Carotene-Donor-Acceptor Complexes in Photosynthesis

The predicted lowering of the excited states of carotenoids may offer a new photosynthetic pathway.

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It has appeared for some time that carotenoids probably play some role in photosynthesis. This is suggested by their universal occurrence as the second major pigment in all autotrophic plants, by their rate of formation parallel to that of chlorophyll in corn seedings (*1*), by the carotene-like changes in the absorption spectrum of *Chlorella* under illumination (*2, 3*), and by their known ability to quench the triplet state of chlorophyll in organic solvents (*4*).

It is not yet settled whether the carotenoids are actually part of the primary photosynthetic sequence. In some organisms they have appeared to be (*5*); and an ether-soluble fraction containing carotenoids is essential for the Hill reaction in isolated chloroplasts (*6*). But here the active component now seems to be vitamin K (*7*). And carotene deficiencies in *Chlorella* and *Chromatium* and several photosynthetic bacteria produce simply an aerobic photosensitivity, so that the cells die under air and light (*8–10*). This suggests that the carotenoids only protect the cell from photooxidation (*9*). Also, they do not appear to play any part in the photoproduction of high-energy phosphate bonds (*10*).

On the other hand, the carotene-free cells have a changed spectrum even in the chlorophyll peaks, indicating that there is normally an association between the two molecules (*2, 9, 10*). Such spectral changes alone could affect the time constants and the photosynthetic pathways through either the singlet or the triplet states of the chlorophyll.

Spectral Shifts

In any case, the evidence for some kind of carotene participation is strong enough that Strehler has asked (*11*) whether light energy might not be transferred sometimes from photoexcited chlorophyll to a carotenoid (rather than in the well-known reverse direction), with the latter then playing some essential role in "mediating" an electron-transfer from a donor to an acceptor molecule in the solution. At first this sounds energetically impossible, since the lowest excited state of chlorophyll is below that of carotene. But carotene is a conjugated chain molecule, and such molecules have the unique spectroscopic property that their spectra and excited states can often be shifted very far by solvent and polarization effects (*12, 13*). Kropf and Hubbard (*14*) have already proposed such shifts for retinene in the complex visual pigment, rhodopsin.

It is the purpose of this article to show that similar spectral shifts should occur for a particular type of carotene complex, and that Strehler's suggestion then opens up certain new possibilities for energy transfer and electron transfer in carotene-containing photosynthetic systems, whether the carotene plays a primary or a secondary role. This adds one more to the numerous theoretical pathways described in the literature (*11*), which have not yet been experimentally excluded. It is worth noting that such energy-shifts and electron-shifts in conjugated-chain complexes might also be important elsewhere in biology.

Nature of the predicted shifts. The spectral shifts depend on the fact that a conjugated chain of N atoms has a maximum wavelength of absorption only when it can be written with two extreme resonance structures that are equivalent (*12*). This "isoenergetic wavelength" is near $\lambda = N(500\ \mathrm{A})$, or at about 11,000 A for a system as long as carotene, with a 22-atom chain (*12, 13, 15*). When the extreme resonance structures are nonequivalent, the peak is shifted (quadratically) to shorter wavelengths and higher energies, reaching its shortest position for a polyene

$$\mathrm{H_2C{=}CH{-}CH{=}CH{-}\ldots{-}CH{=}CH_2}$$

where only one nonionic resonance structure can be written for the ground state. As a result, the carotene peak is actually at about 4800 A. [H. Kuhn showed (*15*) that this increase of energy is due physically to the alternation of single and double bond lengths, which produces a Brillouin gap when one resonance structure is dominant.]

