COMPETITIVE INHIBITION OF 3-KETOSUCROSE FORMATION BY D-GLUCOSE[1]

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A new, crystalline disaccharide was isolated recently from culture media of the crown-gall tumor-inducing organism Agrobacterium tumefaciens grown on sucrose as the carbon source (Fukui, et al., 1963). It was characterized by a variety of physical, chemical and enzymatic techniques and evidence has been presented to prove the structure of the new keto compound to be: α-D-ribo-Hexopyranosyl-3-ulose-β-D-fructofuranoside (the trivial name "3-ketosucrose" will be used throughout this paper). In a continuing study on some of the microbiological aspects of 3-ketosucrose formation it was found that the above conversion from sucrose was strongly inhibited by D- but not by L-glucose. It is the purpose of this communication to describe this effect.

Materials and Methods

Whereas D-glucose and other sugars used were commercial preparations authentic L-glucose was kindly donated by Drs. H. S. Isbell (National Bureau of Standards, Washington) and W. Z. Hassid and E. F. Neufeld (University of California). Strain B_6 of Agrobacterium tumefaciens was used in this study; it was obtained originally from Dr. A. C. Braun, Rockefeller Institute, New York.

The culture medium of McIntire et al. (1942) was used with the omission of zinc ions and the phosphate concentration was tripled over the values given by

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these authors. Sucrose (2%) was used as carbon source and under these conditions the pH of the culture medium did not fall below 6.8 during the growth period. Cells of A. tumefaciens were grown on a rotary shaker at 26° C for approximately 44 hours until the cell count reached 5-7 x 10^9 cells per ml. Cells were usually removed by centrifugation and all analyses were carried out on the supernatant solutions. The medium generally contained 13-16 mg of 3-ketosucrose per ml when cells were grown as stated above.

3-Ketosucrose reacted readily in the Nelson (1944) modification of the micro-Somogyi method at room temperature (23° C) where 95% of maximum color was attained in 2 hours. Sucrose itself was determined by the same method but only after hydrolysis in 0.1 N/H_2SO_4 for 30 min. Values were then obtained as a net difference between the hydrolyzed samples and the unhydrolyzed controls.

Components of the experimental reaction mixture (in 10 ml) were as follows: 1.17 x 10^{-1} M sucrose, 2.5 ml; 1.0 M phosphate buffer (pH 7.0), 1.0 ml; 0.1 M $MgSO_4.7H_2O$, 0.1 ml; A. tumefaciens resting cells, 30 mg wet weight in 1.0 ml; distilled water and inhibitor (where used), to volume. Temperature of reaction, 26° C; time, 60 min. (except where indicated otherwise).

Results

The first striking effect observed was the inhibition by D-glucose (0.2% in the culture medium) of the conversion of sucrose (2%) to 3-ketosucrose when sucrose was used as carbon source in the presence of growing cells. The rate of cell growth, in media containing sucrose compared with others containing both sucrose and D-glucose, was almost the same (8.6 and 8.9 mg/ml, respectively, after 44 hours), but the formation of 3-ketosucrose was completely suppressed in the presence of D-glucose (e.g., 12.6 mg/ml with sucrose alone and < 0.2 mg/ml with sucrose plus D-glucose). From other experiments with intact resting cells it was calculated that the rate of 3-ketosucrose formation/ml/mg wet cells/hour was 1.93 when sucrose was the substrate and 0.0 when sucrose and D-glucose were used together.

No inhibitory effect could be demonstrated when L-glucose was used in place of D-glucose. The relevant data are presented in Fig. 1A. They show that: a) the rate of 3-ketosucrose formation was linear in the first three hours with sucrose (S) as substrate, b) the addition of L-glucose at zero time (S + L - G(0)) had no effect at all on this rate, c) the presence of D-glucose from the start (S + D - G(0)), however, resulted in a dramatic inhibition which could be achieved also by addition of the D-glucose 30 min. after the reaction had been allowed to start (S + D - G (30)). A Lineweaver-Burk (1934) plot of a typical experiment in which D-glucose was used to inhibit the reaction showed clearly that the effect observed was of the competitive type (Fig. 1B).

