PHOTODESTRUCTION OF β-CAROTENE AND PROTECTION OF THE PHOTO-OXYDATION BY CHLOROATRANORINE

por

M. PILAR ESTEVEZ, M. MARTINEZ & C. VICENTE

Abstract. We have described a protection spontaneous system for in vitro β-carotene protection against photooxydation by formation of a complex with chloroatranorine from Evernia prunastri. This system consists in the difficult of epoxydic bounds formation and in the reduction of hidroxylation grade.

Resumen. Se describe un sistema espontáneo de protección in vitro de fotodecoloración de β-caroteno por cloratranorina de Evernia prunastri. Tal sistema radica en el total impedimento de formación de epóxido y disminución del grado de hidroxilación del pigmento.

INTRODUCTION

One of the characteristics of the phycobiont cultures when they are isolated from lichens is to need bright low intensities for their growing under photoergonic regimes (Fox, 1967). This necessity in vivo seems to be resolved by accumulating lichenic compounds in the cortex which stop radiations of a greater energy, or by diminishing the phycobiont cells diameter, which increase its capacity to reflect the incident light. This demand depends of the photooxydation of photosynthetic pigments in high or saturating flow intensities. This phenomenon has effects on chlorophylls (Aronoff & Mackinney, 1943) as much as on carotenes (Zinzou, 1971; Zinzou & Costes, 1973). The content of every unit of weight in these pigments goes from 4 to 10 times lower for lichens than for cormophytes (Wilhelmsen, 1950). So, in this way, it is easy to understand that the lichen needs to protect its pigments against the bright intensities which cause the photooxydation.

This paper is about a protection cell-free system of carotene caused
spontaneously by chloroatranorine, a p-depside of the 3-orcinol series isolated from *Evernia prunastri*.

**Material and Methods**

Chloroatranorine from *Evernia prunastri* according to the method explained before (VICENTE, et al., 1975) and 3-carotene from the same lichen by descending chromatography on Watman paper n.º 1 were isolated. The developing liquid used was ether from oil: acetone (90:10 v/v). In order to obtain the pure pigment the lichenic thallus was washed several times with cold acetone and later with a great deal of deionized water and centrifuged at 17,000 × g at 4º C for 20 minutes the triturates thallus with acetone, being the supernatant reduced to 2.0-3.0 ml in vacuum and darkness. When it is indicated the chromatography is carried out with extracts coming from lichenic thallus which has not been washed before with acetone. The photooxidation experiments have been carried out by lighting volumes of 50 ml of a carotene chloroformic solution. When it is indicated volumes of chloroatranorine were included inside those and kept under conditions of high ventilation. The photodestruction of 3-carotene was appreciated by the decrease of the optical density at 465 nm, the maximum of absorption for the pigment. Conventional filters with a predominant wavelength of 481 (blue), 523 (green), 660 (red) and 730 nm (far-red) were used in order to illuminate with lights of different wavelengths. The far-red spectra are carried out as describes before (ESTEVEZ and VICENTE, 1976).

**Results and Discussion**

3-carotene from *Evernia prunastri* has been identified by its Rf = 0.99 in chromatography on paper and by its spectrum in chloroformic solution, which shows a net maximum at 465 nm (figure 1). However, when the chromatographic separation was carried out from thallus extracts which had not been washed with acetone before, a new compound of a reddish colour, of Rf = 0.94, coexisted with the known compound of Rf = 0.99. This new reddish compound is transformed into dark-yellowish by revealing with ferric chloride. The absorption spectrum of this spot eluated with chloroform showed a maximum strongly attenuated at
Fig. 1.—Absorption spectra for β-carotene (—) from acetone washed thallus; β-carotene (— —) from non-washed thallus and β-carotene + + chloroatranorin (— —) incubated at 37° for 1 hour in darkness.

Fig. 2.—Optical density loss at 465 nm for a formic solution of β-carotene, in the presence (●) or absence (○) of chloroatranorine, irradiated for 1 hour with different white light intensities.
Fig. 3.—Absorption spectra for chloroformic \( \beta \)-carotene solutions irradiated with 4,160 luxes of white light and aeration for (—) 30 minutes; (—•—•) 60 minutes; (+—+—+) 90 minutes; (—•—•—•) 120 minutes and (—•—••—•) 150 minutes.

