The acetate activation reaction serves as a model of ATP- and Mg-dependent reactions in general. The principles which have been applied to the model reaction are equally applicable to the large number of enzyme systems which catalyze the reactions described by Eqs. 1 to 3.

LLOYD L. INGRAHAM
*Western Regional Research Laboratory,
U.S. Agricultural Research Service,
Albany, California*

DAVID E. GREEN
*Institute for Enzyme Research,
University of Wisconsin, Madison*

References and Notes

1. D. E. Green, in *Enzymes in Metabolic Sequences,* edited by D. Greenberg, Ed. (Academic Press, New York, 1954), vol. 1, p. 27.
2. S. P. Colowick, in *Methods in Enzymology,* S. P. Colowick and N. O. Kaplan, Eds. (Academic Press, New York, 1955), vol. 2, pp. 598–616; H. A. Lardy, in *A Symposium on Phosphorus Metabolism,* W. D. McElroy and B. Glass, Eds. (Johns Hopkins Press, Baltimore, Md., 1951), vol. 1, p. 477.
3. The following abbreviations are used: ATP, adenosine triphosphate; ADP, adenosine diphosphate; AMP, adenosine monophosphate; A and B, various reactants; AMP-OAc, mixed anhydride of adenosine monophosphoric acid and acetic acid; AcO^-, acetate ion; CoASH, coenzyme A; CoASAc, acetyl coenzyme A.
4. For an example of reaction 1, see H. Beinert, D. E. Green, H. Hift, R. W. Von Korff, C. V. Ramakrishnan, *J. Biol. Chem.* 203, 35 (1953); for reaction 2, see J. F. Speck, *J. Biol. Chem.* 179, 1405 (1949); for reaction 3, see G. L. Cantoni and J. Durell, *J. Biol. Chem.* 225, 1033 (1957).
5. A. E. Martell and G. Schwartzenbach, *Helv. Chim. Acta* 39, 653 (1956); L. Nannenga, *Federation Proc.* 15, 319 (1956).
6. J. R. Van Qazer and D. A. Campanella, *J. Am. Chem. Soc.* 72, 655 (1950).
7. L. E. Maley and D. P. Mellor, *Australian J. Sci. Research Ser. A* 2, 92 (1949); A. M. Monnier and J. Choteau, *Chem. Abstr.* 43, 4970f (1949).
8. P. C. Sinha and R. C. Ray, *Chem. Abstr.* 37, 6580^5 (1943).
9. A. Albert, *Biochem. J.* 47, 531 (1950); C. B. Monk, *Trans. Faraday Soc.* 47, 297 (1951); A. Albert, *Biochem. J.* 50, 690 (1952); C. B. Murphy and A. E. Martell, *J. Biol. Chem.* 226, 37 (1957).
10. H. Kroll, *J. Am. Chem. Soc.* 74, 2036 (1952); M. Calvin, in *Mechanism of Enzyme Action,* W. D. McElroy and B. Glass, Eds. (Johns Hopkins Press, Baltimore, Md., 1954), p. 221; I. M. Klotz, in *Mechanism of Enzyme Action,* W. D. McElroy and B. Glass, Eds. (Johns Hopkins Press, Baltimore, Md., 1954), p. 257.
11. The entropy of translation is approximately proportional to the logarithm of the molecular weight, so that the translational entropy of AB* is greater than either A or B but less than the sum of the entropies of translation of A and B.
12. Actually the free energy of activation of the chelation process may be quite largely composed of favorable entropy terms [R. G. Charles, *J. Am. Chem. Soc.* 76, 5855 (1954)]. The release of water molecules from the magnesium during the chelation more than makes up for the unfavorable entropy of bringing the reactants together.
13. See, for example, F. H. Westheimer and L. L. Ingraham, *J. Phys. Chem.* 60, 1168 (1956); C. G. Spike and R. W. Parry, *J. Am. Chem. Soc.* 75, 2726 (1953).
14. P. Berg, *J. Biol. Chem.* 222, 991 (1956).

23 May 1958

Pigments in the Flower of "Fu-Yong" (Hibiscus mutabilis L.)

The pigments found in *Hibiscus* flowers are almost exclusively of the flavone type—that is, cannabiscitrin (*H. cannabinus* (*1*), *gossypin* [*H. esculentus* (*2*) and *H. vitifolium* (*3*)], gossypitrin, hibiscitrin, quercetin, sabdaritrin (*H. sabdariffa*) (*4*), and saponarin (*H. syriacus*) (*5*). As far as we are aware, the only *Hibiscus* flower known to contain anthocyanin pigment is *H. rosa-sinensis.* The deep red flower of this plant was reported to contain a delphinidin glycoside (*6*). Recent work, however, has revealed that the coloring matter of the flower is cyanidin diglucoside (*7*). We were much interested to find that no chemical work has been reported on the pigment in the beautiful flower of "Fu-Yong" (*H. mutabilis* L.), one of the most widely cultivated flowers in Chinese and Japanese gardens.

