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METHODS FOR THE QUALITATIVE AND QUANTITATIVE DETERMINATIONS OF TETROSES BY TWO NEW SPECIFIC COLOR REACTIONS*

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Studies of the role of tetrose in the intermediary metabolism and interconversion of sugars in animal and plant cells suggested the development of simple and sensitive color reactions for their qualitative and quantitative determination. The present report deals with two such reactions.

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References p. 188.

PRINCIPLE

When solutions of sugars containing more than 4 carbon atoms in the chain are treated with concentrated H_2SO_4, furfural or its homologues and derivatives are produced, and these show characteristic ultraviolet absorption spectra[1]. When cysteine is added to such reaction mixtures it combines with these compounds and yields colored products with absorption spectra in the visible region. On standing at room temperature these colored products undergo secondary transformations which lead to colored products with different absorption spectra. Tetrose and lower homologues cannot form derivatives of furan and do not yield colored products after addition of cysteine when they are the only sugars in solution. Tetroses, however, can combine with breakdown products of higher sugars to give products with characteristic absorption spectra in the visible part of the spectrum.

1. *Reaction of tetrose with fructose and sulfuric acid*

Procedure. To 0.5 ml of a solution of tetrose containing 100–400 μg/ml are added 0.5 ml of a 0.1% solution of fructose and 4.5 ml of a mixture of one part H_2O and six parts of concentrated H_2SO_4 with thorough cooling in ice water. The mixture is left for a few minutes, then shaken in ice water, and after a few minutes transferred first to tap water and then placed for exactly three minutes in a vigorously boiling water bath. A blank containing water instead of the tetrose solution is run simultaneously. The heated reaction mixture is immediately cooled in tap water and left overnight at room temperature. A yellow color with an absorption maximum at 438 mμ develops (Fig. 1). On the next day, 1.2 ml of H_2O are added with cooling in tap water and the mixture is left for at least eight hours at room temperature.

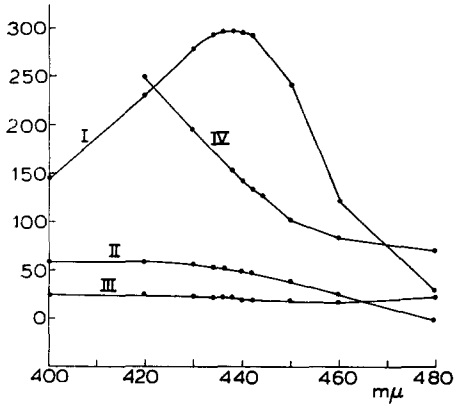

Fig. 1. Absorption spectra of erythrose and other saccharides in the reaction with H_2SO_4. I. Erythrose 2 mg %. II. Equilibrium mixture of R-5-P and its ketopentose-5-phosphate isomers 30 mg % total pentose. III. Fructose 10 mg %. IV. Sedoheptulose diphosphate 6 mg %.

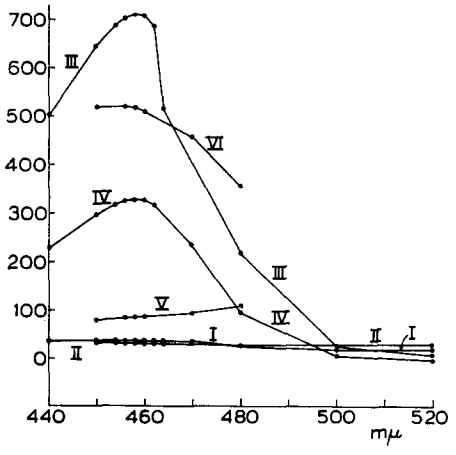

Fig. 2. Absorption spectra in the cysteine H_2SO_4 reaction of various saccharides. I. Equilibrium mixture of Fructose 1.6-diphosphate and triose phosphate 5 mg % total saccharide. II. Equilibrium mixture of R-5-P and its ketopentose isomers 50 mg % total pentose. III. Erythrose-4-phosphate, 2 mg % erythrose. IV. Erythrose 1.6 mg %. V. Sedoheptulose monophosphate 1.5 mg % heptose. VI. R-5-P 170 mg % ribose.

References p. 188.

