INTERACTION BETWEEN CHLOROPHYLL AND CAROTENES WITH DIFFERENT CHROMOPHORIC GROUPS.*

Hedwig Claes

Max Planck Institut für Biologie, Tübingen, Germany
and
Department of Biological Sciences, Fels Fund,
Florida State University, Tallahassee, Florida.

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Carotenoids protect chlorophyll solutions from oxidative bleaching in red light only if the chromophoric group of the polyene contains a minimum number of conjugated double bonds (Claes and Nakayama, 1959 b). The threshold value lies between 7 (ζ-carotene) and 9 (neurosporene) *conjugated* double bonds. The protection does not depend on the *total* number of oxidizable double bonds.

These observations cannot be explained by the assumption that in the presence of oxygen and excited chlorophyll the carotenoids are the preferred oxygen-acceptors, and thus protect chlorophyll or, in vivo, the cell from damage by chlorophyll-sensitized oxidations. We have seen under similar but anaerobic conditions an energy transfer from excited chlorophyll to carotenoids. In presence of chlorophyll and in light not absorbed by the polyenes poly-cis carotenes isomerize in vivo and in vitro. This reaction is inhibited by oxygen (Claes and Nakayama, 1959 a). Here we report on further studies on interactions between excited chlorophyll and various carotenes in vitro.

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Methods.

All pigments used were purified chromatographically. Chlorophyll a was isolated from green leaves. Crystallized preparations of β-carotene, lycopene and neurosporene were available. All other polyenes mentioned were isolated from the mutant strain 5/520 (Claes, 1959) of Chlorella vulgaris. References concerning the structures of the different C_{40} polyenes are found in the earlier publications (Claes and Nakayama, 1959 a and b).

A 750 watt tungsten lamp combined with a 2,8 mm Corning glass colour filter No. 2404 was used as source of red light.

The chlorophyll concentrations were measured at 665 mμ in benzene and at 670 mμ in pyridine. No special care was taken to dry these solvents completely.

In Fig. 1 and 2 relative protection corresponds to the value $\frac{E_3-E_2}{E_1-E_2}.(100)$, where E_1 is chlorophyll concentration before irradiation, E_2 after irradiation without carotene, and E_3 after irradiation in presence of carotene.

Results.

1. Irreversible photoxidation of chlorophyll a in benzene. Fig. 1 shows that with an increasing concentration of β-carotene the protective effect against photoxidation of a 7.10^{-6}M solution of chlorophyll saturates at a molar ratio carotene/chlorophyll$\sim$ 2. The effect saturates and remains strangely incomplete at a carotene concentration which prevents fully the anaerobic photoreduction.

The acyclic polyenes, lycopene with 11 and neurosporene with 9 conjugated double bonds, behave like β-carotene. But, as said above, the protective effect disappears rather

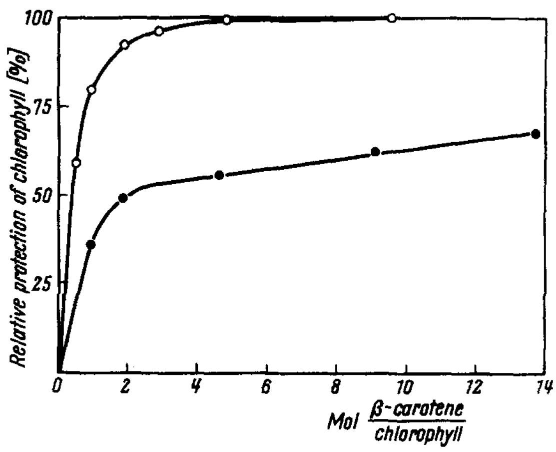

Fig.1. Relative protection of 7·10^{-6}M chlorophyll *a* against photoxydation in benzene (•——•) and photoreduction in pyridine (o——o) by β-carotene.

