Phytochemistry, Vol. 20, No. 6, pp. 1201–1203, 1981.
Printed in Great Britain.

0031–9422/81/061201–03 $02.00/0

COMPARISON OF TRYPTIC PEPTIDE MAPS OF TWO ISOENZYMES OF 6-PHOSPHOGLUCONATE DEHYDROGENASE FROM TOBACCO TISSUE CULTURES

F. AL-QUADAN, S. H. WENDER and E. C. SMITH

Department of Chemistry, Biochemistry Division, University of Oklahoma, Norman, OK 73019, U.S.A.

(*Received* 5 *August* 1980)

Key Word Index—*Nicotiana tabacum*; Solanaceae; tobacco; 6-phosphogluconate dehydrogenase; isoenzymes; peptide mapping.

Abstract—In a previous study by the authors, two isoenzymes of 6-phosphogluconate dehydrogenase were isolated from cultures of tobacco tissue *Nicotiana tabacum* W-38 and shown to be similar in their pH optima and MWs and in their affinities toward 6-phosphogluconate or $NADP^+$. In an attempt to clarify the structural relationships between these two isoenzymes, peptide mapping of trypsin digests of the purified isoenzymes was performed. The maps were found to be similar, with at least 29 peptide groups from the trypsin digestion of each isoenzyme being alike. There were, however, definite minor differences in the peptide maps of the two isoenzymes.

INTRODUCTION

The pentose phosphate pathway, according to Ashihara and Komamine [1], acts as a source of building blocks for the biosynthesis of phenolic compounds in plants. Godin [2] indicated that phenolic accumulation results from the enhancement of the pentose phosphate pathway. Hoover *et al.* [3] demonstrated that certain of these phenolic compounds have either an inhibitory or stimulatory effect on two of four isoenzymes of the first enzyme of the pathway, glucose-6-phosphate dehydrogenase (G6PD) (D-glucose-6-phosphate: $NADP^+$ 1-oxidoreductase, EC 1.1.1.49) isolated from WR-132 tobacco tissue cultures. More recently, Al-Quadan *et al.* [4] reported that the activities of two isoenzymes of the second dehydrogenase of the pathway, namely, 6-phosphogluconate dehydrogenase (6PGD) 6-phospho-D-gluconate: $NADP^+$ 2-oxidoreductase, EC 1.1.1.44) from W-38 tobacco tissue cultures were each affected in a similar manner by certain phenolic compounds as well as by glucose-1,6-diphosphate or 2,3-diphosphoglycerate. Also, both isoenzymes exhibited no major differences in their affinities towards 6-phosphogluconate (6PG) (0.087 and 0.078 mM) or $NADP^+$ (27 and 3.0 μM) and were found to have approximately the same pH optima (pH 7–9) and MWs (69 070–72 000). In this study, we have tried to clarify the structural relationships between these two 6PGD isoenzymes by peptide mapping of trypsin digests of the purified isoenzymes as part of our attempts to explain the role isoenzymes of G6PD and 6PGD play in the synthesis of plant phenolic compounds.

RESULTS AND DISCUSSION

Enzyme purification

Both 6PGD isoenzymes, termed zones I and II, were separated and purified to the stage previously reported by Al-Quadan *et al.* [4]. The most active fractions of each zone obtained from DEAE-cellulose chromatography were pooled separately and concentrated using a Diaflow membrane filter under a stream of nitrogen gas. Of the total enzyme activity recovered, zone I represented 40% while zone II represented 60%.

