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Regulation of mammalian liver uridine diphosphogalactose 4-epimerase by pyrimidine nucleotides

UDPgalactose 4-epimerase which catalyzes the interconversion of UDPgalactose (UDPGal) and UDPglucose (UDPGlc) is an important enzyme in sugar nucleotide metabolism. It enables galactose to enter glucose metabolic pathways and permits glucose conversion to galactose for synthesis of polysaccharides. In mammalian liver the enzyme is thought to be under some degree of metabolic control since it is dependent on NAD^+ for activity and inhibited by NADH[1] and shows competitive product inhibition[2]. Previous observations from this laboratory have indicated that, of a number of purine and pyrimidine nucleotides tested, only UMP, UDP, UDPmannose (UDPMan) and TDPglucose (TDPGlc), significantly inhibited the enzyme[2]. The present report evaluates the nature of the inhibition and the possible regulatory role of these compounds under physiological circumstances.

The enzymatic assay procedure employed which has been described in detail involves the determination of the amount of conversion of UDP[^{14}C]Gal to UDP-[^{14}C]Glc[2]. Using this method with high speed supernatants of rat liver homogenates we have shown the stoichiometric conversion of UDPGal to UDPGlc during short incubation times. All assays were determined using linear initial rates of reaction and protein concentrations within the range of proportionality of the reaction. In the present studies, livers of Sprague–Dawley rats were homogenized in 4 vol. of 0.1 M KCl, diluted 1:6 with quartz-distilled water and centrifuged at 30000 $\times$ g for 30 min at 4°. 20 μl of the clear supernatant containing 60–65 μg of protein were incubated at 37° for 6 min with 0.2 μmole NAD^+, 40 μl 1 M glycine buffer (pH 9.1) and various amounts of substrate UDPGal (0.036 μC UDP[^{14}C]Gal) and inhibitors indicated below brought to a total volume of 0.2 ml with water and to a final pH of 8.65. After ending the incubation by boiling, the amount of UDP[^{14}C]Glu formed was assessed by converting the product to UDPglucuronic acid (UDPGlcUA) with UDPGlc dehydrogenase. Separation of the UDPGal from UDPGlcUA was accomplished by chromatography on polyethyleneimine impregnated cellulose thin layer plates employing a 0.2 M LiCl solvent system and counting separately the areas of the plate containing the substrate and product with a liquid scintillation spectrometer[2]. Data from the inhibition studies were plotted both by the DIXON[3] method and by the method of CLELAND[4] to determine the type of competitive inhibition.

Nucleotides were purchased from Sigma or Calbiochem. UDP[^{14}C]Gal (uniformly labeled, specific activity 276 mC/mmole) was obtained from New England Nuclear Corp.

In the standard assay procedure of adult liver epimerase with 0.1 mM UDPGal as substrate no inhibition was seen with 1 mM AMP, ADP, ATP, ADPGlc, GDPGlc and GDPMan. TDPGlc produced a 50% inhibition at 1 mM. UDPMan, UMP and UDP exhibited about 40% inhibition at 0.25 mM while UTP caused only 10% inhibition at this concentration. Table I shows the inhibitions at various concentrations of uridine nucleotides. UMP and UDP appear to be similar in their potency. Both produced about 20% inhibition at 0.05 mM. UTP did not cause marked inhibition at 0.5 mM. Inhibition of the enzyme prepared from newborn rat liver also occurred, but the inhibition was not as marked as in the adult.

Fig. 1 shows a Lineweaver–Burk plot of the inhibition of adult rat liver epimerase by UMP (A) and of the newborn liver enzyme by TDPGlc (B) and indicates the inhibition to be of the competitive type. Analysis by the method of CLELAND[4] revealed that the inhibition by UMP, UDP and TDPGlc was of the hyperbolic competitive type suggesting an allosteric action. K_i values obtained by plotting the reciprocal of the change in slope *versus* the reciprocal of the inhibitor concentration

TABLE I

INHIBITION OF ADULT RAT LIVER UDPGal 4-EPIMERASE BY NUCLEOTIDES

Incubation conditions as described. UDPGal concentration was 0.1 mM.

Inhibitor concn. (mM)	*Inhibition (%)**		
	UMP	*UDP*	*UTP*
0.05	20	17	
0.10	40	21	
0.15			6
0.20		32	
0.25	48	43	10
0.40		45	
0.50	60		15

* One enzyme preparation was employed for the UDP and UTP study and another for UMP. Values are averages of duplicate determinations.

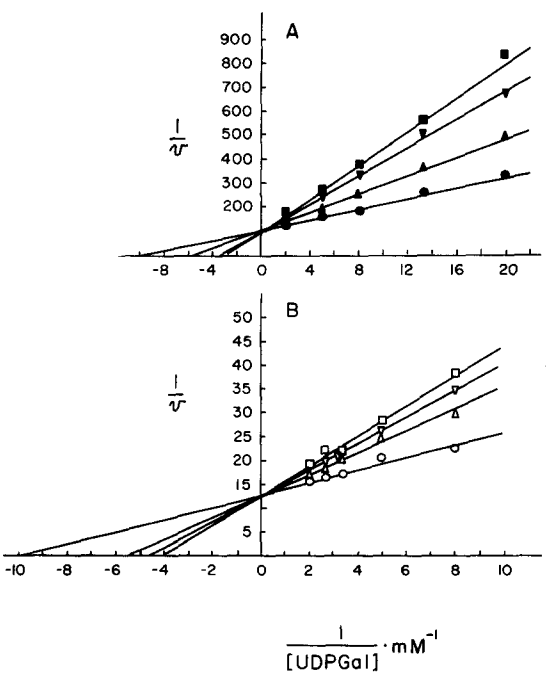

Fig. 1. Reciprocal plots with UDPGal as varied substrate of epimerase. Initial velocities expressed as μmoles UDPGlc formed per min per μg of liver protein. A. UMP as inhibitor of adult enzyme. ●—●, control; ▲—▲, 0.1 mM; ▼—▼, 0.25 mM; ■—■, 0.5 mM. B. TDPGlc as inhibitor of newborn liver enzyme. ○—○, control; △—△, 0.25 mM; ▽—▽, 0.5 mM; □—□, 0.75 mM.