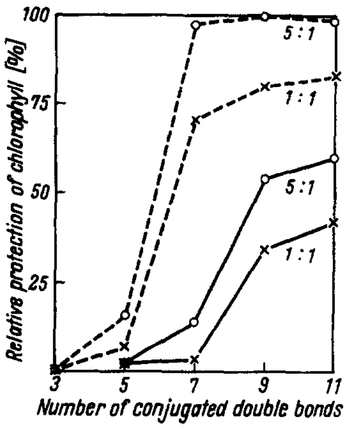

Fig.2. Relative protection of 7·10^{-6}M chlorophyll *a* against photoxydation in benzene (x——x o——o) and photoreduction in pyridine (x---x o---o) by carotenes with different chromophoric groups. The molar ratio carotene/chlorophyll = 1:1 or 5:1 is indicated for each curve.

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suddenly when the carotene molecules contain fewer conjugated double bonds. Simply increasing the concentration of such deficient carotenes does not help. A slight protective effect can be seen with higher concentrations of ζ-carotene (Fig. 2), but not at all with phytofluene which has only 5 conjugated double bonds. The latter polyene was entirely ineffective even at a ratio carotene/chlorophyll = 10.

Carotenes that protect chlorophyll against oxidation thereby also protect themselves. Lycopene, for instance, was stable in the presence of irradiated chlorophyll and oxygen while ζ-carotene was rapidly destroyed under these conditions.

2. Reversible reduction of chlorophyll a. While similar experiments were under way we learned that Krasnovsky et al. (1960) found a carotene inhibition of the photoreduction of chlorophyll by ascorbic acid in pyridine. In our experiments the reduction of chlorophyll (7.10^{-6}M) becomes completely inhibited at a molar ratio β-carotene/chlorophyll = 5 (Fig. 1), in contrast to the results obtained under aerobic conditions. Again, as in photoxidation, the chromophoric group determines whether a particular polyene is effective or not. But the minimum length of a conjugated chain necessary to prevent the photoreduction is shorter, 7 instead of 9 double bonds. ζ-carotene interferes efficiently with chlorophyll sensitized reactions in the absence of oxygen while it does not do so aerobically (Fig.2). Phytofluene (5 conjugated double bonds) becomes slightly effective at rather high concentrations but not phytoene (3 conjugated double bonds) (Fig. 2).

3. Isomerization. The energy transferred from chlorophyll excited by red light to carotenoids permits isomerization in one direction only: cis → trans. If iodine is used as an isomerizing catalyst one obtains a complex equilibrium mixture of isomers independent of the original stereo-configuration of the polyene. But no characteristic changes in the absorption spectrum of all-trans carotenes (Zechmeister et al., 1943) appear when their anaerobic solutions are illuminated in the

presence of chlorophyll.

The rapid isomerization of cis-carotenes (prolycopene and protetrahydrolycopene) is inhibited by oxygen. Similarly p-benzoquinone inhibits if it is substituted for oxygen in an oxygen-free solution of chlorophyll and cis-carotene. The rate of cis-trans isomerization of 6.10^{-6}M carotene by $0,3.10^{-6}$M chlorophyll a in benzene was reduced to 50% or less by 2.10^{-5}M p-benzoquinone.

Conclusions.

The inference from these various results is that the carotenoids interfere in general with chlorophyll sensitized light reactions by removing the energy from the excited chlorophyll molecules. Oxygen need not be involved in the primary process. We assume that oxygen, p-quinone and carotenoids act independently as efficient quenchers of the triplet state of excited chlorophyll in the manner shown by Fujimori and Livingston (1957). When present together they compete for the quenching process. This is in essence the argument used by Calvin in discussing the results of Griffiths et al. (1955). It is thus understandable that chlorophyll sensitized cis → trans isomerizations are slowed down in the presence of either oxygen or quinone and that carotenes inhibit such disparate reactions as photoreduction and photoxidation. The low saturation level, the incomplete protection, by carotene in the case of photoxidation points however to a more complex situation than a simple competition between carotene and oxygen, not to mention the striking influence of the number of conjugated double bonds.

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