LOSS OF C-5 HYDROGEN DURING OXIDATION OF UDP-D-GLUCOSE BY UDP-D-GLUCOSE DEHYDROGENASE

Harry S. PRIHAR and David Sidney FEINGOLD
Department of Microbiology, University of Pittsburgh, School of Medicine, Pittsburgh, PA 15261, USA

Received 24 October 1978
Revised version received 18 December 1978

1. Introduction

UDP-D-Glucose dehydrogenase (UDP-D-glucose: NAD oxidoreductase, EC 1.1.1.22) catalyzes the irreversible NAD^+-linked conversion of uridine 5′-(α-D-glucopyranosyl pyrophosphate) (UDPGlc) to uridine 5′-(α-D-glucopyranosyluronic acid pyrophosphate) (UDPGlcUA) [1]. The first intermediate in the reaction is enzyme-bound uridine 5′-(α-D-glucopyranosyldialdehyde pyrophosphate (UDPGlc-6-CHO) [2], which is formed by abstraction of the pro-*R* hydrogen from C-6 of the D-glucosyl moiety of UDPGlc (fig.1). The UDPGlc-6-CHO is thought to be bound at the catalytic sulfhydryl groups of the enzyme as a hemithioacetal, which is subsequently converted into a thioester by abstraction of the remaining (pro-*S*) hydrogen. The reaction product, UDPGlcUA, would then result from the irreversible hydrolysis of the thioester [3]. We have noticed that there is a decrease of specific activity when UDP-[5-^{3}H]Glc is converted to UDP-[5-^{3}H]GlcUA with UDP-D-glucose dehydrogenase, suggesting that some of the C-5 H exchange with medium H^+ during the course of the dehydrogenation. Details are presented here.

2. Materials and methods

UDP-D-glucose dehydrogenase was prepared from bovine liver essentially as in [4]. D-[3-^{3}H]- and D-[5-^{3}H]glucose were purchased from the Radiochemical Center, Amersham-Searle, Chicago, IL. The position of the label was confirmed by periodate oxidation of the methyl α-D-glucopyranoside of each labeled compound [5]. Paper chromatography was done on Whatman no. 3 paper in solvent A, 7:3 (v/v) ethanol–M-ammonium acetate (pH 7.5); electrophoresis was carried out on Whatman no. 3 paper in

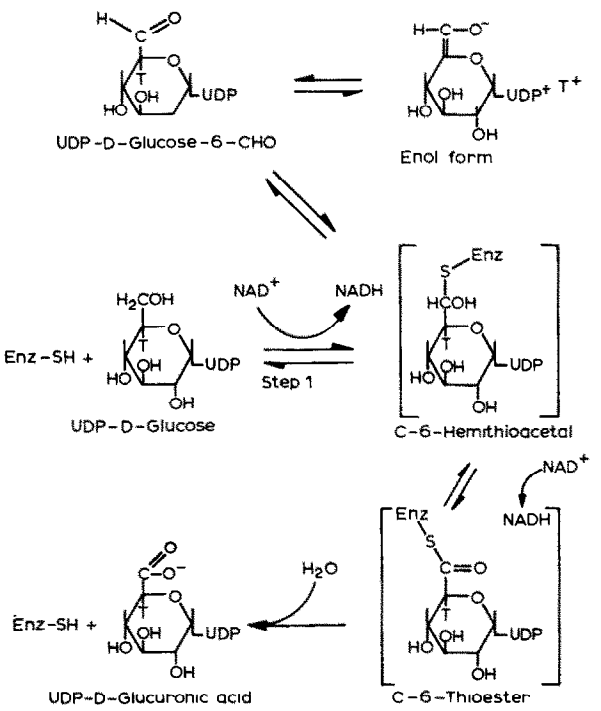

Fig.1. Simplified reaction mechanism of UDP-D-glucose dehydrogenase, showing the proposed enolization of the UDP-glucose 6-aldehyde intermediate. The ^{3}H label is indicated by T; E-SH represents UDP-D-glucose dehydrogenase with a catalytic-site thiol group.