But now suppose that an electron donor molecule, D, approaches one end of the polyene chain while an electron acceptor, A, approaches the other. The resulting system

$$D \cdot {=}{-}{=}{-}\ \ldots\ {-}{=}{-}{=} \cdot A$$

would be a "trimolecular charge-transfer complex," $D \cdot Car \cdot A$, like the bimolecular "charge-transfer complexes," $D \cdot A$, whose interactions and absorption spectra to a $D^+ \cdot A^-$ excited state have been described by Mulliken (*16*). By analogy with the latter case, the ground state of $D \cdot Car \cdot A$ should get an increased contribution from a second resonance structure, a stabilized ionic structure of the form

$$D^+ \cdot {}^-{-}{=}{-}{=}\ \ldots\ {=}{-}{=}{-}^+ \cdot A^-$$

and the latter structure should dominate the first excited state. The plus and minus signs here indicate only the electron shifts and not necessarily the total charge, since the donor and acceptor molecules themselves may be neutral or ions or radicals (*16*).

Any such stabilization of the ground state would be cooperative, and the presence of either D or A would facilitate the acquisition of the other. The trimolecular arrangement would, of course, be competing with ordinary $D \cdot A$ complexes in the same system. But for any $D \cdot Car \cdot A$ complex that is formed, evidently the increased equivalence of the two resonance structures *should shift the carotene absorption peak to much longer wavelengths* (*14*), as is shown in Fig. 1. Depending on the strength of the complex, the peak might lie at any position out to the isoenergetic wavelength at about 11,000 A. [The curve shape should also change as shown (*12, 13*), but this need not concern us here.]

This situation is analogous to the converse case of a donor-acceptor (dipolar)

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molecule in a polarizable solvent, which is realized experimentally with Brooker's merocyanine dyes (*12*). These dyes are of the general type

$$O{=}CH{-}CH{=}CH{-}\ \ldots\ {-}CH{=}CH{-}NH_2.$$

They have a second, dipolar, ionic structure of the form

$$^{-}O{-}CH{=}CH{-}CH{=}\ \ldots\ {=}CH{-}CH{=}NH_2^{+}$$

and the equivalence of the two structures can be changed and the spectra shifted over a range of thousands of angstroms simply by varying the polarity of the solvent; Fig. 1 is in fact based on these spectra. It is therefore not a very great extrapolation to predict a similarly large shift for the present polyene complexes. It would be important to examine some four-component solution systems experimentally to look for such trimolecular complexes and their spectral shifts.

Some support for these predictions may be provided by the blue and green colors of adducts of carotene with Lewis acids or bases, which have been reviewed by Körösy (*17*). The products are complex and unstable mixtures, not well characterized chemically, but they seem to confirm that carotenes can serve either as electron donors or acceptors in bimolecular complexes. The colors might be due either to charge-transfer spectra of the Mulliken kind or to spectral shifts of the carotene peak as described here, but the latter should give a somewhat more limited range of spectral variance, and the comparative constancy of the colors with reagents of various types may therefore favor the latter interpretation.

Possible Photosynthetic Pathway

The part that such shifts in the $D \cdot Car \cdot A$ spectra might play in a photosynthetic mechanism is suggested in Fig. 2. This is an energy level diagram for the singlets (or the triplets) of a system containing many chlorophyll and carotene molecules, as well as donors and acceptors. The first excited states of free or weakly complexed carotenes will lie above those of chlorophyll. But if any carotene is complexed by a sufficiently strong (and sterically suited) donor and acceptor, giving $D \cdot Car \cdot A$, then its first excited singlet (or triplet) state, by our prediction, might be shifted so that it lies below the singlets (or triplets) of the chlorophylls. *In this case, the* D · Car · A *complex will be the energy sink for the excited states of the whole system.*

The light energy absorbed by any molecule in the vicinity will cascade downward by the usual radiationless transitions to the first excited states of the chlorophylls, and thence by the usual spatial energy transfers among these chlorophylls to the unique $D^{+} \cdot Car \cdot A^{-}$ excited state. This process might apply either to the singlet or the triplet states, since, if the wavelength shifts are large enough, the $D^{+} \cdot Car \cdot A^{-}$ triplet can also be brought below the chlorophyll triplet state. [The twisting of the equilibrium configuration of the lowest polyene singlet and triplet (*18*) is a further complication, but it takes some time to accomplish and may not be competitive with fast processes.]

