Tetrahedron Letters Vol. 21, pp 1751 - 1754
© Pergamon Press Ltd. 1980. Printed in Great Britain

0040-4039/80/0429-1751$02.00/0

EVIDENCES OF THE SELF-ASSOCIATION OF ANTHOCYANINS I. CIRCULAR DICHROISM OF CYANIN ANHYDROBASE

Tsutomu HOSHINO* and Ushiho MATSUMOTO
Tokyo College of Pharmacy, Horinouchi 1432-1, Hachioji, Tokyo, 192-03, Japan
Toshio GOTO
Department of Agricultural Chemistry, Nagoya University, Chikusa, Nagoya, Japan

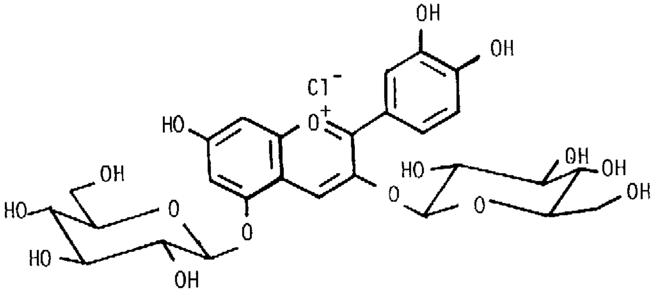
An extraordinarily large CD was observed in the aged aqueous solution of cyanin anhydrobase, indicating self-association of the pigment, which was further supported by the large θ value of the anhydrobase precipitated from the aged solution.

Some indications for the occurrence of self-association of anthocyanins have been appreared,[1] but no convincing evidences so far presented and contribution of the self-association on the flower color variations has not been so widely appreciated as the co-pigmentation and the metal complexing[2]. We have found the phenomenon, through our studies on anthocyanin complexes,[3] that a large circular dichroism (CD) appeared when a molecular complex (commelinin) was formed by mixing awobanin (an anthocyanin) and flavocommelin (a flavone); each of the latter two showed very small CD. Thus, CD is extremely sensitive to molecular asymmetry and, therefore, an effective probe for conformational change during molecular association.

Now we present here first demonstration that the monomer of anthocyanin anhydrobases in neutral solutions has little optical activity, whereas self-associated aggregates show extraordinarily large molecular ellipticities [θ]. Generally, anthocyanins are rapidly transformed in neutral aqueous solutions from colored anhydrobases to colorless pseudobases. Therefore, for rapid measurements of CD spectra in the range of 200-700 nm within 60 seconds, a JASCO J-500C spectropolarimeter equipped with a model DP-500 data processor was employed.

When cyanin chloride (1)[4] was added in phosphate buffer (0.1M, pH 7.0) (final concetration of the pigment : 5×10^{-4} M), the anthocyanin dissolved rapidly as its anhydrobase and then (after ca 60 min) a blue pigment gradually precipitated as floccules. Time courses of visible absorption spectra and optical ellipticities of the solution are shown in Fig. 1.

The θ value increased gradually after dissolution, which suggests slow conformational change of the anhydrobase. Within 2 min after dissolution no orientation of cyanin anhydrobase molecules was observed. From 5 min on, the molecules having fixed conformational arrangement were progressively increased (larger θ) in spite of the decrease in anhydrobase concentration (vis spectr. in Fig. 1), after 60 min the blue precipitates came into sight, indicating the aging effect on the self-association of the anthocyanin anhydrobase to form aggregates.



cyanin chloride (1)