were 0.4 mM for UMP, 0.1 mM for UDP and 0.59 mM for TDPGlc. The inhibition by UDPMan in three separate experiments appeared to be linear competitive with a K_i of 0.2 mM.

Variation in reported values for intracellular concentration of divers nucleotides as well as possible compartmentation of substrates within the cells make firm assignments of physiologic roles for any particular nucleotide tentative. The recent determination of uridine nucleotide levels in rat liver[5,6] indicates the total UTP + UDP + UMP level to be 0.4 μmole/g of wet weight and the UTP + UDP level to be about 0.35 μmole/g of wet weight. These values are within the range of those levels employed to produce the inhibition shown in Table I. Moreover, the K_i for UDP of 0.1 mM and UMP of 0.4 mM approximates the cellular levels reported for these compounds. The amount of inhibition would also be dependent on the enzyme substrate levels but little information is available on liver UDPGal concentration under physiological conditions. The K_m of the liver enzyme for UDPGal is 0.25 mM[2]. The present findings underscore the need for examination of galactose metabolism *in vivo* under circumstances of altered cellular nucleotide levels.

Cellular levels of UDPMan and TDPGlc are probably lower than the K_i value and hence it is unlikely they exert any control of the reaction. UDPGlc itself possibly plays a large role in regulation of epimerization of UDPGal since intracellular levels of this nucleotide[6,7], the normal reaction product, are in the range where significant inhibition is seen *in vitro* and several times the K_i value for UDPGlc[2].

Inhibition of a 250-fold purified preparation of bovine mammary gland UDPGal 4-epimerase by uridine nucleotides has recently been reported to be competitive with K_i values not too dissimilar to those reported here in rat liver[8]. In yeast epimerase preparations UMP has a different effect from that seen with mammalian enzyme, being responsible for the reduction of enzyme bound NAD^+ (ref. 9).

The present observations furnish some insight regarding the stereospecificity of the enzyme. Of the various nucleotides and nucleotide sugars examined as inhibitors, only the pyrimidine compounds with carbonyl functions at positions 2 and 4 and an NH group at position 3 showed inhibition of epimerization. These data support the contention of BUDOWSKY *et al.*[10] using yeast enzyme that the acylamido grouping at position 2 through 4 on the pyrimidine ring is essential for interaction with the enzyme. Indeed, with substitution of the NH_2 group for the carbonyl group at position 4 in cytosine, no competition is observed.

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The effect of thyroxine *in vitro* on the specific activity of the mitochondrial pool of amino acid

Thyroid hormones have been shown to stimulate *in vitro* radioactive leucine incorporation into the protein of mitochondria isolated from normal rats[1,2]; however, this stimulation has not been demonstrated to represent an actual increase in the rate of protein synthesis. Since mitochondria contain a pool of free amino acids[3], it seemed possible that stimulation of radioactive leucine incorporation reflected an increase in the specific activity of the precursor pool of leucine, perhaps through stimulation of uptake of [^{14}C]leucine into mitochondria by thyroid hormones. Several observations have made investigation of this possibility of particular importance. ADAMSON AND INGBAR[4] reported that triiodothyronine (T_3) increased the uptake of certain amino acids (including leucine) and inhibited that of others (including isoleucine) by embryonic chick bone. We described a transport mechanism, activated by ATP, for leucine, isoleucine, valine, and methionine in rat liver mitochondria[5] and also reported that thyroxine (T_4) appeared to stimulate free leucine accumulation in such mitochondria[6]. In the present studies we have examined simultaneously the effects of T_4 *in vitro* both upon [^{14}C]leucine and isoleucine uptake and incorporation into protein and upon concentration of endogenous leucine and isoleucine in mitochondria from normal rat liver.

Mitochondria were prepared from the livers of normal male Sherman rats by homogenization followed by differential centrifugation in 0.25 M sucrose at 0° as previously described[2]. Mitochondria were incubated at 37° with 50 μM T_4 plus 2 mM NaOH or with 2 mM NaOH alone in a medium containing: sucrose, 100 mM; potassium phosphate buffer, pH 7.4, 10 mM; Tris–HCl buffer, pH 7.4, 50 mM; sodium succinate, 10 mM; $MgCl_2$, 10 mM; KCl, 50 mM; [^{14}C]leucine (specific activity 240 mC/mole) or [^{14}C]isoleucine (specific activity 260 mC/mole), 1 or 3 μM; and a mixture of 19 amino acids (*minus* the labelled amino acid), each 5 μM. Mitochondrial protein concentration ranged from 1.0 to 1.2 mg/ml. After precipitation, washing, and heating in trichloroacetic acid, proteins were dissolved in 0.3 ml of formic acid and counted with 56 % efficiency in 10 ml Bray's solution in a Packard liquid scintillation counter. Uptake and identification of free amino acids were determined as previously described[5] with the addition of 100 μM chloramphenicol, which inhibited protein synthesis