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THE PREPARATION OF STABLE MIXED MONOLAYERS OF β-CAROTENE AND THEIR TRANSFER TO GLASS SLIDES

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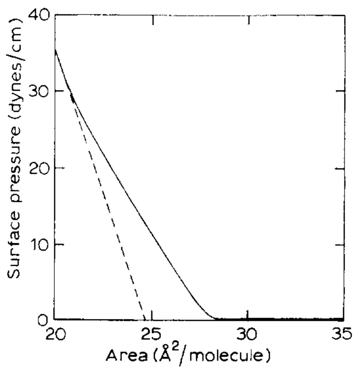
SUMMARY

Stable layers of carotene mixed with arachidic acid have been prepared and transferred uniformly to glass slides. The absorption spectrum of the mixed layers on glass slides has been measured: the extinction coefficient ($1 \cdot 10^5$ $M^{-1} \cdot cm^{-1}$) for carotene at 450 nm in the layers is close to that for carotene in hexane.

In the course of studies on the mechanism of energy transfer between organized layers of photosynthetic pigments we have succeeded in preparing stable monomolecular films of β-carotene mixed with arachidic acid. Recently, it was reported[1] that monolayers of carotene have been prepared at relatively high surface pressures (20–25 dynes/cm), but these layers have not been stable, requiring continuous compression. Also, because of the inherent instability of the monolayer, it was not possible to deposit it uniformly on a hydrophobic quartz slide. It occurred to us that one way of stabilizing the carotene molecule in a monomolecular layer is to add a diluent with a polar head group. We find that a diluent like arachidic acid, with which carotene is miscible, serves to stabilize the carotene at the air–water interface. These mixed layers can, moreover, be readily transferred uniformly to glass slides making it possible to study their optical properties.

When solutions of β-carotene and arachidic acid, mixed in molar ratios up to 1:4 (carotene: arachidic acid), are spread on a $1 \cdot 10^{-4}$ M $CdCl_2$ subphase, the resulting layers are stable at a transfer pressure of 30 dynes/cm. The pressure–area curve for a carotene–arachidic acid (molar ratio 1:4) mixed layer is shown in Fig. 1. By extrapolating the steep region of the curve to zero pressure, the average area per molecule is found to be 24.9 Å^2. In control experiments, we have determined the area per molecule for arachidic acid to be 25.8 Å^2. Assuming that arachidic acid occupies the same area in the mixed layer as it does in pure monolayers, then the area of the carotene molecule is determined to be 21.4 Å^2. A value of 18 Å^2 has been reported[1] for undiluted carotene monolayers although these layers were found to be unstable. The fact that these values are reasonable for carotene, indicates that the film is truly a monolayer.

These layers can be transferred quite readily to glass slides, by an experimental system similar to that of Kuhn and Mobius[2]. In Fig. 2, the absorption spectrum of 20 deposited layers of carotene–arachidic acid (molar ratio 1:8) is presented. Also presented is the spectrum of an equal number of more dilute layers (carotene in ara-



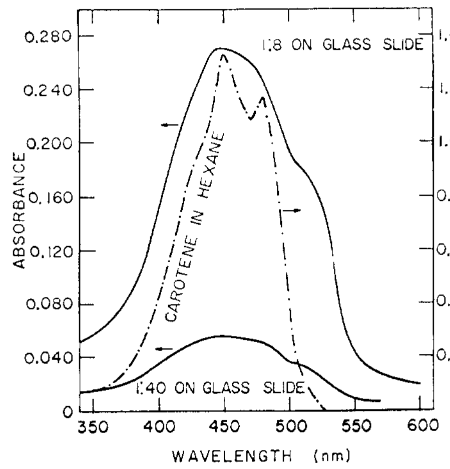


Fig. 1. Pressure-area curve for a mixed monolayer of carotene in arachidic acid (molar ratio 1:4). The layer was spread from a chloroform solution which was 0.62 mM in carotene and 2.54 mM in arachidic acid.