It seems likely that, if its energy is adequate, such a $D^{+} \cdot Car \cdot A^{-}$ excited state could then go easily by a radiationless transition to a separated configuration, in which the D^{+} and A^{-} molecules (ions or radicals, or both) move away and the electrons in the polarized carotene molecule shift back to their free-carotene ground state arrangement, the carotene being a kind of photocatalyst, much as Strehler suggested. He has called it an "electron mediator," a term which emphasizes correctly its intermediate role here and the fact that it is *not* acting as an electron conductor in the metallic sense. (If the spectral shifts are great enough in the bimolecular complexes $D \cdot Car$ or $Car \cdot A$, these could also be energy sinks, with a similar but delayed electron mediation—the carotene storing the excess or defect of charge from the first transfer until it comes in contact with the second reactant, as chlorophyll is sometimes sup-

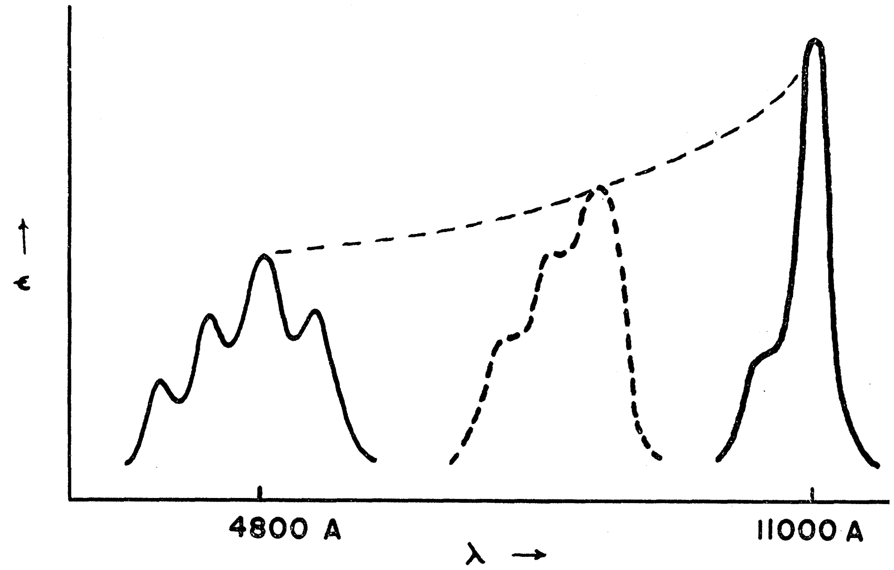

Fig. 1. Predicted change in the spectrum of a 22-atom conjugated chain from the case of one resonance structure dominant (left, carotene) to the hypothetical extreme case with two equivalent "isoenergetic" resonance structures in the ground state (right, very strong $D \cdot Car\ \ A$ complex).

