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SHORT COMMUNICATION

THE FUNCTION OF IRON IN PORPHYRIN AND CHLOROPHYLL BIOSYNTHESIS

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Abstract—Incorporation of ^{14}C-labeled succinate and glycine into chlorophyll precursors and chlorophylls of green and chlorotic leaves demonstrate that iron is necessary for the transformation of coproporphyrin(ogen) into protoporphyrin(ogen).

INTRODUCTION

ALTHOUGH it is well known that plants need iron for greening, the function of iron in this process is still a point of controversy. Since, as a rule, iron-deficient plants are low in protein, it is supposed that as a primary reaction protein metabolism may be inhibited and, as a result, chlorophyll is not stabilized.[1] According to recent experiments, however, iron directly participates in chlorophyll biosynthesis,[2–4] although only a small part of the iron pool in the plant is effective in this process. This fraction is referred to as "active" iron. Oserkowsky[5] plotted the iron extracted with 1 N HCl against chlorophyll content. That iron value at which plotted lines intercept the iron axis was designated inactive iron; the difference to the total iron in the same extract active iron. Another procedure to characterize the iron active in chlorophyll synthesis is the fractionated extraction of leaf material with 0·35 N NaCl, 0·003 M Na-EDTA and 1·5% Triton X-100 solution.[6] It is possible to activate or inactivate parts of the iron pool for chlorophyll biosynthesis by varying the environmental conditions.[7]

Experiments with the chlorophyll mutant xantha$_5$ of *Lycopersicon esculentum* proved that culture media with a low oxidation potential increase the chlorophyll content about 5–10 times.[7] It was possible to demonstrate by extraction procedures and Moessbauer spectrometry that the greening of the mutant is the result of the activated iron metabolism.[7,8] Since the mutant is able to produce high amounts of protein in spite of its low chlorophyll content, it must be assumed that iron is effective while it participates in chlorophyll biosynthesis.[9]

[1] W. BAUMEISTER, *Encyclopedia of Plant Physiology*, Vol. IV, p. 543, Springer-Verlag, Berlin (1958).
[2] J. LASCELLES, *Phys. Rev.* **41**, 417 (1961).
[3] H. V. MARSH, H. J. EVANS and G. MATRONE, *Plant Physiol.* **38**, 632 (1963).
[4] W. P. HSU and G. W. MILLER, *Biochem. Biophys. Acta* **111**, 393 (1965).
[5] J. OSERKOWSKY, *Plant Physiol.* **8**, 449 (1933).
[6] O. MACHOLD, *Flora A* **159**, 1 (1968).
[7] O. MACHOLD, *Flora A* **157**, 183 (1966).
[8] O. MACHOLD, W. MEISEL and H. SCHNORR, *Naturwissenschaften* **55**, 499 (1968).
[9] O. MACHOLD, *Agrochimica* XIII, 64 (1968).

RESULTS AND DISCUSSION

Ninety plants of the normal type Condine Red and ninety of the mutant type xantha$_5$ were incubated with succinate-1,4-^{14}C and glycine-2-^{14}C in a phytotron. The results presented in Fig. 1 are mean values from three different experiments. As compared with the normal plants, the mutant accumulates high amounts of δ-aminolaevulinic acid (δ-ALA). This is also true for uroporphyrin(ogen) (URO) and coproporphyrin(ogen) (COPRO) though the difference between the normal type and the mutant is, in the case of URO, relatively small. From the results it can be concluded that, under conditions where the iron