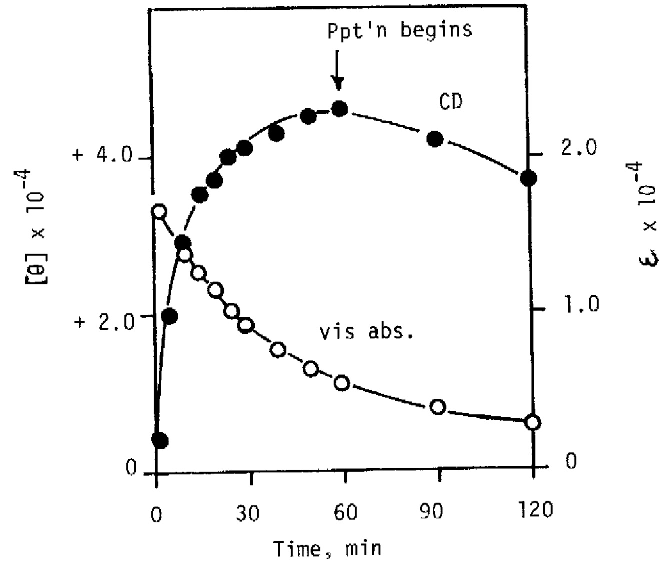


Fig. 1
Time course of visible absorption and CD at λ_{max} of cyanin chloride (5×10^{-4}M) in pH 7.0 phosphate buffer (0.1M).

Thus, the flocculent precipitates must be highly self-associated aggregates of the pigment. Indeed, when the precipitates^5 were dissolved in water (pH 7.0) the solution showed an exceptionally large value of [θ], +260,000 at 630 nm (Fig. 2, dotted line). Stability of aggregates in the solution is greatly high; the ε value at 600 nm was reduced only less than 5% when the solution left at room temp. for 2 hours (Fig. 3). These results suggest that the aggregates formation and the decolorization (hydration) of the monomeric anthocyanin anhydrobases are competitive processes.

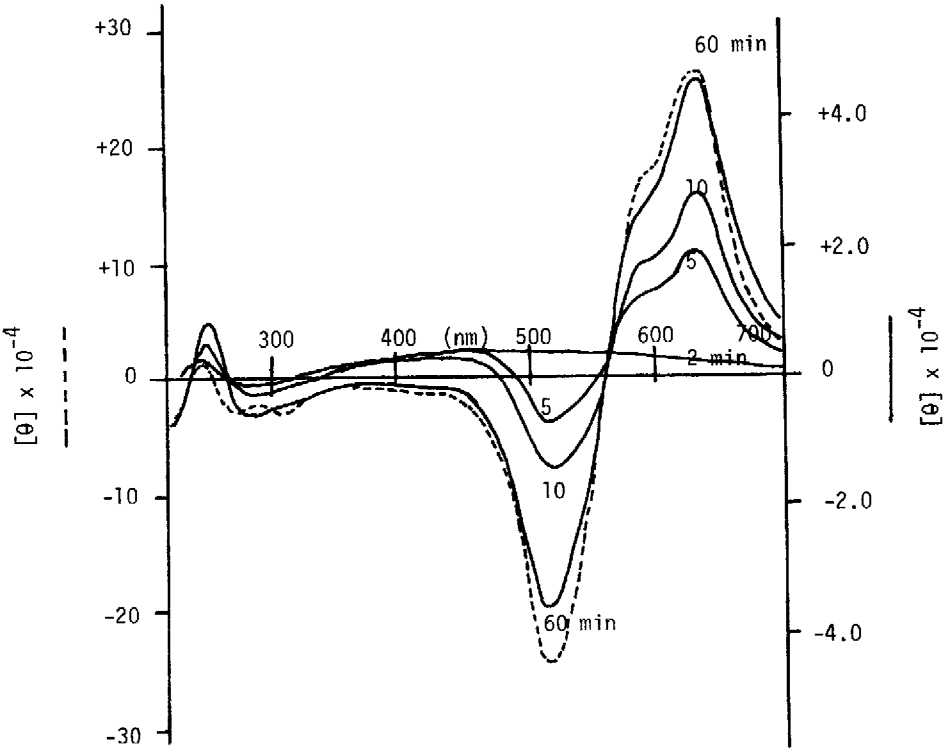


Fig. 2 ——— cyanin chloride (5×10^{-4}M) in phosphate buffer (pH 7.0, 0.1M) 2, 5, 10, 60 min after dissolving.
- - - - - cyanin anhydrobase (aggregated precipitates, 4.6×10^{-4}M) in water (pH 7.0).