Since the outer petals of the flower are faintly pink colored, it was necessary to use a large number of flowers to get a sufficient amount of the pigment solution. The pigments of the flowers were extracted with ethanol hydrochloric acid, concentrated in a vacuum and hydrolyzed. Both paper chromatography and the color reactions of the isolated anthocyanidin fraction showed that its chemical constituent is cyanidin.

The outer petals (500 g) of the flower of *H. mutabilis* L. were repeatedly extracted with 0.01 percent ethanol hydrochloric acid. The combined extracts (500 ml) were concentrated in a vacuum at room temperature until no more solvent could be distilled out. After the concentrate had stood at room temperature for 4 days, the yellow-brown precipitate formed was removed by filtration. The filtrate (5 ml) was further hydrolyzed by adding 1.7 ml of 10 percent hydrochloric acid and refluxing for 30 minutes. (When the filtrate was hydyrolyzed by adding the same volume of concentrated hydrochloric acid and boiling for 2 minutes, a large amount of black tarlike substance was formed.) The resulting brown precipitate was filtered, and the orange-red filtrate was extracted with isoamyl alcohol. The anthocyanidin in the isoamyl alcohol phase was transferred, by extraction with a mixture of 1 percent aqueous hydrochloric acid and benzene (1:1 by volume), into aqueous phase, and then again transferred into isoamyl alcohol phase.

Paper chromatography of the anthocyanidin solution was carried out by using a mixture of glacial acetic acid, 36 percent hydrochloric acid, and water (5:1:5); the chromatograms were developed by the descending method (*8*). From the R_f value of the spot (0.38) and the comparison of this value with the values of cyanidin [0.38, from red rose (*9*)], of pelargonidin [0.51, from *Pelargonium zonale* (*10*)], and of malvidin [0.44, from *Iris kaempherii* (*11*)], the anthocyanidin was found to be cyanidin. The extract of this spot (R_f 0.38) also showed color reactions of cyanidin—that is, it turned red-purple on the addition of sodium acetate and blue on the subsequent addition of ferric chloride.

The brown precipitate obtained was extracted with ether. After the ether was expelled, the yellow residue was dissolved in ethanol. The solution turned to orange-red when it was treated with magnesium powder and hydrochloric acid, showing the existence of flavone (*12, 13*).

PING-YUAN YEH
WEI-FU LING
REIKO TAKASAKA*
*Department of Chemistry, National
Taiwan University, Taiwan, China*

References and Notes

1. K. Neelakantam, P. S. Rao, T. R. Seshadri, *Proc. Indian Acad. Sci.* A14, 105 (1941); *Chem. Abstr.* 36, 7026^4 (1942).
2. T. R. Seshadri and N. Viswanadham, *Current Sci.* (*India*) 16, 343 (1947); *Chem. Abstr.* 42, 2649g (1948).
3. K. V. Rao and T. R. Seshadri, *Proc. Indian Acad. Sci.* A24, 352 (1946); *Chem. Abstr.* 41, 2734i (1947).
4. P. S. Rao and T. R. Seshadri, *Proc. Indian Acad. Sci.* A15, 148 (1942); A16, 323 (1942); *Chem. Abstr.* 36, 7238^9 (1942); 37, 2879^9 (1943); P. R. Rao and T. R. Seshadri, *Proc. Indian Acad. Sci.* A27, 104, 209 (1948); *Chem. Abstr.* 43, 1408a (1949); 44, 1496a (1950); A. G. Perkin, *J. Chem. Soc.* 95, 1855 (1909).
5. T. Nakaoki, *Yakugaku Zasshi* 64, No. 11A, 57 (1944); *Chem. Abstr.* 46, 108d (1952).
6. R. Yamamoto and Y. Oshima, *Sci. Papers Inst. Phys. Chem. Research* (*Tokyo*) 19, 134 (1932); R. Yamamoto and A. Kato, *J. Soc. Tropical Agr.* (*Japan*) 5, 273 (1933).
7. K. Hayashi, *Acta Phytochim.* (*Japan*) 14, 55 (1944); *Chem. Abstr.* 45, 4786d (1951).
8. K. Hayashi and Y. Abe, *Shigen. Kagaku Kenkyusho Ihô* 29, 1 (1953); *Chem. Abstr.* 47, 10070h (1953).
9. R. Willstätter and T. J. Nolan, *Ann. Chem. Liebigs* 408, 1 (1915).
10. R. Willstätter and E. K. Bolton, *ibid.* 408, 42 (1915).
11. G. M. Robinson and R. Robinson, *Biochem. J.* 25, 1687 (1931).
12. K. Hayashi, *Experimental Methods in Plant Pigments* (Nakayama Shoten, Tokyo, 1954), (in Japanese).
13. We wish to thank K. Hayashi (Tokyo Kyoiku University) for his advice, and C. S. Hu (head, department of horticulture), Y. F. Shen (head, department of botany), and T. T. Wang (head, department of forestry of our university for permitting us to collect the flowers at their gardens.
* Present address: Department of Chemistry, University of Louisville, Louisville, Ky.

3 January 1958

* * *