The solution containing zone I was brought to 75% $(NH_4)_2SO_4$ saturation and centrifuged at 23 000 g for 15 min and the resulting pellet was dissolved in 50 mM Tris–HCl (pH 8.5)–30 mM β-mercaptoethanol–10% glycerol. After dialysis, the enzyme preparation was layered on the top of a Sephadex G-150 column, which had been pre-equilibrated with the buffer mentioned above. The enzyme was eluted with this buffer and concentrated using a Diaflow membrane filter. The concentrated solution was again dialysed against the same buffer, and a portion of it was added to a 15 ml $NADP^+$ agarose affinity column which had been pre-equilibrated against the buffer. Ligand concentration varied from 2 to 3.6 μM $NADP^+$/g moist weight of column material in each batch used. Chromatography using 50 mM Tris–HCl–30 mM pyrophosphate–30 mM β-mercaptoethanol–10% glycerol (pH 8.5) was performed according to the procedure of Griffiths *et al.* [5] and Betts and Mayer [6]. The most active fractions were pooled, concentrated as before and dialysed for a short time against deionized water to remove the glycerol. Samples analysed by polyacrylamide gel electrophoresis gave a single narrow band when stained for protein or enzyme activity. The highly purified zone I enzyme preparation was lyophilized for storage. This lyophilized preparation could be stored below 0° for weeks without loss of activity, cf. the relatively short life of the partially purified zone I. The zone II 6PGD which had been separated from the zone I 6PGD by DEAE-cellulose chromatography was purified by exactly the same procedure as outlined above. After purification, it too

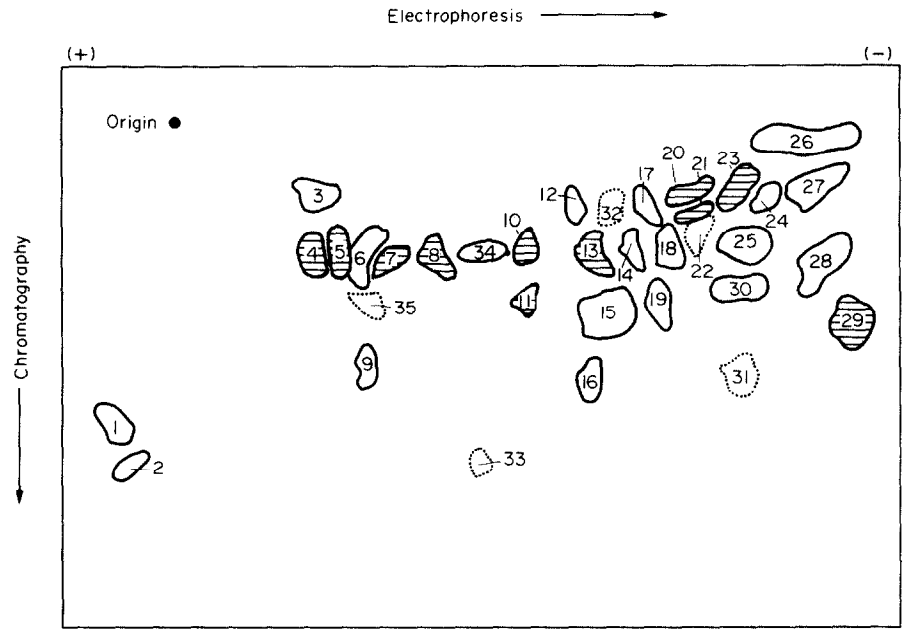

Fig. 1. Tryptic peptide pattern of zone I 6-phosphogluconate dehydrogenase. ▤, Major ninhydrin spots; □, medium intensity ninhydrin spots; ⬚, barely visible ninhydrin spots.

yielded only a single narrow band when stained for protein or enzyme activity after polyacrylamide gel electrophoresis. Both highly purified 6PGD isoenzymes had the same $S_{0.5}$ values for 6PG and $NADP^+$, pH optima, and MWs, as previously reported for the less purified enzymes [4]. Likewise, the effect of phenolic compounds and the effect of sugar phosphate derivatives on the activities of the isoenzymes were similar to those previously reported [4].

Fingerprinting of zones I and II

The highly purified preparations of zone I and zone II 6PGD were subjected to trypsin digestion followed by peptide mapping. The results are shown in Figs. 1 and 2. Most interesting is the fact that these two isoenzymes with similar kinetic properties and MWs also yielded at least 29 peptides that were common to both zone I and zone II. The

