REGULATION OF THE UDP-GLUCOSE-4-EPIMERASE ACTIVITY

IN CHINESE HAMSTER CELLS

Jean-Paul Thirion*

Genetics Department
Weizmann Institute
Rehovot Israel

Received February 14, 1972

SUMMARY

In Chinese hamster cell extracts, the UDP-glucose-4-epimerase (EC 5.1.3.2.) activity is dependent upon the NAD concentration, the activity of the cell UDP-glucose dehydrogenase (EC 1.1.1.22.) and the NAD : NADH ratio. NAD is a cofactor of the enzyme and NADH is a competitive inhibitor. In cells grown in Petridishes the epimerase is synthesized constitutively. There is no enzymatic induction in cells grown with galactose as a source of sugar as observed with wild type E. coli.

INTRODUCTION

A great deal of our understanding of gene regulation in micro-organisms comes from the study of their pathways of carbohydrate metabolism (1) but little is known about gene regulation in mammalian cells. For this reason, we have isolated a galactose (+) cell line to study the UDP-glucose-4-epimerase (EC 5.1.3.2.) (epimerase) which catalyses the following reaction of the galactose pathway UDP-galactose $\rightleftharpoons$ UDP-glucose (EC 5.1.3.2.)

We have found that the epimerase in Chinese hamster cells grown "in vitro", is synthesized constitutively. Cells grown on galactose have approximately the same amount of epimerase as cells grown on glucose. There is no enzymatic induction. We thus propose that the epimerase activity in Chinese hamster cells is under the control of the NAD : NADH ratio.

*Present address : Institut de Biologie Moléculaire de la Faculté des Sciences de Paris, 9, quai Saint Bernard - Paris 5ème

MATERIALS AND METHODS

Reagents. (^{14}C) UDP-galactose (289 mC per mM) was from the Radiochemical Centre (Amersham) ; UDP-galactose, NAD, NADH, galactose ("glucose free") which was found to contain only 0.008 % of glucose, UDP-glucose dehydrogenase and yeast epimerase were from Sigma Co., N-glycylglycine and xylene cyanol were from BDH Chemical Limited.

Cell growth and media. Clone C12 from an established Chinese hamster cell line derived from bone marrow, can grow either on galactose or on glucose as a source of carbohydrate (to be published). Cells were grown in Dulbecco's medium (2) with glucose (or galactose when indicated) supplemented with 10% foetal calf serum (Grand Island Biological Company) which had been dialysed exhaustively (the amount of glucose remaining in the serum is 4×10^{-5} mg/cc). Ten cm diameter Petri dishes with about $2\text{-}5 \times 10^{6}$ cells were washed three times with phosphate buffered saline and drained (3). One ml of water was added per dish and the cells were alternatively frozen at -20°C and thawed (three times). They were then lysed at 0°C in a homogenizer and the lysates cleared of debris by a low speed centrifugation.

Epimerase assay. The enzyme assay previously described (4) was modified to account for the UDP-glucose dehydrogenase (EC 1.1.1.22.) in the cell extracts.

Except when indicated, 60 µl of either Chinese hamster cell extract or yeast epimerase were incubated at 37°C for up to 30 min at pH 8.7 with 6 µmoles sodium N-glycylglycinate, 40 mµ moles UDP-galactose, 0.4 mµ mole (^{14}C) UDP-galactose and 400 mµ moles Nad in 40 µl of water. The reaction was stopped by heating in boiling water for two minutes then cooled at 0°C (first step). About 150 units of yeast UDP-glucose dehydrogenase, 20 mµ moles NAD, 12.5 µ moles sodium N-glycylglycinate (pH 8.7) in 0.1 ml water were then added at 0°C. Incubation was resumed at 37°C until no more UDP-glucuronate was formed (about 20 min.) (second step). The protein concentration was measured by the Lowry procedure (5) using serum albumin as a standard.

(^{14}C) UDP-glucuronate was separated from (^{14}C) UDP-galactose by paper electrophoresis (fig. 1). The radioactive areas were cut out and counted in a liquid scintillation counter. The amount of labelled UDP-glucuronate was a measure of the epimerase activity.

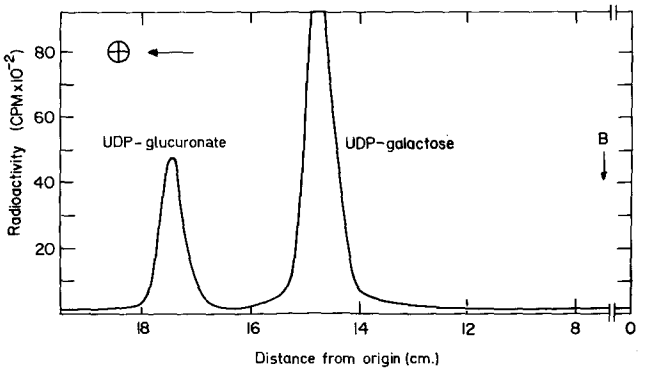

Fig. 1 - Electrophoretic separation of UDP-galactose from UDP-glucuronate. After step two, about 10 µl of the enzymatic reaction mixture mixed with a small quantity of xylene cyanol were electrophoresed on Whatman 3 MM in 0.05 M pyrimidium acetate (pH 6.5) for 40 min. at 50 volts/cm. (^{14}C) UDP-glucuronate was separated from (^{14}C) UDP-galactose. Arrow B indicates the position of migration of xylene cyanol. Radioactivity (————) was as detected by a Packard 7201 radioautogram scanner.

