Note

The Occurrence of Ferredoxin-sulfite Reductase in Barley Roots

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Most information on the sulfate assimilation, namely cysteine biosynthesis, by higher plants has been derived from experiments using photosynthetic tissues and reduction of sulfite, catalyzed by sulfite reductase (SiR), is generally considered to be the final step of sulfate reduction in chlorophyllous tissues.¹⁻¹⁰⁾

Recently ferredoxin-dependent SiR[hydrogen-sulfide: ferredoxin oxidoreductase, EC 1.8.7.1] has been prepared to a highly purified state from spinach leaves and partially characterized.⁹⁾

Apart from this leaf SiR, we wish to report the occurrence of that enzyme in non-green tissues, *i.e.* barley roots. Seeds of *Hordeum vulgare* L. var. Sekitori No. 2, kindly supplied by Mr. Susumu Miwa, Chiba Prefectural Agricultural Experiment Station, were sterilized in diluted Uspulun solution, and they were grown in 1/2 Hoagland solution at room temperature under white fluorescent light of 3500 lux. To get SiR preparations, roots from 8-d-old barley seedlings were homogenized with mortar and pestle in the same volume of 100 mM Tris-HCl buffer, pH 8.5. After removing the precipitate from homogenate by centrifugation, the supernatant was concentrated by ammonium sulfate precipitation (80% saturation). The resulting sediment, collected by centrifugation, was dissolved in 100 mM Tris-HCl buffer, pH 8.5, and dialyzed overnight against the same buffer. The procedures used for preparing spinach ferredoxin and chloroplast grana were the same as described in previous paper.⁷⁾ SiR activity was demonstrated by the method, similar to that previously described for leaf enzyme with slight modifications.⁹⁾ The reaction mixture included, in a total volume of 2.4 ml, spinach chloroplast grana equal to 105 μg chlorophyll, 96 μmol of Tris-HCl buffer, pH 8.5,

FIG. 1. Sulfite Reduction by Barley Root SiR in the Presence of Ferredoxin.

Root SiR preparation equivalent to 1.68 mg of protein and 0.512 mg of ferredoxin were used throughout. Other conditions as described in text.

FIG. 2. Effect of Ferredoxin Concentration on Activity of Root SiR.

Root SiR preparation equivalent to 2.25 mg of protein was used. The assay conditions were the same as in Fig. 1 except for varying concentrations of ferredoxin.

25.5 μmol of sodium ascorbate, 0.2 μmol of 2,6-dichlorophenol indophenol, 7.2 μmol of sodium sulfite

FIG. 3. Effect of Sulfite Concentration on Activity of Root SiR.

The same amount of SiR preparation as described in Fig. 2 was used. The assay conditions were the same as in Fig. 1 except for varying concentrations of sulfite.

and appropriate amounts of spinach ferredoxin and barley root SiR preparation. The reaction was carried out in Warburg vessels at 37°C under strict anaerobiosis, which was assured by heating the grana at 55°C for 5 min to destroy oxygen evolution activity.⁷⁻⁹ Illumination was by a 300-W tungsten lamp providing 20,000 lux. The reaction was initiated by the addition of sodium sulfite from the first side arm, stopped by the addition of 0.1 ml of 12 N H₂SO₄ from the second side arm and sulfide formed was analyzed by the method described by Tamura *et al.*⁹ Protein was determined by the method of Lowry *et al.*¹¹ using bovine serum albumin as the standard.

Figure 1 shows the time course curve of ferredoxin dependent sulfite reduction in the presence of barley root SiR under the assay conditions described above. Saturation curves for ferredoxin and sulfite are shown in Figs. 2 and 3 respectively. The enzyme has a relatively broad area of optimum activity occurring between pH 8.0 and pH 8.5 in Tris-HCl buffer. Under optimal conditions, ferredoxin-sulfite reductase activity was

found to be directly proportional to increased amounts of enzyme added up to 3 mg of protein in the reaction mixture. Methyl viologen, reduced by *Desulfovibrio desulfuricans* hydrogenase system described by Tamura,²⁰ could replace reduced ferredoxin, but the efficiency was much lower.

Whereas no evidence exists as yet for the presence of ferredoxin in root tissues of higher plants, the occurrence of a significant level of ferredoxin-sulfite reductase in barley roots may suggest the presence of an endogenous electron carrier, which is replaceable with ferredoxin. If a ferredoxin-like electron carrier truly exists in barley roots, sulfite reduction in this tissue could proceed by a system analogous to that elaborated by Tamura for sulfite reduction in the dark by leaf extracts, involving NADPH and ferredoxin-NADP reductase.⁹ We are now trying to purify and characterize the root sulfite reductase in comparison with leaf enzyme, presumably involving in sulfate assimilation by root tissues in higher plants and further to isolate the physiological electron carrier in that tissue.

REFERENCES

- 1) G. Tamura, *Bot. Mag. (Tokyo)*, **77**, 239 (1964).
- 2) G. Tamura, *J. Biochem.*, **57**, 207 (1965).
- 3) G. Tamura, K. Asada and R. S. Bandurski, *Plant Physiol.*, **42**, S-36 (1967).
- 4) A. Schmidt and A. Trebst, *Biochim. Biophys. Acta*, **180**, 529 (1969).
- 5) G. Tamura, *Tech. Bull. Fac. Hortic. Chiba Univ.*, **18**, 61 (1970).
- 6) A. Schmidt and J. D. Schwenn, Abstract of Papers, 2nd International Congress on Photosynthesis, Stresa, 1971 p. 507.
- 7) G. Tamura and S. Itoh, *Agric. Biol. Chem.*, **38**, 225 (1974).
- 8) G. Tamura, "Shokubutsu-Koso Tanpakushitsu Kenkyuho," ed. by Y. Morita *et al.*, Kyoritsu Shuppan, Tokyo, 1976, p. 356.
- 9) G. Tamura, T. Hosoi and J. Aketagawa, *Agric. Biol. Chem.*, **42**, 2165 (1978).
- 10) M. Masada, K. Fukushima and G. Tamura, *J. Biochem.*, **77**, 1107 (1975).
- 11) O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).