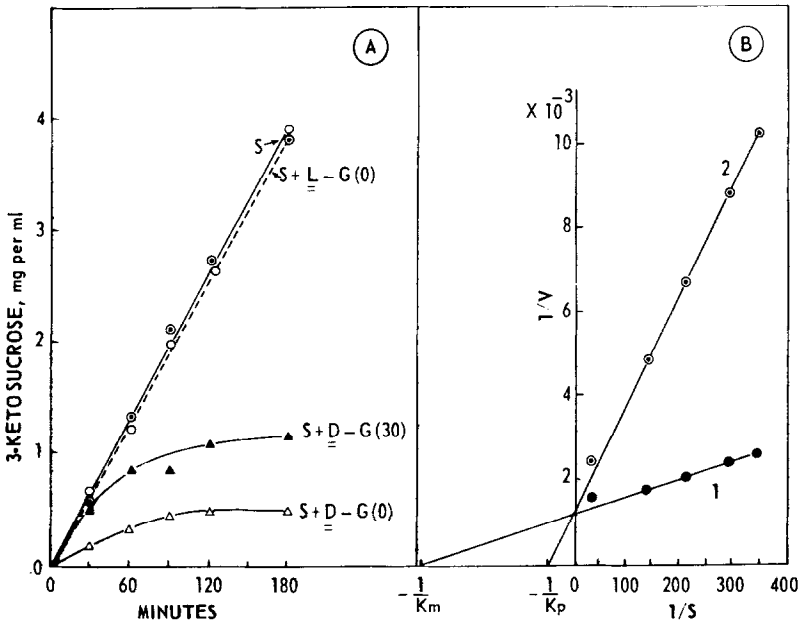

Fig. 1. A) Effects of 5.5×10^{-3} M D- and of L-glucose on the rate of conversion of sucrose to 3-ketosucrose in the presence of resting cells of A. tumefaciens (B_6).

B) Lineweaver-Burk plot of the above reaction in the absence (1) and in the presence (2) of 2.8×10^{-3} M D-glucose.

A comparison with other carbohydrates or carbohydrate-containing substances as potential inhibitors is given in Table I. All data in the Table

pertain to additions of the potential inhibitors at zero time, i.e. at the same time at which the substrate (sucrose) was added. Pre-incubation with D-glucose for 30 min. prior to sucrose addition increased the inhibition by 5.5×10^{-3} M D-glucose to 100%. On the other hand, pre-incubation with 2:4-dinitrophenol did not change the effectiveness of the latter significantly. Also, when the concentration of D-galactose and of α-methyl-D-glucoside was each raised ten fold, a small inhibitory effect of approx. 10% was observed.

Table 1

Effects of some carbohydrates and of 2:4-dinitrophenol on the conversion of sucrose to 3-ketosucrose by A. tumefaciens

Compound	Concentration (molar)	Inhibition[x] (per cent)
D-Glucose	5.5×10^{-3}	85
D-Glucose	5.5×10^{-4}	45
L-Glucose	1.1×10^{-2}	0
D-Galactose	5.5×10^{-3}	0
α-Methyl-D-glucoside	5.5×10^{-3}	0
Phloridzin	1.0×10^{-3}	28
D-Xylose	6.6×10^{-2}	0
D-Fructose	2.9×10^{-2}	0
2-Deoxy-D-glucose	6.1×10^{-3}	0
2-Deoxy-D-ribose	6.2×10^{-3}	0
2:4-Dinitrophenol	1.0×10^{-3}	32
2:4-Dinitrophenol	1.0×10^{-3}	58[xx]

[x]All results pertain to a 60-min. reaction time except the one marked [xx] which refers to an experimental time of 120 min.

The results described above were obtained in studies with intact cells. When the conversion of sucrose to 3-ketosucrose was carried out with a cell-free extract of A. tumefaciens (prepared by sonic oscillation at 10 KC at a power output of 0.9 Amp. for 10 min.) no inhibition could be demonstrated with 5.5×10^{-3} M D-glucose or with 1.0×10^{-3} M 2:4-dinitrophenol as the intended inhibitors.

Discussion

The stereospecificity of D-glucose as a competitive inhibitor of the conversion of sucrose to 3-ketosucrose shown above to occur only with intact cells but not with cell-free preparations gives rise to speculation on the possible mechanism of the D-glucose effect with respect to the entry of sucrose into the bacterial cell. The similarity of the effect compared to the action of 2:4-dinitrophenol used under the same experimental conditions reinforces the view that the probable action of these inhibitors is on the entry process. Work is now in progress which is designed to provide an answer to this important question.

References

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