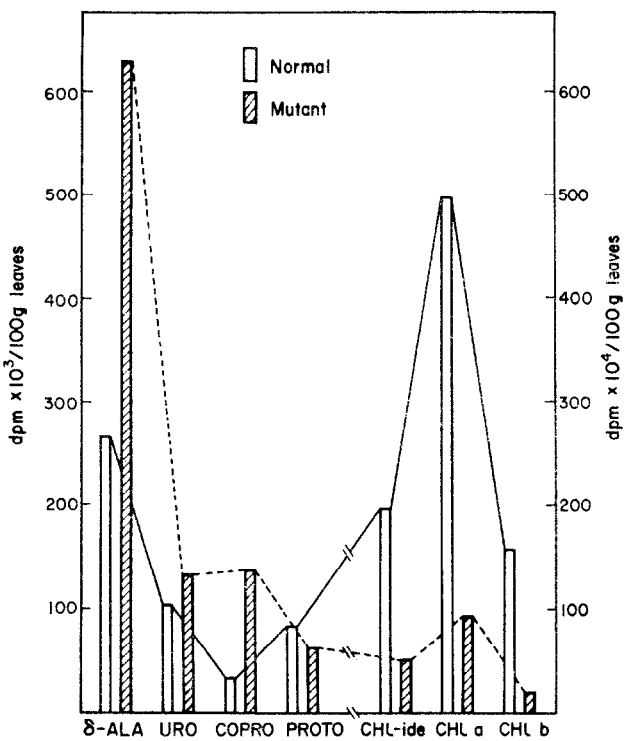
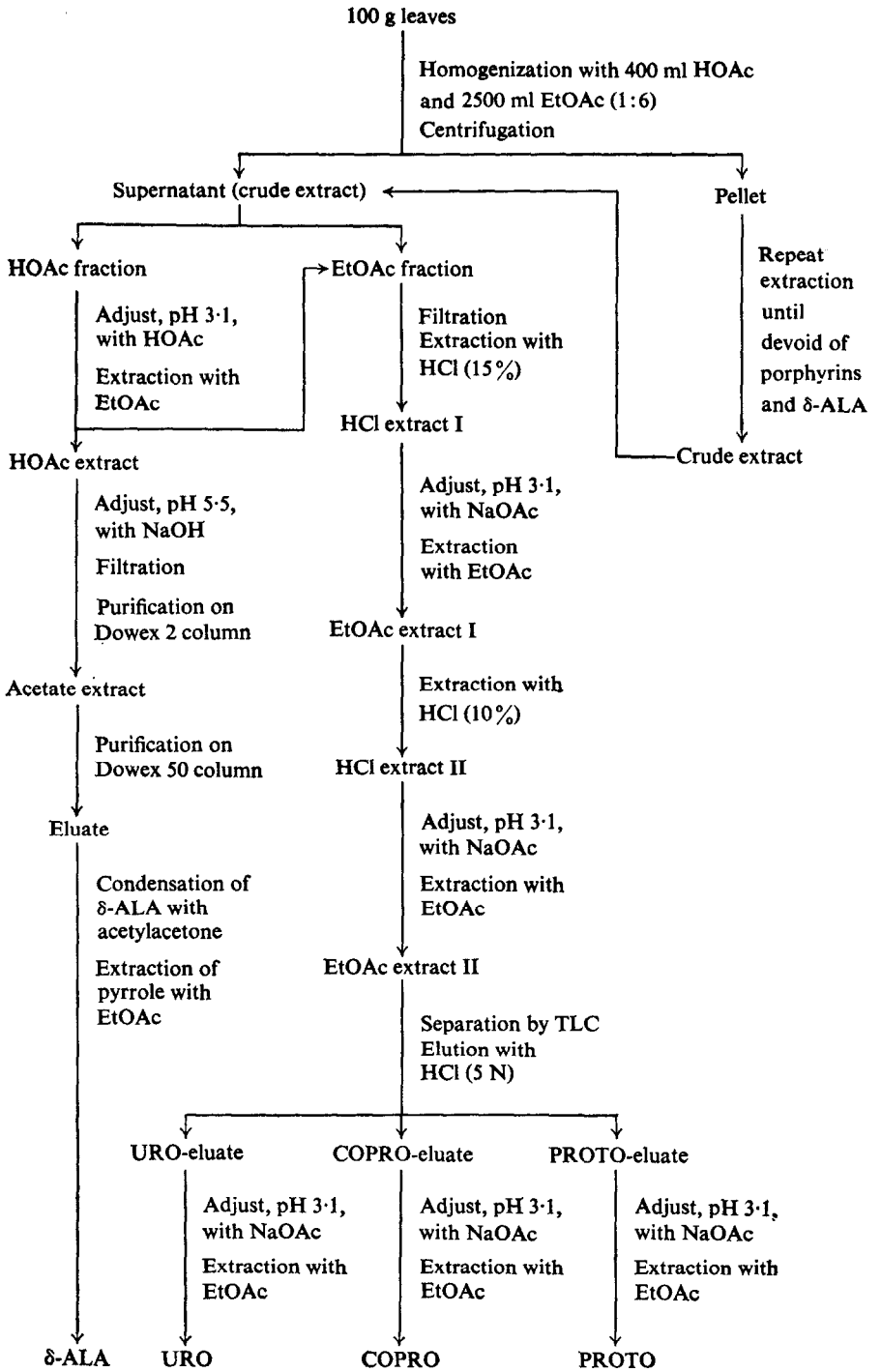


FIG. 1. THE INCORPORATION OF SUCCINATE-1,4-^{14}C AND GLYCINE-2-^{14}C INTO CHLOROPHYLL PRECURSORS AND CHLOROPHYLLS IN NORMAL AND MUTANT TOMATO PLANTS 3 hr AFTER APPLICATION.

metabolism of the mutant is inactivated for chlorophyll biosynthesis, all precursors up to COPRO accumulate. Starting with protoporphyrin(ogen) (PROTO) the relations are inverted. The mutant incorporates significantly less succinate-1,4-^{14}C and glycine-2-^{14}C into PROTO and chlorophylls than the normal plants. Thus, it is evident that the transformation of COPRO into PROTO is inhibited in the mutant.

