phil. Trans. R. Soc. London Ser. B* **273**, 227–253.
4. French, C. S., Brown, J. S. & Lawrence, M. C. (1972) *Plant Physiol.* **49**, 421–429.

5. Clayton, R. K. (1973) *Annu. Rev. Biophys. Bioeng.* **2,** 131–156.
6. Katz, J. J. & Norris, J. R. (1973) in *Current Topics in Bioenergetics,* ed. Sanadi, D. R. (Academic Press, New York), Vol. 5, pp. 41–75.
7. Kok, B. (1957) *Nature* **179,** 583–584.
8. Murata, N. & Takamiya, A. (1969) *Plant Cell Physiol.* **10,** 193–202.
9. Hiyama, T. & Ke, B. (1971) *Arch. Biochem. Biophys.* **147,** 99–108.
10. Philipson, K. D., Sato, V. L. & Sauer, K. (1972) *Biochemistry* **11,** 4591–4595.
11. Norris, J. R., Uphaus, R. A., Crespi, H. L. & Katz, J. J. (1971) *Proc. Natl. Acad. Sci. USA* **68,** 625–628.
12. Norris, J. R., Sheer, H., Druyan, M. E. & Katz, J. J. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 4897–4900.
13. Shipman, L. L., Cotton, T. M., Norris, J. R. & Katz, J. J. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 1791–1794.
14. Closs, G. L., Katz, J. J., Pennington, F. C., Thomas, M. R. & Strain, H. H. (1963) *J. Am. Chem. Soc.* **85,** 3809–3821.
15. Katz, J. J., Closs, G. L., Pennington, F. C., Thomas, M. R. & Strain, H. H. (1963) *J. Am. Chem. Soc.* **85,** 3801–3809.
16. Shipman, L. L., Janson, T. R., Ray, J. G. & Katz, J. J. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 2873–2876.
17. Fong, F. K. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 3692–3695.
18. Fong, F. K. & Koester, V. J. (1976) *Biochim. Biophys. Acta* **423,** 52–64.
19. Boxer, S. G. & Closs, G. L. (1976) *J. Am. Chem. Soc.* **98,** 5406–5408.
20. Willstätter, R. & Hocheder, F. (1907) *Justus Liebigs Ann. Chem.* **354,** 205–258.
21. Fischer, H. & Bäumler, R. (1929) *Justus Liebigs Ann. Chem.* **474,** 65–120.
22. Weller, A. & Livingston, R. (1954) *J. Am. Chem. Soc.* **76,** 1575–1578.
23. Conant, J. B., Hyde, J. F., Moyer, W. W. & Dietz, E. M. (1931) *J. Am. Chem. Soc.* **53,** 359–373.
24. Pennington, F. C., Strain, H. H., Svec, W. A. & Katz, J. J. (1964) *J. Am. Chem. Soc.* **86,** 1418–1426.
25. Willstätter, R. & Stöll, A. (1910) *Justus Liebigs Ann. Chem.* **378,** 18–72.
26. Fischer, H. & Lambrecht, R. (1938) *Z. Physiol. Chem.* **253,** 253–286.
27. Stöll, A. & Wiedermann, E. (1933) *Helv. Chim. Acta* **16,** 183–206.
28. Staab, H. A. (1962) *Angew. Chem. Int. Ed. Engl.* **1,** 351–367.
29. Shaw, J. E., Kunerth, D. C. & Sherry, J. J. (1973) *Tetrahedron Lett.,* 689–692.
30. Gerlach, H. & Thalmann, A. (1974) *Helv. Chim. Acta* **57,** 2661–2663.
31. Wieland, T., Faesel, J. & Faulstich, H. (1968) *Justus Liebigs Ann. Chem.* **713,** 201–208.
32. Isenring, H., Zass, E., Smith, K., Falk, H., Luisier, J. & Eschenmoser, A. (1975) *Helv. Chim. Acta* **58,** 2357–2367.
33. Katz, J. J., Norman, G. D., Svec, W. A. & Strain, H. H. (1968) *J. Am. Chem. Soc.* **90,** 6841–6845.
34. Seely, G. R. & Jensen, R. G. (1965) *Spectrochim. Acta* **21,** 1835–1845.
35. Cotton, T. M., Trifunac, A. D., Ballschmiter, K. & Katz, J. J. (1974) *Biochim. Biophys. Acta* **368,** 181–198.
36. Thornber, J. P. (1975) *Annu. Rev. Plant Physiol.* **26,** 127–158.
37. Kok, B. (1961) *Biochim. Biophys. Acta* **48,** 527–533.