Fig. 2. Absorption spectra of slides containing 20 layers of carotene in arachidic acid. The spectrum of carotene, 9.91 μM in *n*-hexane[3], is included for comparison purposes.

chidic acid, molar ratio 1:40). The ratio of absorbances of the two layers is the same (5) as the ratio of concentrations of carotene in arachidic acid. Included for comparison purposes is the spectrum of carotene in hexane solution. The spectrum of the carotene layers appears to possess a less fine structure in the region of maximum absorption (450–490 nm). It should be noted that the spectrum obtained for the carotene layers is quite similar to that obtained by Schmidt *et al.*[4,5] for superimposed mixed monolayers of lutein in cadmium arachidate. Lutein differs in structure from carotene in that the former compound possesses two hydroxyl groups, which would be expected to stabilize such a molecule at the air–water interface. The extinction coefficient at 450 nm for absorption of the carotene in the film for carotene in arachidic acid (at a surface pressure of 30 dynes/cm) is calculated to be $1 \cdot 10^5$ $M^{-1} \cdot cm^{-1}$ based on a molecular area of 20 Å² and a layer thickness of 25 Å. This value is in agreement with that obtained for solutions of carotene in *n*-hexane. The fact that this agreement holds, that the films are both stable and transferable, and that the ratio of absorbances of two different concentrations of carotene is the same as the ratio of concentrations, is, we believe, evidence that the carotene is uniformly distributed in the layer.

The layers on the slide appear to decompose with time. Eventually, the absorption spectrum in the visible region vanishes. This process appears to be first order as shown in Fig. 3 in which the disappearance of carotene absorbance at 450 nm is plotted semilogarithmically as a function of time. The half-time for decomposition is 2.6 h.

One of the postulated functions of carotene in the chloroplast is that it transfers light excitation energy to chlorophyll[6]. It seems clear from the foregoing spectral results that if carotene serves this function, it must do so by absorbing light at relatively short wavelengths (around 450 nm) and by transferring the excitation energy

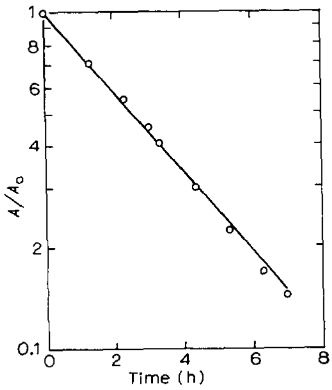


Fig. 3. Disappearance of carotene absorption at 450 nm plotted semilogarithmically as a function of time. The slide contained 20 deposited layers of carotene in arachidic acid (molar ratio 1:8).

to chlorophyll at much higher wavelengths (approx. 650 nm). It is unlikely that energy transfer could occur as a long-range phenomenon, *i.e.* resonance transfer, because carotene, even when fixed in an organized layer presumably minimizing dissipative intermolecular collisions, is non-fluorescent at room temperature. At 77 °K, only a small fluorescence is observed at 510 mm when exciting light at 450 nm is used. It would be expected that, in the absence of an acceptor, fluorescence of the organized layers would be observed which would overlap with an absorption band of chlorophyll *a*. It seems, therefore, that any energy-transfer process between β-carotene and chlorophyll must occur at short range in agreement with the conclusions of Sineschekov *et al.*[7].

REFERENCES

1 Leblanc, R. M. and Orger, B. H. (1972) *Biochim. Biophys. Acta* 275, 102–104
2 Kuhn, H. and Mobius, D. (1971) *Angew. Chem. Int. Ed. Engl.* 10, 621–637
3 Lang, L. (1961) *Absorption Spectra in the Ultraviolet and Visible Region*, Vol. 1, p. 59, Academic Press, New York
4 Schmidt, S., Reich, R. and Witt, H. T. (1971), *Proc. 2nd Int. Congr. on Photosynth.*, p. 1087, W. Junk, N.V., The Hague
5 Schmidt, S. and Reich, R. (1972) *Ber. Bunsenges. Phys. Chem.* 76, 1202–1208
6 Vernon, L. P. and Seely, G. R. (1966) *The Chlorophylls*, p. 614, Academic Press, New York
7 Sineschekov, V. A., Litvin, F. F. and Das, M. (1972) *Photochem. Photobiol.* 72, 187–197