THE PRESENCE OF IRON IN PHOTOSYNTHETIC PYRIDINE NUCLEOTIDE REDUCTASE

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San Pietro and Lang(1) has described the presence of a soluble protein factor in chloroplast extract catalyzing the reduction of triphosphopyridine nucleotide by the illuminated chloroplasts. This protein, named by them as photosynthetic pyridine nucleotide reductase (PPNR), appears to be identical with "methaemoglobin reducing factor", a naturally occurring catalyst of reduction of various haem proteins in the Hill reaction, described previously by Davenport et al(2).

PPNR is reddish brown in color, and its absorption increases towards the shorter wavelengths, showing four absorption maxima at 465, 422, 337 and 276 mμ. Davenport and Hill have noted that the disappearance of visible absorption caused by acid or heat treatment of the enzyme was always accompanied by a deterioration of the enzymic activity(3). They have assumed that the color may arise from its content of flavonoid pigment, no haem nor flavin being detected in the enzyme. The present paper will report the presence of iron as essential constituent of the PPNR obtained from spinach leaves. Experimental evidence is also described to show the correlation between the content of iron and the absorption spectrum.

PPNR was extracted from spinach leaves and fractionated with acetone as described by San Pietro and Lang(1). Further purifi-

cation was carried out, using a column of diethylaminoethyl cellulose as described by Hill and Bendal(4). The preparation employed in the present work was electrophoretically homogeneous, and in its absorption spectrum, the relative heights of the absorption maxima at 337, 422 and 465 mμ to that at 267 mμ were 0.62, 0.44 and 0.40, respectively.

It was noticed that storage of PPNR for several weeks at 0°C caused a gradual disappearance of brownish color accompanied by a loss of the enzymic activity. The discolored enzyme thus obtained by aging was found to turn its color to deep red on addition of o-phenanthroline, with which the fresh PPNR preparation exhibited no appreciable change in absorption spectrum over the visible region. Similar decolorization and inactivation of the enzyme caused by acid or heat treatment was always accompanied by the appearance of iron capable of reacting with the chelator. These facts indicate the presence of iron in PPNR, and its liberation on denaturation due to aging or other treatments.

The iron content of PPNR was, therefore, determined with the preparation from which iron had been released by incubating in 1 % hydrochloric acid at 80°C for 10 minutes. The iron was converted into ferrous state with hydroxylamine or hydroquinone as reducing agent and estimated spectrophotometrically with o-phenanthroline. Our preparation of spinach PPNR was found to contain 1.2 % of iron, from which a minimum molecular weight of 4,600 was calculated. On the basis of molecular weight of 17,000 described by Appella and San Pietro(5), it was assumed that PPNR contains about four atoms of iron in one molecule of the protein.

If the determination of iron was carried out in the presence of reducing agents, a small but significant portion of iron was found in a form incapable of forming complex with the chelator.

This finding suggests that, at least, certain portion of the iron in the molecule of PPNR is present in ferric state. A quantitative calculation in this respect, however, will be inadequate, since no special care was taken in this experiment to prevent the released iron from possible valency change during the acid treatment of the protein.

Spectrophotometric investigation revealed that the addition of sodium hydrosulfite was quite ineffective in inducing any significant change in the absorption spectrum of PPNR. In the presence of hydrogen peroxide, a gradual decolorization occurred, but this was found to be due to the liberation of iron from the protein. Of interest is the fact that a similar decolorization accompanied by release of the metal was observed in the presence of p-chloromercuribenzoate. Since the absence of haem in PPNR has been confirmed by Davenport and Hill(3), it is reasonable to assume that the iron is directly combined with the protein through sulfhydryl linkage. In order to investigate the role of iron in PPNR, simultaneous determination of changes in color, iron content and enzymic activity was performed on the enzyme dialyzed against 0.05 M phosphate buffer of pH 7.0 in the presence and absence of 0.01 M of KCN. As will be seen in Fig. 1, enzymic activity decreased most rapidly, while decline of color and iron content was relatively slow and paralleled with each other. This similarity in time course of disappearance of color and iron suggests that the iron constitutes, at least, one of the chromophores of the enzyme. Elucidation of the role of iron in the catalytic activity of PPNR, however, still requires further investigation.

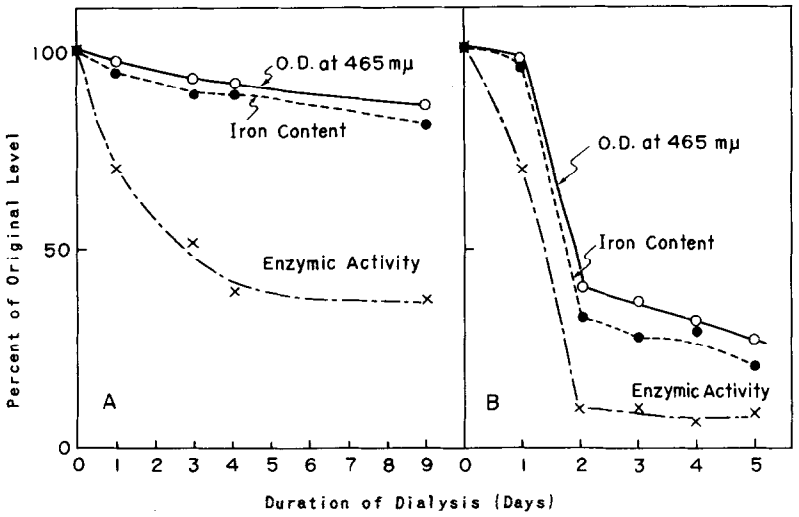

Fig. 1 Effects of dialysis on the color, iron content and enzymic activity of spinach PPNR. Fresh PPNR was dialyzed at 4°C against 0.05 M phosphate buffer of pH 7.0 (A), and the same buffer containing 0.01 M KCN (B). At suitable intervals, aliquots were taken to determine optical density at 465 mμ, iron content and enzymic activity. All values were corrected for dilution and indicated as percent of corresponding values in the original enzyme.

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