solvent B, 0.05 M ammonium formate (pH 3.6). Radioactive compounds were located with 4 π strip counter, freed from ammonium salts by washing with absolute ethanol, and eluted with water. Radioactivity was measured in a Searle Analytic, Chicago, IL, Mark III liquid scintillation system Model 6880 in aquasol, a dioxane-based scintillation mixture from New England Nuclear, Boston, MA. Specifically ^{3}H-labeled and D-[U-^{14}C]glucose was converted into labeled UDPGlc as in [6]. UDPGlc was converted into UDPGlcUA with UDP-D-glucose dehydrogenase in the presence of a large excess of NAD^+ in 0.1 M glycylglycine buffer (pH 8.7). UDPGlc and UDPGlcUA were purified by paper chromatography in solvent A followed by high voltage paper electrophoresis in solvent B. Uridine 5′-(methyl-α-D-glucopyranosyluronate pyrophosphate) (UDPGlcUAOMe) was prepared from UDPGlcUA as in [7] and purified by high-voltage electrophoresis in 0.1 M ammonium formate buffer (pH 4.0). Uridine 5′-(α-D-galactopyranosyl pyrophosphate) (UDPGal) was prepared from UDPGlc with UDP-D-glucose 4-epimerase (EC 5.1.3.2) (Sigma Chem. Co., St Louis, MO). Residual UDPGlc in the equilibrium mixture was converted into UDPGlcUA by exhaustive treatment with UDP-D-glucose dehydrogenase and NAD^+, and the UDPGal was isolated by paper chromatography followed by electrophoresis. Uridine 5′(α-D-galactopyranosyldialdehyde pyrophosphate) (UDPGal-6-CHO) was prepared from UDPGal as in [2], using D-galactose oxidase (EC 1.1.3.9).

3. Results

The reaction of UDP-D-glucose dehydrogenase with a mixture of UDP-[U-^{14}C]-Glc and UDP-[5-^{3}H]Glc (^{3}H/^{14}C ratio 5.32) and NAD^+ was studied as a function of time. The ^{3}H/^{14}C ratio for UDPGlcUA isolated after 5 min reaction (4.17) did not change after 24 h continued reaction. The ^{3}H/^{14}C ratio for unreacted UDP-Glc (5.32) remained constant throughout the course of the reaction. Under identical conditions a mixture of UDP-[U-^{14}C]Glc and UDP-[3-^{3}H]Glc (^{3}H/^{14}C ratio 1.29) gave UDPGlcUA with ^{3}H/^{14}C ratio 1.24; the ratio for UDPGlc isolated from the reaction mixture also was identical to that of the substrate. That the ^{3}H originally present at C-5 had exchanged with medium H^+ was shown by quantitative recovery of the label in the aqueous portion of the reaction mixture, obtained by passage through a small column of mixed-bed ion-exchange resins (AG 501–X8 (D) 20–50 mesh, Bio-Rad Labs, Richmond, CA). The enzyme-catalyzed loss of the C-5 H was independent of pH in the range 5.5–8.7. Identical results were obtained when the reaction was done at pH 5.5, 6.2, 7.5 and 8.7.

Since a (thiol) ester is considered to be an intermediate in the dehydrogenation [3], doubly-labeled UDPGlcUA was converted into UDPGlcUAOMe to determine whether 5-H is lost from the ester. When a mixture of UDP-[U-^{14}C]GlcUA and UDP-[5-^{3}H]GlcUA (^{3}H/^{14}C ratio 0.56) was converted into UDPGlcUAOMe, there was no change in the ^{3}H/^{14}C ratio (final ^{3}H/^{14}H ratio 0.58), indicating that there was no loss of C-5 ^{3}H during esterification or from the ester itself.

