Absorption and Breakdown of Iron–Ethylenediamine Tetraacetic Acid by Tomato Plants

Jacobson[1] was the first to show that plants could utilize iron supplied as the ferric chelate of ethylenediamine tetraacetic acid (Fe–EDTA). Subsequent workers[2,3] have concluded from experiments with isotopically labelled chelating agents that the iron was released after the uptake of the entire molecule by plant roots. Tiffin and Brown[4], however, showed that, at least initially, iron was absorbed by roots from a phenolic analogue of Fe–EDTA while most of the chelating agents remained in the culture solution. The absorption of Fe–EDTA was therefore further examined, using the iron chelate labelled in both portions of its molecule.

Tomato plants were raised from seed germinated in acid-washed sand and grown in an iron-free nutrient solution in the glasshouse. Ten days after the first true leaves had appeared, three plants of uniform size were transferred to each of six culture dishes. The nutrient solution included 1 p.p.m. of iron as Fe–EDTA, prepared from equimolar amounts of ferric chloride labelled with iron-59 and EDTA labelled with carbon-14 in the 2-position of the acetate group. The stability of the iron chelate in the nutrient solution was checked by keeping a further replicate free from plants under the same experimental conditions. During the experiment light was excluded from the nutrient solution and its *p*H was maintained at 5–6.

After 10, 17 and 24 days the plants and nutrient solution in two dishes were taken for isotope analysis. The plants in each dish were bulked, divided into tops and roots and dried at 65° C. overnight. For iron-59 assay, the material was digested with nitric and perchloric acids and the γ-radiation measured in a scintillation counter. For carbon-14, the plant material was oxidized with a Van Slyke acid mixture, the carbon-14 was collected as barium carbonate and counted under an end-window Geiger–Müller tube[5]. Solutions were evaporated to dryness in the presence of inactive carrier before oxidation and assay. No iron-59 was present in the barium carbonate precipitates. The iron-59 counts were converted to μgm. iron; carbon-14 results were expressed as μc. since the chemical form of this isotope was uncertain (Table 1).

Almost all the added iron was recovered either from the solutions or from the plants; by contrast the carbon-14 recovered fell steadily to about 60 per cent of its original value after 24 days, indicating decomposition of the EDTA. Both isotopes were almost completely recovered from the solution in which no plants were grown. The loss of carbon-14 was therefore not due to decomposition of the Fe–EDTA in the nutrient solution, but to absorption and breakdown in the plants.

After 10 days the plants had absorbed about 41 per cent of the added iron but only 26 per cent of the carbon-14. As there was no decomposition in the nutrient solution these figures imply that the plant roots had absorbed both iron and iron chelate. Later results showed that while the rate of uptake of iron decreased with time, that of the carbon-14 (EDTA) continued steadily. The EDTA liberated by the absorption of iron probably chelated other metals, for example, copper, zinc and manganese, in the nutrient solution. Subsequent absorption of these chelates would explain the steady increase in carbon-14 content and the progressive increase in the ratio of carbon-14 to iron in the plant tops.

This is the first report of experimental evidence that EDTA is broken down during the utilization by plants of the iron chelate, although it has been suggested previously that the iron might be released during breakdown of the chelate in leaves by sunlight[6].

D. G. Hill-Cottingham
C. P. Lloyd-Jones

Research Station,
Long Ashton,
Bristol.

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A Lipoprotein Staining Method for Zone Electrophoresis

In an attempt to overcome some of the well-known disadvantages of the commonly used lipoprotein stains, a new method, based on the use of Schiff's stain after preliminary ozonization, was developed. It does not depend on the use of lipid stains, and, consequently, washing the stain out of the background is not necessary. Furthermore, it can be used equally well on cellulose acetate material and on filter paper.

The electrophoretic separation is performed in the usual manner, but a somewhat larger sample than that used for ordinary protein separation is recommended. When the separation is completed, the

Table 1

Duration of sampling date	Nutrient solution		Plant roots		Plant tops		Total recovery as percentage of original solution	
	μgm. Fe	μc. C-14	μgm. Fe	μc. C-14	μgm. Fe	μc. C-14	Fe	C-14
0 days (original solution)	200	9·04					100	100
10 days	122·7 111·5	6·98 6·29	14·6 15·0	0·30 0·40	55·6 65·0	0·11 0·11	96·5 95·8	81·8 75·2
17 days	75·0 90·0	5·47 5·92	22·9 31·3	0·82 0·59	93·4 68·9	0·24 0·17	95·7 95·1	72·2 73·9
24 days	81·0 84·6	3·87 4·49	30·0 29·4	0·81 0·57	81·1 70·6	0·35 0·63	96·1 92·3	55·6 62·9
24 days*	197·8	9·11	—	—	—	—	98·9	100·8

* This dish of nutrient culture solution contained no plants.