RESULTS

The activation of Chinese hamster UDP-glucose-4-epimerase by NAD.

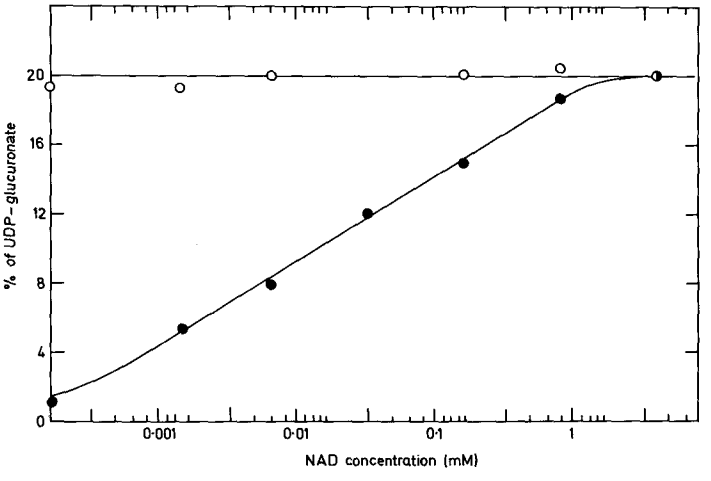

Fig. 2 - Epimerase activity as a function of the initial NAD concentration. The epimerase assays were carried out as described in Materials and Methods but with different initial concentrations of NAD during step one of the assay. The incubation time was 30 min. for step one. The yeast epimerase assays were carried out with 60 µl of yeast epimerase in water substituted for 60 µl of Chinese hamster extract. (——— • ———) Percentage of radioactivity in the form of (^{14}C) UDP-glucuronate after incubation with the Chinese hamster epimerase and, (——— o ———) with the yeast epimerase.

NAD is a cofactor of the epimerase in liver (6) and also of the epimerase in Chinese hamster cells as shown by the following experiments. When the assay was carried out as described in Materials and Methods, with different initial concentrations of NAD and with an incubation period of 30 min. for the first step, the Chinese hamster epimerase activity (fig. 2 closed circles) increased logarithmically with the initial NAD concentration and then reached a plateau, but the yeast epimerase activity (open circles) was independent of the addition of NAD.

The activity of Chinese hamster UDP-glucose dehydrogenase and the reduction of NAD during the first step of the assay.

Since NAD was a cofactor of the epimerase, it was important that no other reaction which could lower the amount of NAD should take place during the determination of the epimerase activity (step one of the assay). When this step was monitored at 340 mμ, a reduction of NAD was observed. Therefore, we have investigated this phenomenon. We found that the Chinese

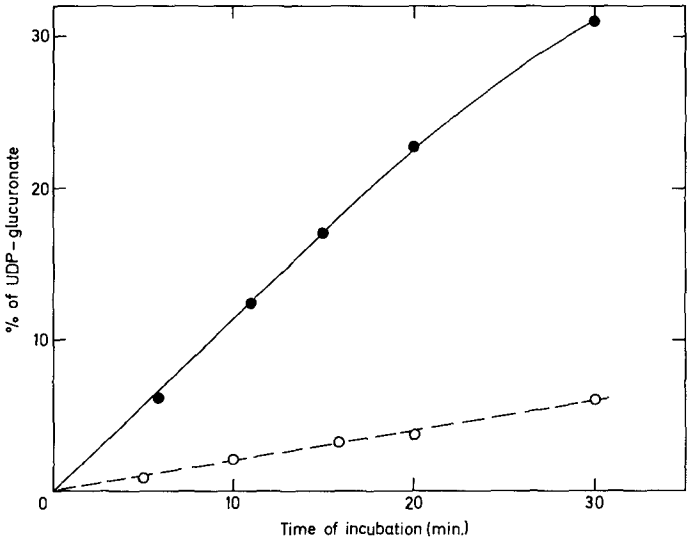

Fig. 3 - UDP-glucose dehydrogenase activity in cell extracts during step one of the epimerase assay.

