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August 1956

ABSTRACT

The absorption spectra of α - and β -carotene crystals suspended in water have been measured, and compared with the absorption spectra of benzene extracts made from the same suspensions. The usual three absorption bands were found to be shifted in the suspensions towards shorter wave lengths than in the extracts, and an additional longer-wave-length band was found in each of the suspensions of crystals.

ABSORPTION SPECTRA OF SUSPENSIONS OF CAROTENE CRYSTALS*

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The absorption spectra of carotenes in a suspension of the crystals was studied in order to obtain information regarding the state of carotenes in condensed systems (including *in vivo* plant material). In 1938, P. Karrer and W. Straus¹ observed the spectra of colloidal suspensions of carotenes in water, which were prepared by adding an acetone solution of carotenes to water. According to these authors, such suspensions of α -carotene showed a band between 510 and 490 m μ , in addition to the other bands of shorter wave length. The corresponding band of a suspension of β -carotene was found between 506 and 540 m μ . The positions of the maxima were found to vary in the ranges cited above with different suspensions. The wave lengths of these bands are rather long compared with the wave lengths of the bands of α - and β -carotenes in true solutions. The question arises whether the longest-wave-length band in these suspensions corresponds to the longest-wave-length band in solution, or whether it is a band additional to those which are found in solution. Recently, K. Shibata, A.A. Benson, and M. Calvin² developed a new technique for observing the absorption spectra of scattering translucent materials, using an opal glass. This method was applied in observing the spectra of suspensions of carotenes.

METHODS AND MATERIALS

The suspension of the crystals of carotenes was prepared in the following way. The crystals first were suspended in water with the aid of a drop of an aqueous soap solution. The suspension thus obtained was sonicated for 10 minutes at 0°C with 9-kc supersonic vibration. In this process, the size of the crystals was reduced and the soap prevented the aggregation of the disintegrated particles. Only the upper level of the sonicated suspension was used for observing the spectrum. The sample thus prepared was stable in that no noticeable sedimentation of the particles occurred during observation of the spectrum.

The absorption spectra were determined with a Cary Recording Spectrophotometer Model 11. A sheet of opal glass was attached to both sample and reference cells.² The thickness of the cells was 1 cm. Since the height of the absorption band depends on the particle size, the height was not determined on the molecular basis (molecular extinction coefficient) of carotene. The height in Figs. 1 and 2 is expressed in absorbance of the

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** On leave from the Tokugawa Institute for Biological Research in Tokyo and Waseda University in Tokyo, Japan.

¹ P. Karrer and W. Straus, *Helv. Chim. Acta* 21, 1624 (1938).

² Shibata, Benson, and Calvin, *Biochim. et Biophys. Acta* 15, 461 (1954).

Fig. 1. Absorption spectra of α -carotene.

—— crystal suspension in water
----- benzene extract

Fig. 2. Absorption spectra of β -carotene.

———— crystal suspension in water
----- benzene extract

particular suspension. To make sure that carotenes were not chemically altered in the process of sonication, carotenes were extracted by benzene from the suspensions. The results showed no indication of destruction of the carotenes. The spectra of these benzene extracts were also useful for comparison with the spectra of the corresponding suspensions.

RESULTS AND DISCUSSION

In Figs. 1 and 2, the solid curves are the spectra of the suspensions of α - and β -carotenes, respectively. The broken curves in these figures show the corresponding spectra of the benzene extracts. The absorption maxima of the suspension of α -carotene were 515, 482, 455, 428 m μ , although the fourth band appeared only as a shoulder. There seemed to be a fifth band at shorter wave lengths, but the position was not clear enough to locate it. In the benzene extract, the positions of the bands were 488, 458, and 434 m μ . From the shape and the relative heights of the peaks in the absorption spectra of both suspension and extract, it is clear that the first band at 515 m μ is an additional band, which appears only in the spectrum of the suspension of the crystals.

A similar study on β -carotene (Fig. 2) shows absorption maxima of 528, 486, 460, and 430 m μ in the spectrum of the suspension, and 492, 463, and 436 m μ in the spectrum of the benzene extract. The similarity of the shapes of both solid and broken curves in the region below 500 m μ indicates that the band at 528 m μ is an additional one.

By changing the period of sonication, the effect of particle size on the absorption spectrum of the suspension of β -carotene was studied. Decreasing the period made the relative heights of the peaks similar in the spectrum. This is to be expected due to the flattening effect caused by increasing the particle size.³ The positions of the second, third, and fourth peaks remained the same with increasing particle size, while the position of the maximum of the first peak varied from 528 to 540 m μ . That the maximum of only the first band is affected by the particle size agrees with the interpretation that this band arises as a result of the state of the carotenes in suspensions.

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³ L. N. M. Duysens, *Biochim. et Biophys. Acta* 19, 1 (1956).