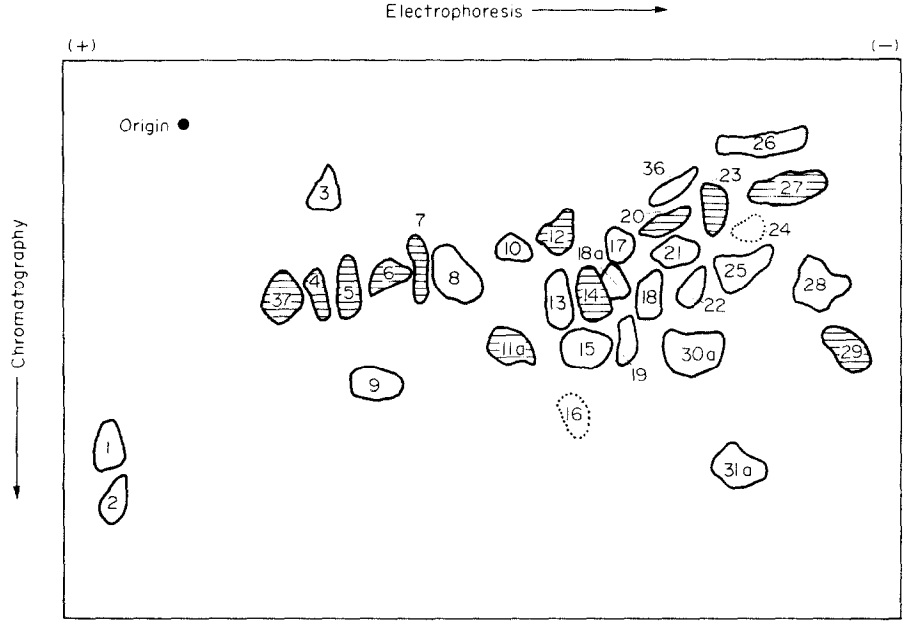

Fig. 2. Tryptic peptide pattern of zone II 6-phosphogluconate dehydrogenase. ▤, Major ninhydrin spots; □, medium intensity ninhydrin spots; ⬚, barely visible ninhydrin spots.

peptides have been put into groups to facilitate comparisons. A match group is defined as a group that has all its members in one zone overlapping the members of the other zone. To determine these matching or overlapping peptides the technique of Kim *et al.* [7] was used. Peptides 1–10, 12–16, 17–19, 20–22, 23–25 and 26–29 can be classified as match groups, and are most likely identical peptides. Thus, the two isoenzymes should have remarkably similar amino acid composition and sequence. However, there are several distinct differences between the two isoenzymes. Looking to the left of peptides 4–8 on the chromatogram of zone I, it is clear that the peptide numbered 37 from zone II was absent in the peptides from zone I, while peptide 34 located on the chromatogram to the right of peptide 8 was found among the peptides from zone I but not from zone II. Several peptides were located in different positions. For example, peptide 11 from zone I migrated more slowly than did 11a from zone II; peptide 31a in zone II migrated faster than peptide 31 of zone I; and peptide 30, located between peptides 19 and 28 in zone I, occupied a different position than peptide 30a from zone II. In summary, it can be concluded that although the two 6PGD isoenzymes are indeed very similar, there are definite minor differences in their peptide maps. The similarities in the peptide maps are not surprising for these isoenzymes, since the two isoenzymes have similar kinetic properties and MWs. Still to be answered, however, is why the tissue cultures elaborate, in almost equal activities, two different isoenzymes with such similar properties.

EXPERIMENTAL

Disc gel electrophoresis. Enzyme solns were analysed for isoenzyme components by polyacrylamide gel electrophoresis according to the method of Ornstein and Davis as outlined by Al Quadan *et al.* [4]. Isoenzymes were visualized according to the procedures described by Schnarrenberger *et al.* [8] and Al Quadan *et al.* [4]. Proteins were visualized using Coomassie Brilliant Blue [4].

Two-dimensional chromatography and high voltage electrophoresis. These procedures were performed according to a slight modification of the methods of Helinski and Yanofsky [9]. Descending chromatography was done using a solvent system of *iso*-BuOH–HCO_2H (90%)–H_2O (70:0.9:2.1) for *ca* 10 hr with methyl red as the indicator. High voltage paper electrophoresis was performed for 1.25 hr at 2.4 kV. The solvent consisted of pyridine–HOAc–H_2O (1:10:300), pH 3.7. Quinine sulfate was used as an indicator. The chromatogram was developed by spraying with 0.5% ninhydrin in Me_2CO followed by heating at 100° for 15 min. Samples were prepared as described by Kim *et al* [7].

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