Phytochemistry, 1972, Vol. 11, pp. 1595 to 1596. Pergamon Press. Printed in England.

THE IMPORTANCE OF INHIBITING POLYPHENOL OXIDASE IN THE EXTRACTION OF FRACTION 1 LEAF PROTEIN

W. T. JONES and J. W. LYTTLETON

Applied Biochemistry Division, Department of Scientific and Industrial Research, Palmerston North, New Zealand

(*Received* 24 *October* 1971)

Abstract—The interaction between polyphenol oxidation products and Fraction 1 protein produces a modified protein of increased electrophoretic mobility. A method is described for the extraction of red clover leaf Fraction 1 protein free of polyphenol oxidation products by inhibiting polyphenol oxidase with sodium diethyldithiocarbamate.

INTRODUCTION

THE SOLUBLE leaf proteins of higher plants may be classified on the basis of their size into two groups; namely Fraction 1 and Fraction 2 proteins.[1] Fraction 1 protein is an apparently homogeneous protein of high molecular weight, which constitutes the major protein in the chloroplasts of green leaves.[2] It has been identified with the enzyme ribulose diphosphate carboxylase.[3,4] Although Fraction 1 has similar properties whatever its origin, recently Fraction 1 proteins isolated from different plant species have been shown to migrate with different electrophoretic mobilities on acrylamide gel electrophoresis.[5]

During work at this laboratory designed to eliminate phenolic oxidation products from red clover Fraction 1 protein, it was observed that the presence of these oxidation products modified the properties of the isolated protein, and the present paper describes the properties of such modified proteins.

RESULTS AND DISCUSSION

Fraction 1 protein prepared from red clover in the presence of sodium diethyldithiocarbamate (SDDC), a polyphenol oxidase inhibitor, exhibited different physical properties from the protein isolated in its absence. In the latter case, solutions of the isolated protein were brown in colour, with an $A\frac{280\text{ nm}}{260\text{ nm}}$ of 1·4, and preparative ultracentrifugation gave a pellet which was exceedingly difficult to disperse, as found previously by Cohen *et al.*[6] By contrast, when SDDC was used, the Fraction 1 protein gave solutions which were colourless ($A\frac{280\text{ nm}}{260\text{ nm}}$ 1·75–1·85) or pale yellow at concentrations above 20 mg/ml, while the pelleted material dissolved readily. When analysed by gel electrophoresis a mixture of two samples of Fraction 1 protein, prepared with and without SDDC, resolved into two clearly

[1] S. J. SINGER, J. EGGMAN, J. M. CAMPBELL and S. WILDMAN, *J. Biol. Chem.* **197**, 233 (1952).
[2] J. W. LYTTLETON and P. O. P. TS'O, *Arch. Biochem. Biophys.* **73**, 120 (1958).
[3] J. MAYAUDON, *Enzymologia* **18**, 345 (1957).
[4] P. W. TROWN, *Biochemistry* **4**, 908 (1965).
[5] J. H. WILSON and D. R. MACALLA, *Can. J. Biochem.* **46**, 44 (1968).
[6] M. COHEN, W. R. GINOZA, W. R. HUDSON and S. G. WILDMAN, *Science* **124**, 1082 (1956).

separated components. Electrophoresis of each sample separately indicated that the protein prepared in the absence of the inhibitor had the higher electrophoretic mobility.

It is suggested that the enhancement of mobility is due to phenolics which have become bound to the protein after oxidation and these react with amine and sulfhydryl groups on the protein molecule.[7] On gel electrophoresis at pH 8·9, the phenolic groups ionize, giving a greater negative charge, hence greater mobility to the protein. The differences in electrophoretic mobility of Fraction 1 protein isolated from different plants may not be found in any structural differences but arise from differences in the phenol oxidation products which may be adsorbed to the Fraction 1 protein molecule during isolation.

Goldthwaite and Bogorad[8] studied Fraction 1 preparations from a range of dicotyledon species and reported similar electrophoretic migration rates, a result in disagreement with those of Wilson and MacAlla.[5] This discrepancy could be explained by the fact that in the former case the plants were extracted into buffer containing 2% polyvinylpyrrolidone, a known phenolic adsorbent and inhibitor of polyphenol oxidase.[9,11] This would therefore support the hypothesis proposed in the present paper to explain differences in Fraction 1 protein mobility. It is concluded, therefore, that it is important to inhibit the formation of polyphenol oxidation products when isolating Fraction 1 protein.

EXPERIMENTAL

Acrylamide gel electrophoresis. Acrylamide gel electrophoresis was performed using a vertical gel.[10] The electrode buffer was 0·1 M Tris/0·1 M Glycine pH 8·9, and the gel buffer was 0·05 M Tris/0·05 M Glycine. Protein bands were located in the gel by immersion in a 0·025% solution of Coumassie brilliant blue in MeOH–HOAc–H_2O (25:7:68) for 15 min. Gels were destained in this solvent until the background was colourless and either scanned in a densitometer or photographed.

Extraction and isolation of fraction 1 protein. Leaves were infiltrated under vacuum with phosphate buffer (ionic strength, I = 0·2, pH 7·5) containing 1 mM SDDC and 1 mM mercaptoethanol. In control preparations the SDDC was omitted. After crushing the leaves into buffer in a Pirie[12] type disintegrator, Fraction 1 protein was isolated.[13]

[7] W. S. PIERPOINT, *Rothamsted Experimental Station*, part 2, p. 199 (1970).
[8] J. J. GOLDTHWAITE and L. BOGORAD, *Anal. Biochem.* **41**, 57 (1971).
[9] J. W. ANDERSON, *Phytochem.* **7**, 1973 (1968).
[10] M. S. REID and R. L. BIELESKI, *Anal. Biochem.* **22**, 374 (1968).
[11] J. R. L. WALKER and A. C. HULME, *Phytochem.* **4**, 677 (1968).
[12] N. W. PIRIE, *J. Agric. Eng. Res.* **1**, 81 (1956).
[13] W. T. JONES and J. W. LYTTLETON, *N.Z. J. Agric. Res.* (1972), (in press).

Key Word Index—Trifolium pratense; Leguminosae; red clover; Fraction 1 protein; polyphenol oxidase; electrophoresis.