To test the possibility that the loss of 5-H occurs during the conversion of UDP-Glc into the dialdehyde intermediate, UDP-Glc-6-CHO, the analogous compound, UDPGal-6-CHO, was prepared from a mixture of [U-^{14}C, 5-^{3}H]UDPGlc (initial ^{3}H/^{14}C ratio 5.38) as in [2]. The ^{3}H/^{14}C ratio for UDPGal-6-CHO obtained from the UDPGal (^{3}H/^{14}C ratio 5.32) by the action of D-galactose oxidase was 2.11, showing that 60% of C-5 ^{3}H had been lost by exchange with medium H^+ during the preparation of UDPGal-6-CHO from UDPGal. When UDPGal-6-CHO was redissolved in water and re-isolated by lyophilization, further loss of ^{3}H occurred, indicating that the C-5 H of UDPGal-6-CHO readily exchanges with water H^+.

4. Discussion

Although admittedly the comparison between UDPGlcUAOMe and enzyme-bound thioester is somewhat tenuous, the observed stability of the C-5 H in UDP-[5-^{3}H]GlcUAOMe makes it seem unlikely that the thioester between UDPGlcUA and enzyme is involved in the exchange reaction. On the other hand, the extensive exchange with medium H^+ of the C-5 H in UDPGal-6-CHO suggests that free UDPGlc-6-CHO is the active species involved in loss of ^{3}H during the enzymatic conversion of UDP-[5-^{3}H]Glc into UDP-[5-^{3}H]GlcUA. A plausible mechanism for the

exchange to occur is via facile keto-enol tautomerism between C-5 and C-6 in UDPGlc-6-CHO (fig.1). Since the enzyme-bound hemithioacetal is less likely to tautomerize, exchange would mainly occur with the free aldehyde. The limited extent of exchange observed, 30% of the ^3H initially present, can be explained by assuming that the enzyme-bound UDPGlc-6-CHO is in equilibrium with free aldehyde during the reaction, and the relative rates of dissociation and tautomerization are such that only 30% of the exchange of the C-5 H with medium H^+ occurs.

UDP-D-glucose dehydrogenase has been purified to homogeneity from beef [4], rat liver [8] and *Escherichia coli* [9] and its presence has also been demonstrated in many other organisms. Although studies of the reaction mechanism have been done only with the beef-liver enzyme, UDPGlc-6-CHO is doubtless involved in the reaction catalyzed by all UDP-D-glucose dehydrogenases. However, since the loss of C-5 H during the reaction depends on the relative rates of formation and utilization of the intermediate, as well as its affinity for the enzyme, it cannot be predicted a priori whether or to what extent the C-5 H would exchange with medium H^+ during UDPGlcUA formation catalyzed by enzymes from other sources.

Acknowledgements

This work was supported by grants from the National Institutes of Health (no. AM 18160, AM 15332). We are grateful to Dr James S. Franzen and Mr Paul Marchetti for helpful discussions and advice and to Mrs Marie Bowser for technical assistance.

References

[1] Strominger, J. L., Maxwell, E. S., Axelrod, J. and Kalckar, H. M. (1957) J. Biol. Chem. 224, 79–90.
[2] Nelsestuen, G. L. and Kirkwood, S. (1971) J. Biol. Chem. 246, 3828–3834.
[3] Ordman, A. B. and Kirkwood, S. (1977) J. Biol. Chem. 252, 1320–1326.
[4] Zalitis, J. and Feingold, D. S. (1969) Arch. Biochem. Biophys. 132, 457–465.
[5] Prihar, H. S., Meganathan, R. and Feingold, D. S. (1978) Carbohyd. Res. 67, 271–274.
[6] Wright, A. and Robbins, P. W. (1965) Biochim. Biophys. Acta 104, 594–596.
[7] Gebb, C., Baron, D. and Grisebach, H. (1975) Eur. J. Biochem. 54, 493–498.
[8] Kelkar, S. M. and Nadkarni, G. B. (1977) Indian. J. Biochem. 14, 305–309.
[9] Schiller, J. G., Lamy, F., Frazier, R. and Feingold, D. S. (1976) Biochim. Biophys. Acta 453, 418–425.