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SHORT COMMUNICATION

USE OF TWEEN 80 IN FERREDOXIN EXTRACTION FROM GYMNOSPERMS

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Abstract—The problem of extracting and the quantitative determination of ferredoxin from leaves of *Pinus pinaster*, *P. pinea*, *Thuja orientalis* and *Taxus baccata* has been examined. Positive results were obtained only by adding Tween 80 to extracting medium.

DURING our researches on the enzymes in Conifers,[1,2] we considered it desirable to extend our study to the extraction and quantitative determination of ferredoxin. A preliminary account has been published elsewhere.[3] Ferredoxin has been extracted from bacteria,[4,5] algae[6,7] and some Angiosperms.[8–10] Nothing has so far been reported on its extraction from Gymnosperms, perhaps because the presence of substances such as resins and tannins make the preparation of active enzyme extracts from adult tissues difficult. Goldstein and Swain examined a number of detergents and polymers for the reactivation of enzyme proteins precipitated by tannins, and obtained the best results by use of Tween 80.[11] For this reason, we have examined the use of this substance for the preparation of ferredoxin from conifer leaves. Results of experiments with four species are summarized in Table 1, and it can be seen that by addition of Tween 80 to the extracting medium, ferredoxin activity was detectable in the extract. In extracts prepared without the detergent, no protein was extracted. Moreover, the use of the polymer at the concentrations used by us, does not interfere with the determination of proteins by the biuret method. Comparative values for ferredoxin and chlorophyll content of the leaves are shown in Fig. 1.

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TABLE 1. FERREDOXIN CONTENT IN TWEEN 80 EXTRACTS OF CONIFER LEAVES*

Species	Protein content in ferredoxin extract (mg/ml)	Ferredoxin activity (μmoles NADPH/hr/ml)	Specific activity of ferredoxin (μmoles NADPH/hr/mg protein
Pinus pinaster	1·80	38 9	21·60
Pinus pinea	1·60	37·4	23·40
Thuja orientalis	1·80	45·4	25·20
Taxus baccata	0·70	21·0	30·00

* Without Tween 80, no protein was extracted from any species.

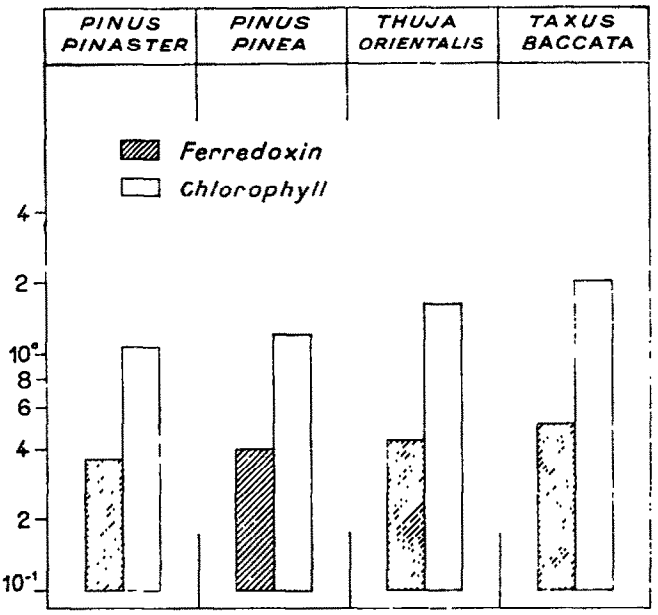

FIG. 1. COMPARATIVE VALUE OF FERREDOXIN AND CHLOROPHYLL CONTENT IN *Pinus pinaster*, *P. pinea*, *Thuja orientalis* AND *Taxus baccata*.
The extracts were prepared by addition of Tween 80. The values are expressed on logarithmic scale. Ferredoxin activity is expressed as μmoles of NADP reduced/min/mg protein. Chlorophyll content is expressed as mg/g fresh wt.

EXPERIMENTAL

Leaves from adult trees of *Pinus pinaster*, *P. pinea*, *Thuja orientalis* and *Taxus baccata* were washed in distilled water and kept for 2 hr at 0° to swell before extraction. Chloroplasts were prepared from fresh leaves of *Spinacea oleracea* by the method of Davemport.[10]

The chlorophyll content of spinach preparations was determined after extraction with 80% acetone, according to MacKinney.[12]

Protein content of the extracts was estimated by the biuret reagent, according to the method of Beisenherz *et al.*[13]

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Ferredoxin was extracted as follows: the leaves were homogenized in a Waring Blendor for 5 min at 0° with 5 times their weight of an ice-cold solution containing 0·05 M TRIS pH 8 and Tween 80 1% (v/v). The dark green homogenate was filtered through a double layer of cheesecloth and glass wool. The filtrate was treated with cold acetone as described by San Pietro and Lang.[14] The final supernatant, dialized for 6 hr against 0·005 M TRIS pH 8 in the cold, was used for ferredoxin assay.

Photochemical activity measurements were carried out in 3 ml silica cells of 1 cm light path. Reduction of NADP was followed as an increase in extinction at 366 nm, 25° by the method of Davemport[10] in an Eppendorf recording photometer, operating with selected spectral lines from a mercury lamp and equipped with an interference filter. $\epsilon_M^{366m\mu}$ for NADP is taken as $3{\cdot}3 \times 10^3$.[15] The reaction mixture contained: 0·5 mM NADP, 100 mM TRA buffer pH 7·6, 10 mM Mg SO_4, spinach chloroplasts (chlorophyll content 220 μg), ferredoxin extract (40 or 80 μl) and water to a final volume of 2·5 ml. The samples were illuminated for 4 min using as light source a 500-W projector lamp at a distance of 36 cm. The incident light was filtered through heat-absorbing glass and 1 cm-layer of 5% (w/v) cupric sulphate. Blanks (containing all components except ferredoxin extract) were prepared to check possible endogenous activity of spinach chloroplast ferredoxin. Furthermore, since the high content of spinach chloroplasts made solutions opalescent, the reaction mixtures were centrifuged at 5000 rev/min for 10 min before readings were taken.

Chemicals. TRA (Triethanolamine-hydrochloride, buffer), NADP were purchased from C. F. Boehringer & Soehne G.m.b.H., Mannheim, Germany. Tween 80 was purchased from Fluka AG.

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