A COLOR REACTION OF PENTOSE PHOSPHATE ESTERS SUBSTITUTED IN POSITION 5*

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The differentiation between pentose phosphate esters substituted in position 5 and other phosphate esters of pentoses, as well as free pentoses, by color reactions, was based so far on the difference in the rate which the color in Bial's orcinol reaction is developed by these different compounds. This procedure is not usable in the case of ketopentose phosphates as the difference in the rate of the color development in this case is too small for accurate determinations.

The present paper deals with a modification of the carbazole reaction of carbohydrates in which pentose substituted by phosphate in position 5 gives a completely different absorption spectrum from other pentose phosphate esters, as well as free pentoses and other saccharides. This reaction can be used to detect small amounts of free pentose esters or pentose esters not substituted in position 5 in presence of much larger amounts of esters or nucleotides of pentose substituted in position 5.

EXPERIMENTAL

1. *Procedure*

To 1 ml of a solution containing 0.1 to 1 μmole/ml of ribose-5-phosphate (R-5-P) or a related nucleotide substituted in position 5, or 0.02–0.2 μmole/ml of keto-pentose-5-phosphate are added with shaking 5 ml of 75 volume % sulphuric acid and 1 ml of a freshly prepared solution of carbazole in absolute ethanol. The reaction mixture is repeatedly shaken, immersed in a vigorously boiling waterbath, and then, after exactly 2 minutes, cooled in tap water. The water in the bath must be boiling so vigorously that the boiling does not stop after immersion of the test tubes. Solutions of adenosine-5-phosphate (Ad-5-P) either free or in combined form in coenzymes, or adenosine-polyphosphates produce a greenish blue color, while all other pentoses and their esters produce a purple color.

2. *Absorption spectra*

The reaction product from Ad-5-P and its derivatives has a characteristic absorption curve with 2 maxima, one at 710 and the other, less pronounced, at 600 mμ (Fig. 1). Between the two maxima is a minimum at 630 mμ. The absorption curve of the purple color from free pentose and adenosine-3-phosphate (Ad-3-P), on the other hand, shows one single absorption maximum at 540 mμ. The optical density at 710 mμ remains fairly constant, but the absorption at the lower wavelengths increases in intensity for hours. To obtain as great a difference as possible between the absorption spectrum of the two classes of nucleotides, it is necessary to carry out the spectrophotometric measurements not later than 5 to 10 minutes after the end of the heating period and in as short a time as possible. Adenosine-polyphosphates (ATP and ADP) as well as dinucleotides (coenzyme I and coenzyme II) show a slightly different behavior from Ad-5-P itself in so far as the ratio D_{540}/D_{710} in these compounds is somewhat higher than that for Ad-5-P itself. As will be demonstrated in a subsequent paper, this is due to the fact that the phosphate on carbon 5 in these

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compounds is much more rapidly split off by the acid than from Ad-5-P. With these compounds, therefore, a greater amount of ribose itself or of adenosine will be present in the reaction mixture and produce a higher D_{540}. The same is true of the two ketopentose phosphates. D_{540}/D_{710} for

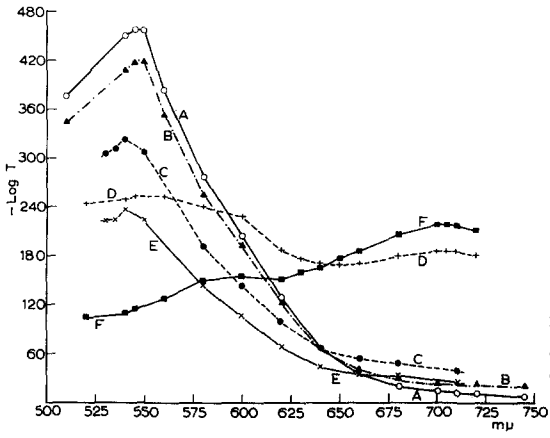

Fig. 1. Absorption spectra in the carbazole reaction of various pentoses and their esters. A = ribulose 0.1 μmole/ml. B = xylulose 0.08 μmole/ml. C = ribose 0.25 μmole/ml. D = ribulose 5-phosphate 0.12 μmole/ml. E = adenosine-3-phosphate. F = adenosine-5-phosphate.

ribulose-5-phosphate was found to be 1.47 and for xylulose-phosphate 1.28 (Table I). In the latter case the determination was carried out indirectly on a mixture of the two ketopentose-5-phosphates obtained from a pure natural mixture of ribose-5-phosphate isomers from a human hemolysate. The lower value of this ratio for xylulose-5-phosphate agrees with the lower hydrolysis constant of this ester as compared to that of ribulose-5-phosphate. The values for ketopentose-5-phosphate, however, are still far below the values for Ad-3-P or the three ketopentoses. The ratios D_{540}/D_{710} for xylulose and ribulose and xylulose-1-phosphate exceed those for ketopentose-5-phosphate about as much as values for free aldopentose those of their 5-phosphate esters.

