Note

o-Aminodiphenyl-acetic Acid Test as a Spectrophotometric Method for Determination of Pentose Phosphate Esters

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The *o*-aminodiphenyl-acetic acid test¹⁾ has been conventionally used as a spectrophotometric method for specific determination of aldohexoses and -pentoses in which development of brown, green, and yellow colors depends on the kind of aldose forming a corresponding glycosylamine (Schiff base) with the reagent. Thus, the method is used to determine aldoses even in a mixture containing ketoses which react little or not at all with the reagent. Interestingly, a greenish yellow color unusual for an aldopentose (*e.g.*, L-arabinose) developed when this colorimetric method was used on a cell-free extract of *Escherichia coli* IFO 3545 grown on L-arabinose under hypertonic conditions with 5% sodium chloride (Tanaka, Sunada, Muroi, Taniguchi and Oi, unpublished results). L-Ribulose-5-phosphate was detected as the substance accumulated in the bacterial cell due to regulation of L-arabinose metabolism by the presence of 5% sodium chloride in the medium.^{2,3)} This strongly suggested reactivity of a ketopentose derivative such as its phosphate ester with *o*-aminodiphenyl although the conformation of the ketose itself is not involved in the Schiff base-forming reaction with the reagent. This report is concerned with the usefulness of colorimetric determination of several pentose phosphate esters including L-ribulose-5-phosphate by this method.

The following chemicals were purchased from Sigma Chemical Co.: D-ribulose-5-phosphate; D-xylulose-5-phosphate; D-fructose-6-phosphate; and D-fructose-1,6-diphosphate. L-Arabinose, D-xylose, D-ribose, and D-fructose were the products of Wako Pure Chemical Industries, Ltd. L-Ribulose was prepared by chemical isomerization of L-arabinose as described previously.³⁾ L-Ribulose-5-phosphate was prepared by enzymic phosphorylation of L-ribulose by L-ribulokinase as previously described.³⁾ *o*-Aminodiphenyl (for biochemistry) was obtained from Wako Pure Chemical Industries, Ltd. and was directly used without recrystallization. The reaction was done by heating a mixture of the sample solution (0.4 ml)

containing each sugar or its phosphate ester and 2 ml of 0.4% *o*-aminodiphenyl in glacial acetic acid at 100°C, as described by Timell *et al.*¹⁾ The absorbance of each solution was measured or calibrated using a Shimadzu UV-260 spectrophotometer with 1 cm glass cells.

As shown in Fig. 1A, L-arabinose with brownish yellow color gave an absorption optimum at 380 nm in the reaction, while L-ribulose-5-phosphate gave a characteristic absorption optimum at 650 nm in addition to that around 380 nm and had a greenish yellow color. Although the reaction with L-arabinose required less than 45 min, the reaction with L-ribulose-5-phosphate required 100 min of heating for the maximum absorbance both at 380 nm and 650 nm. The relationship between absorbance and concentration of L-ribulose-5-phosphate was linear between 6.25 and 300 µg per 0.4 ml at 380 nm and 12.5 and 400 µg per 0.4 ml at 650 nm (Fig. 1B).

Table I summarizes the reactivity of other hexoses or pentoses as well as their phosphate esters in the reaction. Aldopentoses tested did not show significant absorbance at 650 nm and the reactions with ketoses¹⁾ were fundamentally negative as reported by Timell *et al.* However, phosphate esters of ketopentoses such as D-ribulose-5-phosphate and D-xylulose-5-phosphate gave the same type of positive reaction as L-ribulose-5-phosphate with another absorption optimum at 650 nm. Phosphate esters of ketohexoses (*e.g.*, D-fructose-6-phosphate and D-fructose-1,6-diphosphate) were less reactive and absorbance at

TABLE I. REACTIVITY OF SEVERAL SUGARS AND SUGAR PHOSPHATE ESTERS IN THE *o*-AMINODIPHENYL-ACETIC ACID TEST^a

Sugar ^b	Concentration (µg/0.4 ml)	Absorbance at	
		380 nm	650 nm
L-Arabinose	100	0.94	0.06
D-Xylose	100	1.02	0.06
D-Ribose	100	0.97	0.07
L-Ribulose	200	0.08	0.01
D-Fructose	200	0.14	0.01
D-R5P	100	0.78	0.52
D-Ru5P	200	1.24	0.55
L-Ru5P	200	0.88	0.48
D-Xu5P	200	1.65	0.56
D-F6P	200	0.28	0.03
D-FDP	200	0.12	0.02

^a The mixture with each free sugar and sugar phosphate ester was heated for 45 min and 100 min, respectively at 100°C.

^b The following abbreviations were used: D-R5P, D-ribose-5-phosphate; D-Ru5P, D-ribulose-5-phosphate; L-Ru5P, L-ribulose-5-phosphate; D-Xu5P, D-xylulose-5-phosphate; D-F6P, D-fructose-6-phosphate; D-FDP, D-fructose-1,6-diphosphate.

FIG. 1. Absorption Spectra of L-Arabinose, L-Ribulose, and L-Ribulose-5-phosphate (A) and Relationship between Concentration of L-Ribulose-5-phosphate and Absorbance (B) in *o*-Aminodiphenyl-acetic Acid Test. In (A), the mixture with L-arabinose or L-ribulose was heated for 45 min at 100°C, while that with L-ribulose-5-phosphate was heated for 100 min at 100°C. The absorbances (650 nm) of L-arabinose and L-ribulose were kept unchanged at a level negligible for the absorbance of L-ribulose-5-phosphate even if they were heated for more than 100 min. a, blank with distilled water; b, L-ribulose (200 µg); c, L-arabinose (100 µg); d, L-ribulose-5-phosphate (200 µg). In (B), each mixture with a different concentration of L-ribulose-5-phosphate was heated for 100 min at 100°C and absorbance was measured at 380 nm (●) and at 650 nm (○).

650 nm was negligible. D-Ribose-5-phosphate was found to be a sugar phosphate ester showing the highest reactivity.

Bial's orcinol test⁴⁾ is known to yield a positive reaction with D-xylulose-5-phosphate and D-ribulose-5-phosphate, giving rise to the same absorption spectrum as L-arabinose and D-ribulose. Dische and Borenfreund⁵⁾ reported that D-ribose-5-phosphate could be determined in the same way as aldopentoses by a modified phloroglucinol test in the presence of ketoses and aldohexoses and -heptoses. Phosphate esters of ketosugars were also negative in this reaction. Accordingly, the reactivity of the above sugar phosphate esters in either the orcinol or phloroglucinol test seems to fundamentally depend on those of corresponding free sugars without a phosphate moiety. These free sugars should interfere with the colorimetric determination of their phosphate esters when present at an

appreciable level. On the contrary, the reactivity of L-ribulose-5-phosphate was quite different from those of free sugars such as L-ribulose and L-arabinose in the *o*-aminodiphenyl-acetic acid test. The mechanism of the characteristic color development is unknown but seems to be very complex, because the presence of a phosphate moiety enabled the molecular structure of ketopentose to react with the reagent and to form a glycosylamine with characteristic absorption spectrum in the reaction (Fig. 1). This was also true for the phosphate esters of other ketopentoses. Thus, the *o*-aminodiphenyl-acetic acid test is useful for the determination of phosphate esters of pentoses when it is done using the absorbance at 650 nm which is free from the influence of aldoses at the same level. Therefore, this method can be used for the determination of phosphate esters such as L-ribulose-5-phosphate and D-xylulose-5-phosphate occurring in the metabolic pathways

of L-arabinose or D-xylose of *E. coli*.

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