It is possible to overcome the inhibition by activating the iron metabolism of the mutant.[7] In another experiment, plants of the mutant type were cultivated in solutions with different redox potentials. In the solution with a high oxidation potential the plants contained 0·13 mg chlorophyll/g fresh leaves whereas, after cultivation in a solution with a low potential, this value was increased ten-fold (Table 1). Plants raised in solutions of high and low oxidation potential were also incubated with succinate-1,4-^{14}C and glycine-2-^{14}C. The data of



SCHEME 1. EXTRACTION AND ISOLATION OF δ-AMINOLAEVULINIC ACID AND PORPHYRINS.

Table 1 show an accumulation of COPRO in comparison to PROTO under conditions where iron is inactivated. Only relatively small amounts of labeled precursors are incorporated into chlorophylls. The activation of iron leads to a decreased relation of COPRO to PROTO and an increased incorporation into chlorophylls.

TABLE 1. THE INCORPORATION OF SUCCINATE-1,4-^{14}C AND GLYCINE-2-^{14}C INTO PORPHYRINS AND CHLOROPHYLLS IN CHLOROTIC AND GREEN LEAVES OF THE TOMATO MUTANT XANTHA$_5$ 3 hr AFTER APPLICATION

State of iron	Chlorophyll content (mg/g leaves)	Radioactivity (dpm × 10^3 100 g leaves)	
		COPRO:PROTO	Chlorophyll a+b
Inactivated	0·13	3·2	551
Activated	1·36	1·6	1834

The experiments demonstrate that iron is necessary for the transformation of coproporphyrin(ogen) into protophoryrin(ogen). These results agree with those of Lascelles on microorganisms and of Hsu and Miller on higher plants.[2,4]

EXPERIMENTAL

The shoots with four leaves were cut from 40-day-old plants and placed in small beakers with 3 ml of Hoagland solution (1/5 strength) containing [11] μc succinate-1,4-^{14}C and glycine-2-^{14}C. After 3 hr incubation at 25° the leaves were homogenized and extracted with 1·3 N HOAc and EtOAc at 2° to isolate δ-aminolaevulinic acid and porphyrins (Scheme I). To avoid loss of labeled precursors, carrier δ-ALA (8 μg/100 g fresh leaves) and carrier porphyrins (70 μg each/100 g fresh leaves) were added to the leaf homogenate before extraction. After purification of the HOAc extract, δ-ALA was allowed to react with acetylacetone at pH 4·6. To control the reaction, it was compared with δ-ALA produced by Schuchard, Munich. The resulting pyrrole was identified with a modified Ehrlich's reagent.[10]

Porphyrins were isolated and purified from the EtOAc fraction by alternated extraction with HCl and EtOAc.[11] After concentration, the porphyrins were separated on TLC plates (solvent 2,6-lutidine/water saturated with ammonia) by the method of Mundschenk.[12] Coproporphyrin III (A grade) and protoporphyrin IX (B grade) of Calbiochem were used to identify the labeled porphyrins. To identify uroporphyrin, this compound was isolated from prophyria urine and characterized by spectrophotometric examination of its Soret maximum.

The chlorophylls were extracted from 100-mg leaf samples with acetone and separated by TLC.[13,14] To produce chlorotic leaves, iron was inactivated by cultivating the mutant plants in an aerated solution with nitrate as nitrogen source. Shifting the plants to a solution with ammonium increased the active iron content as well as the chlorophyll content. The state of iron in the leaves was characterized by a fractionated extraction procedure and by Moessbauer spectrometry.[7,8]

[10] D. MAUZERALL and S. GRANICK, *J. Biol. Chem.* **219**, 435 (1956).
[11] E. J. B. DRESEL and J. E. FALK, *Biochem. J.* **63**, 72 (1956).
[12] H. MUNDSCHENK, *J. Chromatog.* **25**, 380 (1966).
[13] A. HAGER and F. MEYER-BERTENRATH, *Planta* **69**, 198 (1966).
[14] C. J. SELISKAR, *Anal. Biochem.* **17**, 174 (1966).