2. *Reaction of tetrose with fructose, sulfuric acid and cysteine*

Procedure. To the mixture of tetrose solution and H_2SO_4 prepared and heated as described above, is added immediately after cooling 0.1 ml of a 3% solution of cysteine HCl. The mixture is left at room temperature overnight and then 1.2 ml of water is added to it. The yellow color is replaced by a greenish-yellow one with an absorption maximum at 458 mμ (Fig. 2). The maximum color is obtained after about 10 hours and the color remains constant for many hours. The spectrophotometric readings are carried out against a corresponding blank, which shows a faint purple color.

3. *Specificity of the two reactions*

All three tetroses (erythrose, threose and erythrulose) produce in both reactions almost identical absorption curves. Higher saccharides also influence the secondary cysteine reaction of fructose in a similar way like tetroses and, therefore, yield various colors which are much less intense than that produced by tetroses and differ completely in their absorption curves. Of the lower homologues of tetrose, only glycolic aldehyde produces the same form of absorption curve as the tetroses, but the molar extinction coefficient is only about 1/20 of that from erythrose (Table 1). Trioses and their phosphates do not produce any color whatsoever; formaldehyde yields an uncharacteristic brown color, with almost uniform absorption between 400 and 500 mμ, which does not interfere with the reaction of tetroses.

4. *Influence of the concentration and nature of tetrose on intensity of the reaction with cysteine*

As can be seen from Table I, the optical densities at 458 mμ (*D* 458) and the differences *D* 458–*D* 480 are proportional to the concentration of erythrose in the range between 10 and 40 μg/ml. Erythrose-4-phosphate has the same molar extinction coefficient as erythrose, as shown by the fact that dephosphorylation by prostate phosphatase of this ester does not significantly change its reactivity. It was, therefore, used for the

TABLE I

OPTICAL DENSITIES AT 458 AND 480 mμ AND THEIR DIFFERENCES IN THE FRUCTOSE CYSTEINE H_2SO_4 REACTION OF SOLUTIONS OF ERYTHROSE AND OTHER SUGARS AT VARIOUS CONCENTRATIONS

Expt. No.	*Substance*	*Concentration* μg/ml	*Optical densities*		*D 458 – D 480*
			D 458	*D 480*	
I	Erythrose	40	1.135	0.312	0.824
	Erythrose	20	0.598	0.165	0.432
	Erythrose	10	0.296	0.083	0.213
	Threose	20	0.720	0.192	0.528
	Erythrulose	20	0.750	0.207	0.543
II	Erythrose	20	0.600	0.164	0.436
	Erythrose	10	0.300	0.083	0.217
	Erythrose	5	0.152	0.042	0.110
	Glycolic Aldehyde	100	0.325	0.090	0.235
	Sedoheptulose	25	0.079	0.105	—0.026
III	Fructose	100	0.035	0.061	—0.026
	Ribulose-5P & Ribulose-5P	1200 800	0.046	0.030	0.016

References p. 188.

standardization of erythrose solutions. Threose (prepared from its isopropylidene derivative) and erythrulose (prepared and assayed in Dr. HORECKER'S laboratory) showed extinction coefficients higher by 21% and 26% respectively than that of erythrose*.

5. *Effect of alkali on the two color reactions of tetrose and other sugars*

When solutions of tetroses are incubated for 15 minutes with 1 *N* HaOH at room temperature, 40% of erythrose, and about 80% of erythrose-4-phosphate is destroyed. The products of this breakdown do not contribute significantly to the two color reactions, and after the alkali treatment the absorption curves of tetroses in these reactions do not change materially as far as their form is concerned. This alkali treatment degrades also other saccharides, particularly keto sugars, and in this case some of the reaction products show a positive absorption in the reaction with fructose and sulfuric acid with and without cysteine. This leads to a significant change in the form of their absorption curve which is particularly pronounced in the case of ketopentose-5-phosphates. The difference absorption curves determined by subtracting the optical densities at various wave lengths in the two color reactions carried out with solutions before and after treatment with alkali, therefore, do not differ significantly in their form in the case of tetroses. In the case of other sugars, these difference curves become negative as illustrated in Fig. 3 and 4. The difference absorption curves in the two color reactions are, therefore, in general even more characteristic for tetroses, as compared with other sugars, than the simple absorption curves.