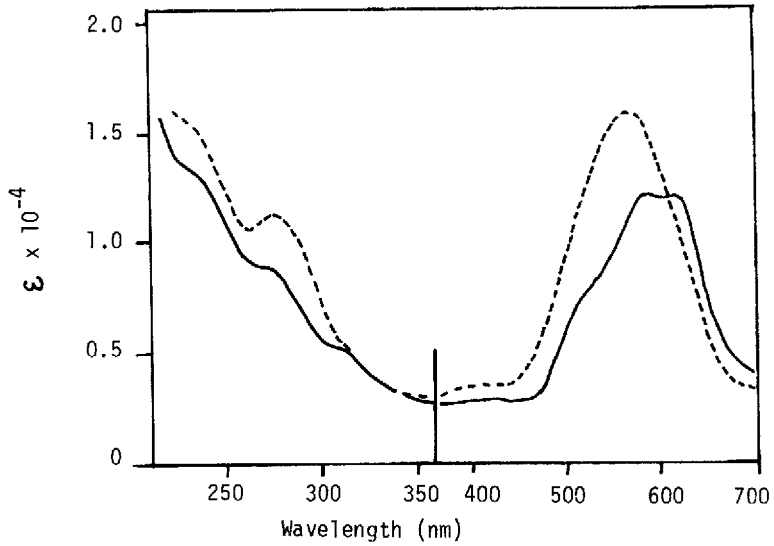


Fig. 3
- - - - - - within 2 min after dissolving cyanin chloride (5×10^{-4}M) in pH 7.0 phosphate buffer (0.1M).
——— aggregated precipitates of cyanin anhydrobase (4.6×10^{-4}M) dissolved in water (pH 7.0).

The characteristic CD was lost by addition of DMSO or urea, which acted to disorder "structured water" surrounding the aggregates, thus indicating the driving force for the self-association would be mainly the hydrophobic interactions among the aromatic nuclei stacked parallel to each other. This concept is further supported by a remarkable decrease of the ε value by the aggregates formation as shown in Fig. 3.

Unlike cyanin (cyanidin-3,5-diglucoside) (and other anthocyanidin-3,5-diglucosides such as delphin, malvin and pelargonin), cyanidin-3-glucoside shows little concentration dependence of its θ values, suggesting that the glucose moiety at 5-position has an important role in the self-association of anthocyanin. Indeed, malvidin-3-glucoside behaved similarly with cyanidin-3-glucoside, whereas malvidin-5-glucoside (anhydrobse precipitated from neutral solution) showed a large optical ellipticity.

Origin of the large optical ellipticities would come from molecular dissymmetry of each of the anthocyanin molecules whose conformation is fixed in some way,[3b] or form a helical conformation of thread-likely aggregated anthocyanin molecules. Further studies on the clarification of the origin are in progress.

Acknowledgements ——— We thank Mr. T. Yamada and Miss. M. Nishizawa for their technical assistance.

REFERENCES AND FOOTNOTES

1. S. Asen, R. N. Stewart, and K. H. Norris, Phytochemistry, **11**, 1139 (1972); ibid., **14**, 2677 (1975).
2. (review) C. F. Timberlake and P. Bridle, "The Anthocyanins", in J. B. Harborne, T. J. Mabry and H. Mabry Ed., "The Flavonoids", Chapman and Hall, London, 1975, pp 214-266.
3. (a) T. Goto, T. Hoshino, and M. Ohba, Agric. Biol. Chem., **40**, 1593 (1976). (b) T. Goto, T. Hoshino, and S. Takase, Tetrahedron Lett., 2905 (1979). (c) T. Hoshino, U. Matsumoto, and T. Goto, Phytochemistry, in press.
4. Cyanin chloride was obtained as follows: shisonin (cyanidin 3-p-coumaroylglucoside-5-glucoside) isolated from Perilla ocimoides was partially hydrolyzed by a mild acid treatment and chromatographed on an Avicel column with n-BuOH-2N HCl (1:1) as an eluent.
5. Precipitates of cyanin anhydrobase were collected by centrifugation of the solution (2 ml) after allowing to stand for 2 hours. To the ppts. was added 0.5 ml of water and the mixture centrifuged. The pH of the supernatant was 7.0. The concentration of anthocyanin was determined spectrophotometrically after dilution of the supernatant with 3N HCl.

(Received in Japan 1 February 1980)