Fig. 4.—Optical density loss at 405 nm for a chloroformic solution of \( \beta \)-carotene, in the presence (●) or absence (○) of chloroatranorine, irradiated for 1 hour with different qualities of light.
465 nm and a main maximum at 275 nm (figure 1). The nature of these maxima together with the phenolic structure of the compound, which had been showed by its reactivity with the ferric chloride, seemed to indicate that the spot of Rf 0.94 included carotene and a lichenic substance into its composition.

1 mg of β-carotene and 5 mg of chlороatrànorine were incubated in a final volume of 1 ml in chloroformic solution at 37° for 1 hour in darkness. So, in this way, a spot of the same characteristics (Rf = 0.94 and able to develop colour with ferric chloride) appeared by descending chromatography. The absorption spectrum of this spot, as figure 1 shows, was able to resemble to the isolated complex from the lichen thallus. As it had been previously suggested (Vicente, 1975) that such compounds were photostables, the irradiation of chloroformic solutions of carotene and chloroatrànorine in the same proportions with white lights of different intensities of bright flow took place. As figure 2 shows, the descending of the optical density which is attributed to the photooxydation of the β-carotene beings instantly and becomes maximum under bright flow of 4,160 luxes. The photobleaching is accompanied with a progressive bleaching at 465 nm, as figure 3 shows. However, the chlороatrànorine presence in the mixtures of reaction makes ready the photodecolouring of β-carotene in ranging proportions between 25 and 75 % depending on the incident bright flow.

The dependence between destruction-protection and the quality of the irradiating light is still more significant. As figure 4 shows, the protection as opposed to photooxydation has not effect for blue lights having partial effects for green lights and total effect for red and far-red lights. There is no photodestruction of β-carotene when chlороatrànorine is present for the last ones.

Undoubtedly a complex β-carotene-chlороatrànorine of a photostable nature is formed. There is also the possibility of forming complexes with other lichenic substances as it derives from the evaluated difference at the maximum of absorption on the ultraviolet zone of the spectrum for the complex formed in vitro and the complexes espontaneously formed by extracting thallus without being previously washed. The complex is photostable according to the intensity of the incident bright flow and the energy of such light. It is characteristic of it that greater photostability is reached with wavelengths longer than 500 nm. There is a reasonable doubt about the physiological meaning of this protection system since as figure 1 shows, the formation of the complex is joined
Fig. 5.—Far-red spectrum for the chloroatranorine.

Fig. 6.—Far-red spectrum for the oxidized β-carotene from Eternia prunastri.
to a loss of photoreceptivity at 465 nm. However, the fact that the p-depsides can be preferentially synthesized by the phycobiont cells set us thinking of this system being functional as the last resort for growing lichens under saturating intensities of light.

Fig. 7.—Far-red spectrum for the hypothetic complex β-carotene-chloroatranorine.

The characteristics of the far-red spectrum for the chloroatranorine have been described previously (ESTEVEZ & VICENTE, 1976, and figure 5). The far-red spectrum for a β-carotene isolated from Evernia prunastri washed with acetone for remove the lichen substances shows several interesting facts (figure 6). A great peak at 3,450 cm⁻¹ is indicative of an O-H strain vibration, being due to the presence of a free -OH. The peaks at 900 and 800 cm⁻¹ are peculiar of the epoxyde bound

\[
\begin{array}{c}
\text{O} \\
\text{C} \quad \text{C}
\end{array}
\]

The spectrum of the hypothetic complex β-carotene-chloroatranorine shows great differences with this (figure 7). The 3,450 cm⁻¹ band is minimized, being indicative of a slight hydroxylation. The maxima at 1,460-1,420 cm⁻¹ are difficult to interpret that, due to CH deformations, are characteristics of both -CH₃ and >CH₂ groups. Nevertheless, the peak near 720 cm⁻¹ is peculiar of an oscillation effect in
>CH₂, being indicative that the principal chain of the β-carotene molecule is being saturated. This peak is not observed for the oxidized β-carotene. The main characteristic of the complex is the extinction of the epoxide band at 900 cm⁻¹ although persists the band at 800 cm⁻¹. However, this fact is not revealing that the chloroatranorine presents a peak for the same length. The presence of the drug is determined by the appearance of the peaks at 1664 cm⁻¹ (-COO-CH₃) and 2850 cm⁻¹ (-O-CH₃). The organic chlorine has been not detected. The hypothesis is that chloroatranorine provokes changes in the β-carotene structure by whole or partial union whose effects are the saturation of the chain redounding to an effective protection to the oxidation remarkable by the absence of the epoxidic bounds and the reduction of the hydroxylation process.

REFERENCES


Cátedra de Fisiología Vegetal
Facultad de Biología
Universidad Complutense de Madrid