Cell extracts (42 μg of protein) were incubated for different times and the incubation mixture was heated in boiling water and cooled (step one) and then reincubated as in step two. After steps one and two, samples were electrophoresed as described in fig. 1. (---- o ----) Percentage of radioactivity in the form of (^{14}C) UDP-glucuronate after step one ; (---- ● ----) Percentage of radioactivity in the form of (^{14}C) UDP-glucuronate after step two.

hamster UDP-glucose dehydrogenase, present in the cell extracts, converted some of the UDP-glucose, produced by the epimerase reaction, into UDP-glucuronate. During this reaction UDP-glucose + 2 NAD $\rightleftharpoons$ UDP-glucuronate + 2 NADH (EC 1.1.1.22.), two moles of NAD were reduced for every mole of UDP-glucuronate produced, thereby decreasing the NAD concentration.

When the Chinese hamster epimerase assay was carried out as described in Materials and Methods, the amounts of (^{14}C) UDP-glucuronate in the incubation mixture after steps one and two were as shown in Figure 3 (open circles and closed circles, respectively). During step one, the cell UDP-glucose dehydrogenase catalysed the production of UDP-glucuronate from UDP-glucose and NAD. During this step, the initial NAD concentration was 4 mM ; the amount of NAD reduced after 30 min. was 4.8 mμ moles corresponding to 6 % (or 2.4 mμ moles) of UDP-glucuronate (fig. 3).

Inhibition of the epimerase reaction by NADH.

NADH was produced during step one. Therefore, we have investigated whether or not NADH inhibited the Chinese hamster epimerase. When the epimerase reaction was carried out for an incubation period of 30 min. with either 2.5 mM or 0.25 mM of NAD and with different concentrations of NADH during the firs step, the epimerase was inhibited (table 1). This inhibition was a function of the NAD : NADH ratio rather than the NADH concentration (see particularly ratios of 10 and 1 of table 1) and was therefore competitive. If this were so, then competitive inhibition of the reaction E + NAD $\rightleftharpoons$ E-NAD should be relieved by the addition of substrate. Indeed, this was found to be the case with 2.5 mM of NAD ; the NAD : NADH ratio remained greater than 140 throughout step one and no inhibition was detected.

Constitutive synthesis of Chinese hamster epimerase.

With NAD concentrations greater than 2.5 mM, the inhibition of NADH was relieved and the enzyme was fully active. Therefore, to study whether or not the Chinese hamster epimerase was inducible, we chose a concentration of 4 mM NAD for the first step of the assay. Cells were grown either in glucose or in galactose. In glucose, we found a specific activity of 620 μmoles of UDP-galactose converted into UDP-glucose per hour per mg of protein at 37°C (620 units). This was not very different from the 680 units found for the cells grown in galactose. This indicated that the epimerase was synthesized constitutively.

TABLE 1

Epimerase activity as a function of the NAD and NADH concentrations

Initial NAD concentration (μM)	2500	2500	2500	2500	2500	250	250	250	250	250
Initial NADH concentration (μM)	0	2.5	25	250	2500	0	0.25	2.5	25	250
NADH (μM) produced during the first step	18	18	17	7.2	1.5	14.7	14.7	11	5.9	1.3
Interval of variation of the NAD:NADH ratio during the first step	140-∞	120-1000	60-100	10	1	17-∞	21-1000	22-100	8.2-10	1
% of UDP-glucuronate after the 2nd step	18.0	18.1	17	7.2	1.5	14.7	14.7	11	5.9	1.3
% of inhibition when compared to 18 and 14.7 for 2500 μM and 250 μM of NAD respectively	0	0	5	60	92	0	0	25	60	91

The epimerase assay was carried out as described in Materials and Methods, incubation was for 30 min and with different initial concentrations of NAD and NADH during step one of the assay.

DISCUSSION

In wild type E. coli, NAD is tightly bound to the epimerase and there is no competitive inhibition by NADH. The reaction UDP-glucose $\rightleftharpoons$ UDP-galactose is regulated by the amount of epimerase, the synthesis of which is either induced or repressed (7). In Chinese hamster cells grown in Petri dishes and possibly in the animal the epimerase is synthesized constitutively. The results show that the reaction is controlled by the ratio of NAD to NADH and not by the amount of epimerase.

ACKNOWLEDGMENT

The author is grateful to Dr Sachs and Dr Vinocour for helpful discussions and for their hospitality at the Institute.

REFERENCES

1 - G. Buttin
Advances in Enzymology, vol. 30, Interscience Publishers, New-York, London and Sydney, (1968), p. 81.

2 - J. D. Smith, G. Freeman, M. Vogt and R. Dulbecco.
Virology, 12, (1960) 185.

3 - R. Dulbecco and M. Vogt,
J. Exp. Med., 99, (1954) 167.

4 - E. S. Maxwell, K. Kurahashi and H. M. Kalckar,
Methods in Enzymology, vol. 5, Academic Press, New-York and London (1962), p. 174.

5 - O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall
J. Biol. Chem., 193, (1951) 265.

6 - E. S. Maxwell,
The enzyme, vol. 5, Academic Press, New-York and London (1961) p. 443.

7 - G. Buttin
J. Mol. Biol., 7, (1963), 164.