GLUCONEOGENESIS FROM D-TAGATOSE BY ISOLATED RAT AND HAMSTER LIVER CELLS

Robert ROGNSTAD

Cedars-Sinai Medical Research Institute, Los Angeles, California 90029, USA

Received 10 February 1975

1. Introduction

D-Tagatose, the ketose related to D-galactose, has been shown recently to be a good substrate for beef-liver fructokinase [1]. We have investigated whether tagatose is further metabolized by the liver, and have found that it is a rather good gluconeogenic substrate in rat and hamster liver cells.

2. Methods

Isolated rat or hamster liver cells were prepared from 24-hr fasted animals by the procedure of Berry and Friend [2] with minor modifications (omission of hyaluronidase and use of Krebs-Henseleit [3] buffer). Incubations were run in 25 ml Erlenmeyer flasks in 2 ml of Krebs-Henseleit buffer with 5% CO_2/95% O_2 in the gas phase. Previously described methods were used for determination of tritium yields in glucose [4] and water [5]. Enzymic analysis of glucose was carried out according to Slein [6], with the substitution of NAD^+ for $NADP^+$, and with the use of glucose 6P dehydrogenase from *Leuconostoc mesenteroides*. Hexokinase (Sigma Type C-302) free of glucose 6P isomerase was used. After the reaction had gone to completion, glucose 6P isomerase was added to analyze for fructose. These methods were also used to test for possible glucose or fructose impurities in tagatose. Glycerol was determined according to Wieland [7].

D-Tagatose was obtained from Sigma Chemical Co. (St. Louis, Mo. USA). An earlier study [8] of liver fructokinase had stated that apparent activity towards tagatose as substrate was in actuality due to a 25% impurity of fructose in the commercial (from a different source) tagatose used. The tagatose obtained from Sigma contained no glucose or fructose by enzymic assay. The more recent study on beef-liver fructokinase by Raushel and Cleland [1] which reports activity towards tagatose, used tagatose also obtained from Sigma.

D-[1R,S-T]tagatose was synthesized by reduction of D-galactosone (200 μmol) with $NaB[T]H_4$ (about 10 μmol) in 1 ml of water at pH 7. The aldehyde group is reduced much faster than the ketone group [9]. D-Galactosone was made by scaled-down versions of standard procedures involving the preparation of D-galactose phenylosazone [10], followed by nitrous acid treatment to form the osone [11]. The D-[1R, S-T]tagatose was purified on a 1 × 20 cm column of Dowex 1 × 8 (borate), being eluted with 0.035 M $(NH_4)_2B_4O_7$, and further purified by paper chromatography using phenol/water (80/20, by vol.) as solvent.

3. Results and discussion

Hamster liver cells were incubated with D-tagatose in concentrations from 2 mM to 20 mM (table 1). At 2 mM, over half of the substrate was converted into glucose in 2 hr. The rates of gluconeogenesis were nearly the same during the first and second hours. The rate of gluconeogenesis from 20 mM D-tagatose was about twice that from 20 mM D-galactose. This suggested that an isomerization of tagatose or a tagatose phosphate to galactose or a galactose phosphate, was not involved in the gluconeogenic pathway. Since tagatose has been shown to be a substrate for fructokinase, with a K_m only slightly higher, and a V_{max}

Table 1
Gluconeogenesis from D-tagatose or D-galactose by isolated hamster liver cells.

Gluconeogenic substrate (Concentration)	Glucose formation (μmol) 1 hr	2 hr
Endogenous	0.4	0.7
D-Tagatose (2 mM)	1.9	3.1
D-Tagatose (5 mM)	2.7	5.0
D-Tagatose (10 mM)	3.1	5.6
D-Tagatose (20 mM)	3.1	5.7
D-Galactose (20 mM)	1.5	3.1

Isolated hamster liver cells (about 20 mg dry weight) were incubated in 2 ml Krebs-Henseleit buffer with the substrates shown.

about the same as that of fructose [1], it seemed likely that this might be the initial step in the pathway. Lardy [12] has noted that tagatose 1,6-diP is slowly cleaved by rabbit-muscle aldolase to D-glyceraldehyde 3P and dihydroxyacetone P. By analogy alone (no direct tests of this have yet been made), it is possible that aldolase B of the liver may be able to cleave D-tagatose 1P to D-glyceraldehyde and dihydroxyacetone P. If the gluconeogenic pathway proceeded through the triose phosphate stage, extensive isotopic equilibration of the triose phosphates would cause labelling of C-6 of glucose from a tagatose originally labelled on C-1. Unfortunately no D-[1-^{14}C]tagatose was available for this purpose, but we were able to prepare D-[1R,S-T]tagatose from a precursor on hand. It should be noted that in the reduction of the aldehyde group of galactosone with tritiated borohydride, approximately equal labelling is expected of the two hydrogens on C-1 of the tagatose formed.

Rat liver cells were incubated with D-[1R,S-T]tagatose, again at several concentrations, and D-[IT]galactose was included for comparison (table 2). Again, at 20 mM substrate concentration, D-tagatose produced glucose at more than twice the rate from D-galactose. Periodate degradation of the glucose formed from D-[1R,S-T]tagatose showed nearly two-thirds of the tritium on C-6, the rest undoubtedly largely on C-1. The results are what would be expected from a pathway involving partial isotopic equilibration of the triose phosphates, followed by the loss of most of the tritium in one of the two stereospecific positions on C-1 of fructose 6P in the glucose 6P isomerase reaction [13]. The glucose formed from D-[IT]galactose, on the other hand, had only 10% of the tritium on C-6. The accepted gluconeogenic pathway from D-galactose proceeds through glucose 6P. The limited amount of labelling on C-6 can be related to F6P $\rightleftarrows$ FDP futile cycling, evidence for which was previously presented by Clark et al. [14] when galactose is the gluconeogenic substrate, with the use of [5T]glucose.