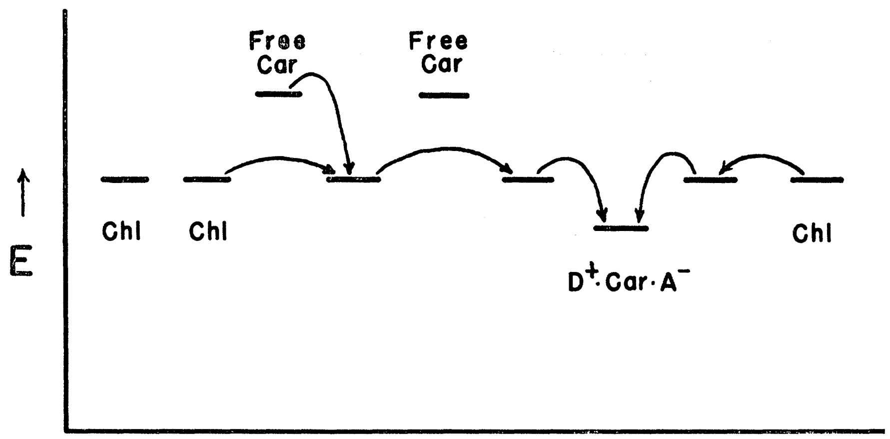

Fig. 2. The $D \cdot Car \cdot A$ complex as the possible energy sink for the excited states (singlets or triplets) of a large chlorophyll-carotenoid system containing donors and acceptors.

posed to do. But the shifts would be smaller and the reactions less rapid and specific than in the trimolecular case.)

There are two auxiliary possibilities that also deserve mention. First, if its energy is inadequate for separation of D^+ and A^-, the $D^+ \cdot Car \cdot A^-$ excited complex might store the energy for a short time in the triplet state, awaiting the arrival of a second quantum, just as chlorophyll itself is assumed to do in the Franck two-quantum hypothesis (*19*). The $D^+ \cdot Car \cdot A^-$ triplet might also be able to satisfy the requirements for the "first reservoir" of Bassham and Shibata (*20*). Second, the question is still under debate as to what the initial donors and acceptors are in photosynthesis; but if chlorophyll itself were the donor or acceptor in a carotene mechanism (*11*), the shift in the chlorophyll spectrum (*2, 9, 10*) and the quenching of its triplet (*4*) in the presence of carotene would both be accounted for. But the acceptability of the main mechanism described here is independent of whether these auxiliary possibilities prove acceptable or not.

Special features of the pathway. Note that the postulated energy-transfer steps here are of types that can have a quantum efficiency near unity; but the energy is not transferred to inadequate donors or acceptors or to random $D^+ \cdot A^-$ complexes in the solution. It is not transferred to just any carotene molecules, but only to those that are ready for the reaction. The reactants are preset, and the energy does not have to be held for the completion of diffusion-controlled steps. (While one quantum is being utilized at one site, these steps can be proceeding in parallel, as D and A molecules are joining up at other carotene sites.) The D^+ and A^- products of the reaction are also separated in space, so that the probability that they will both return or recombine to give a back reaction is smaller than it would be for a bimolecular electron transfer. If the carotenoids are part of the primary sequence, these features of the proposed process would be especially interesting in the light of the well-known evidence that there is a "photosynthetic unit," in which all of some hundreds of chlorophyll molecules can transfer their absorbed energy to a single reaction site; for any such unit would seem to require just this kind of efficiency and specificity.

Possible Tests

It is not easy to say what experiments would be best to test this picture. If there is only one $D \cdot Car \cdot A$ complex per photosynthetic unit at a given time, its spectrum would probably be too weak to detect directly, especially since we cannot predict exactly where its absorption maximum would be. Flash studies and paramagnetic studies might be similarly hampered. And while the fluorescence and phosphorescence of the chlorophylls would be quenched by this process, this is necessarily true of any efficient photochemical process. Moreover, complexes are always labile, and their weak binding energy in the ground state makes it hard to do convincing tracer or substitution experiments.

Probably the best tests will be the examination of simple polyene solution systems. If $D \cdot Car \cdot A$ complexes with a shifted spectrum can be found there, a number of energy-transfer experiments suggest themselves. Such studies will at least confirm or refute the possibility of applying this picture to photosynthesis in living systems, and they may suggest more direct ways to check the mechanism. Obviously they may also be relevant to the spectral shifts and transfer processes of the polyene visual pigments (*21*).

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21. I am indebted to Dr. B. L. Strehler (now of the Gerontology Branch, NIH, Baltimore City Hospital) for calling my attention to the problem of explaining theoretically how a chlorophyll-carotene system might work.

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