TABLE I

OPTICAL DENSITIES AT 710 AND 540 mμ AND THEIR RATIOS IN THE CARBAZOLE REACTION OF VARIOUS PENTOSES AND THEIR ESTERS. READINGS 10–30 MINUTES AFTER HEATING

Expt. No.	*Substance*	*Concentration μmoles/ml*	$D_{710} \times 1000$	$D_{540} \times 1000$	$D_{540}/_{710}$
I	Adenosine-5-phosphate	0.5	345	176	0.51
	Adenosine-3-phosphate	0.5	49	415	8.5
	Ribose	1.0	116	940	8.4
II	Ribose-5-phosphate	0.4	265	129	0.48
	Ribulose-5-phosphate	0.33	346	548	1.30
	Adenosine-3-phosphate	0.8			
III	Ribulose-5-phosphate	0.14	90	129	1.43
	Ribulose	0.13	17	451	26.5
	Xylulose	0.12	20	511	25.5
	Xylulose-1-phosphate	0.13	30	570	19.0
IV	(a) Adenosine-5-phosphate	0.5	320	170	0.53
	(b) Adenosine-5-phosphate	0.25	163	86	0.54
	(c) Adenosine-3-phosphate	0.5	42	425	10.1
	Adenosine-5-phosphate (a) + adenosine-3-phosphate (c)		356	560	1.58

3. *Specificity*

The reaction with carbazole is a general reaction of carbohydrates. Glycolic aldehyde, triose and hexoses also give strong colors with absorption maxima at 620, 600 and

560 mμ respectively. The different classes of sugars, therefore, can be distinguished by the form of the absorption curve of their colored products.

4. *Detection of pentose substituted in position 5 by the carbazole reaction*

With R-5-P, Ad-5-P, R-3-P, as well as ribulose-5-phosphate, D_{710} is proportional to concentration. Mixtures of R-5-P and ribulose-5-phosphate, as well as mixtures of Ad-5-P and Ad-3-P, showed additive behavior.

The great differences in the value of D_{540}/D_{710} between pentose substituted in position 5 and that of derivatives of ribose substituted in other positions, as well as free ribose and ketopentose, make it possible to detect 3-esters in presence of an excess of 5-esters. Because of the rapid changes at room temperature in the value of this ratio for various esters, it is always necessary to make the readings of the experimental sample simultaneously with those of standard samples of esters assumed to be present in the experimental mixtures. It is possible under these conditions to detect about 10% of ribose not substituted in position 5 in presence of 90% of R-5-P or Ad-5-P. The lack of specificity of the reaction, however, imposes severe limitations on this use of the reaction. In general it can be applied only to solutions in which no significant amounts of sugars other than pentoses are present. Because of small differences in the value of D_{540}/D_{710} between adenosine-5-phosphate and its polyphosphate and dinucleotides, moreover, the presence of these compounds in larger amounts must be taken into account. At present the reaction seems to be of advantage only for the study of isomerization processes in which an interconversion of various types of pentose phosphate esters takes place.

5. *Influence of SH groups on the carbazole reaction of nucleotides*

SH compounds like cysteine and glutathione interfere with the carbazole test insofar as the difference in the absorption curve between ribose-5-phosphate and ribose-3-phosphate becomes less pronounced. The optical density at the same time increases considerably. These compounds can be eliminated by incubation in 0.5 N Na_2CO_3 for one hour at room temperature under shaking. This treatment, however, cannot be applied in presence of ketopentoses, and for nucleotides and aldopentoses, the standard solutions must be treated like the experimental samples.

SUMMARY

1. A color reaction is described which differentiates between pentose-5-phosphates and free pentose and other pentose esters.

2. The application of this reaction for the detection of pentose-5-phosphates in presence of other pentose esters is discussed.

REFERENCE

1. Z. DISCHE, *Biochem. Z.*, 189 (1927) 77.

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