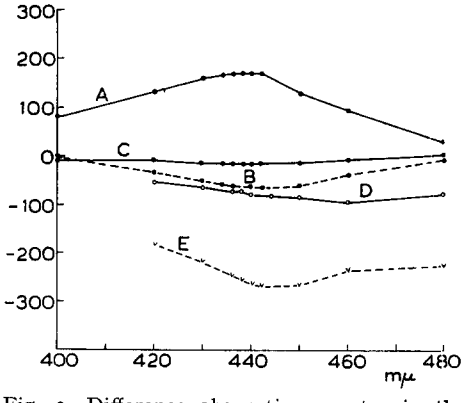

Fig. 3. Difference absorption spectra in the H_2SO_4 reaction of various saccharides treated and not treated with alkali. A. Erythrose 2 mg %. B. Equilibrium mixture of R-5-P and its ketopentose-5-phosphate isomers 30 mg % total pentose. C. Fructose 10 mg %. D. Sedoheptulose diphosphate 6 mg % heptulose. E. Xylulose-5-phosphate 6 mg % pentose.

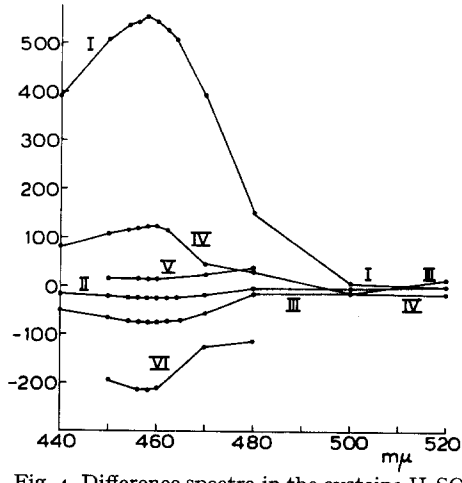

Fig. 4. Difference spectra in the cysteine H_2SO_4 reaction of various saccharides before and after treatment with alkali. I. Erythrose-4-phosphate 4 mg % erythrose. II. Equilibrium mixture of fructose 1.6-diphosphate and triose phosphate 5 mg % total saccharide. III. Equilibrium mixture of R-5-P and its ketopentose isomers 30 mg % total pentose. IV. Erythrose 1.6 mg %. V. Sedoheptulose monophosphate 1.5 % heptose. VI. R-5-P 170 mg % ribose.

* We are greatly indebted to Dr. HENRY Z. SABLE for a preparation of erythrose; Dr. CLINTON E. BALLOU for a preparation of erythrose-4-phosphate; Dr. NELSON K. RICHTMYER for a preparation of isopropylidene threose; and Dr. GILBERT ASHWELL and Dr. B. L. HORECKER for a preparation of erythrulose.

References p. 188.

6. *Detection of tetrose by the two color reactions*

To detect tetroses by their absorption curves in the reaction with fructose and sulfuric acid alone, or with the addition of cysteine solutions which contain other sugars in large excess, it is obviously necessary to determine first quantitatively all the sugar components in the solution and their contribution to the absorption of the unknown in the two reactions. The use, however, of the reaction with fructose and sulfuric acid without addition of cysteine may, in the case of extracts from living tissues, give doubtful results because many organic substances when heated with H_2SO_4 produce yellow and brown colors which will distort the absorption curves from tetrose. This difficulty can in general be met by determining the difference absorption curves before and after treatment with 1 *N* NaOH. The absorption due to reactions of other substances than sugars with H_2SO_4 can be expected not to be significantly influenced by alkali at room temperature. The changes in the absorption by sugars due to alkali are small compared with their initial absorption as well as with the change in absorption of tetroses.

7. *Quantitative determination of tetrose in tissue extracts*

Sugars present in mixture with tetroses in large excess of the latter may depress by a few per cent the color produced by tetrose. In determining, therefore, tetrose in tissue extracts, it is advisable to use an internal standard. If deproteinization of the tissue is carried out with trichloroacetic or perchloric acid, the blank should contain approximately the same amounts of these acids as does the unknown. A solution containing 1 μmole of erythrose per ml shows a D 458 between 0.08 and 0.1 in various determinations, depending upon the temperature and time of reading. To achieve the maximum reproducibility and agreement between duplicate samples, it is necessary to make readings at 458 mμ as well as at 480 mμ, and use the difference D 458–D 480 as measure of the concentration of tetrose. This difference is only about 20% smaller than E 458 itself. This difference, furthermore, is almost negligible for hexoses and pentoses. For heptoses, however, D 458–D 480 is negative and can be significant. In presence of significant amounts of heptose, therefore, the latter must be separately determined and the result corrected for the negative absorption due to this sugar.

SUMMARY

Two specific color reactions of tetroses, which permit the quantitative determination of between 5 and 50 μg of these sugars are described.

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