Another conceivable pathway might involve formation and cleavage of D-tagatose 1,6-diP, but no

Table 2
Gluconeogenesis from D-[1R,S-T] tagatose or D-[1T]galactose by isolated rat liver cells

Gluconeogenic Substrate (Concentration) Endogenous	Total glucose formation (μmol/2 hr)	Glucose formation from substrate (μmol/2 hr)	Isotopic yields (% of added T) Glucose	Water	Residual substrate	% of T on C-6 of glucose
D-[1R,S-T] Tagatose (2 mM)	5.6	2.6	31	25	40	68
D-[1R,S-T] Tagatose (5 mM)	9.3	6.3	31	21	39	65
D-[1R,S-T] Tagatose (10 mM)	14.4	11.4	32	20	45	62
D-[1R,S-T] Tagatose (20 mM)	19.6	16.6	25	13	53	64
D-[1T] Galactose (20 mM)	9.0	6.0	4	12	80	10

Isolated rat-liver cells (about 50 mg dry weight) were incubated for 2 hr in 2 ml of Krebs-Henseleit buffer. Glucose formation from the added substrate assumes that endogenous glucose formation proceeds at the same rate as in the absence of added substrate.

Table 3
Effect of ethanol on tagatose metabolism

Substrate (Concentration)	Glucose Formation (μmol/2 hr	Glycerol Formation (μmol/2 hr
Endogenous	3.2	0
D-Tagatose (20 mM)	24.4	0.1
D-Tagatose (20 mM) Plus Ethanol (20 mM)	16.4	1.2

Rat liver cells (about 50 mg dry weight) were incubated for 2 hours with the substrates shown.

enzyme system in the liver has been reported which would convert tagatose 1P into tagatose 1,6-diP (or phosphorylate tagatose to tagatose 6P). Bissett and Anderson [15,16] have recently shown that certain bacteria contain enzymes for an alternate pathway of galactose metabolism, involving isomerization of galactose 6P to tagatose 6P, conversion into tagatose 1,6-diP, by an enzyme distinct from phosphofructokinase, and cleavage to the triose phosphates by an aldolase distinct from fructose 1,6-diP aldolase. In order to establish whether D-glyceraldehyde is formed in the metabolism of D-tagatose by the liver, we have incubated D-tagatose in the presence of ethanol (table 3). It is known that ethanol causes a certain amount of glycerol production from the D-glyceraldehyde formed from fructose, when ethanol and fructose are simultaneously metabolized [17]. Metabolism of D-tagatose and ethanol also results in a small amount of glycerol formation. This result is consistent with the pathway proposed involving aldolase cleavage of tagatose 1P, but does not prove that this is the sole pathway. Further studies on enzyme specificities and on isolation of intermediates in large-scale incubations with ^{14}C-labelled tagatose will be required to outline fully the pathway followed.

Acknowledgement

This work was supported by U.S. Public Health Service Grant No. AA1226.

References

[1] Raushel, F. M. and Cleland, W. W. (1973) J. Biol. Chem. 248, 8174–8177.
[2] Berry, M. N. and Friend, D. S. (1969) J. Cell. Biol. 43, 506–520.
[3] Krebs, H. A. and Henseleit, K. (1932) Z. Physiol. Chem. 210, 33–66.
[4] Rognstad, R., Clark, D. G. and Katz, J. (1974) Eur. J. Biochem. 47, 383–388.
[5] Rognstad, R. and Clark, D. G. (1974) Eur. J. Biochem. 42, 51–60.
[6] Slein, M. W. (1965) in: Methods of Enzymatic Analysis (Bergmeyer, H. U., ed.) pp 117–123, Academic Press, New York.
[7] Wieland, O. (1965) in: Methods of Enzymatic Analysis (Bergmeyer, H. U., ed.) pp 211–214, Academic Press, New York.
[8] Adelman, R. C., Ballard, F. J. and Weinhouse, S. (1967) J. Biol. Chem. 242, 3360–3365.
[9] Bayne, S. and Fewster, J. A. (1956) Advan. Carbohydrate Chem. 11, 43–96.
[10] Richtmyer, N. K. (1963) in: Methods in Carbohydrate Chemistry (Whistler, R. L. and Wolfrom, M. L., eds.) Vol. II, pp 127–131. Academic Press, New York.
[11] Henseke, G. (1956) Chem. Ber. 89, 956–963.
[12] Lardy, H. A. (1951) in: Phosphorus Metabolism (McElroy, W. D. and Glass, B., eds.) p. 116, Johns Hopkins, Baltimore.
[13] Rose, I. A. and O'Connell, E. C. (1961) J. Biol. Chem. 236, 3086–3092.
[14] Clark, M. G., Kneer, N. M., Bosch, A. L. and Lardy, H. A. (1974) J. Biol. Chem. 249, 5695–5703.
[15] Bissett, D. L. and Anderson, R. L. (1974) J. Bact. 117, 318–320.
[16] Bissett, D. L. and Anderson, R. L. (1974) J. Bact. 119, 698–704.
[17] Damgaard, S. E., Lundquist, F., Tonnesen, K., Hansen, F. V. and Sestoft, L. (1973) Eur. J. Biochem. 33, 87–97.