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THE COMPETITIVE INHIBITION OF *p*-NITROPHENYL-β-D-GLUCOPYRANOSIDURONIC ACID SYNTHESIS BY ALIPHATIC ALCOHOLS *IN VITRO*

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Abstract—The primary aliphatic alcohols, methanol, ethanol, propanol and butanol were found to compete with *p*-nitrophenol for the uridine diphosphate glucuronic acid transferase preparation from guinea pig liver. The K_i values correlate with the water–olive oil partition coefficients of these alcohols. The inhibitory action increases exponentially with the length of the alkyl chain and linearly with the lipid solubility.

ALCOHOLS are metabolized *in vivo* to a certain degree via the β-D-glucopyranosiduronic acid pathway. Both methanol and ethanol and also other primary aliphatic alcohols are excreted as D-glucuronic acid conjugates after oral administrations to rabbits. The excreted β-D-glucopyranosiduronic acid fraction increases with the branching of the molecule and the lengthening of the carbon chain. If the oxidative metabolism of ethanol is depressed by tetraethylthiuram disulphide, the excretion of the conjugated fraction increases.[1–3]

The UDPGA (uridine diphosphate glucuronic acid) transferase activity of liver microsomes has been found to be composed of several different enzymes with different properties and specificities.[4–8] In the present study the effect of some primary aliphatic alcohols on the conjugation of a phenolic compound was explored in the presence of UDPGA and a UDPGA transferase preparation from guinea pig liver in order to study the mechanism of the inhibition.

METHODS

The UDPGA transferase preparation was obtained from the liver of adult male guinea pigs by separating the microsomal fraction using the method developed by Mills and Smith.[9] It was stored in melting ice, and it was discarded after two days. The different alcohols were added to the reaction mixture to a final concentration indicated in Fig. 1. The reaction mixture[10] contained in a total volume of 300 μl 0·27 mM of UDPGA (98%, Sigma Chemical Co, St. Louis), 3·3 mM of dipotassium ethylendiamine tetraacetic acid, 0·13 mM or 0·067 mM of *p*-nitrophenol, 0·167 M potassium phosphate buffer pH 7·5 and the liver preparation (microsomes from 50 mg of fresh liver). The incubation (38°) was stopped by adding after 7·5 min, 0·5 ml of 0·4 N trichloracetic acid, and the amount of free *p*-nitrophenol was measured.[10] Ethanol (AaS, for spectrometry) was obtained from Oy Alkoholiliike Ab, Helsinki, *p*-nitrophenol and methanol, propanol and butanol (*pro analysi* or for chromatography) from Merck AG, Darmstadt.

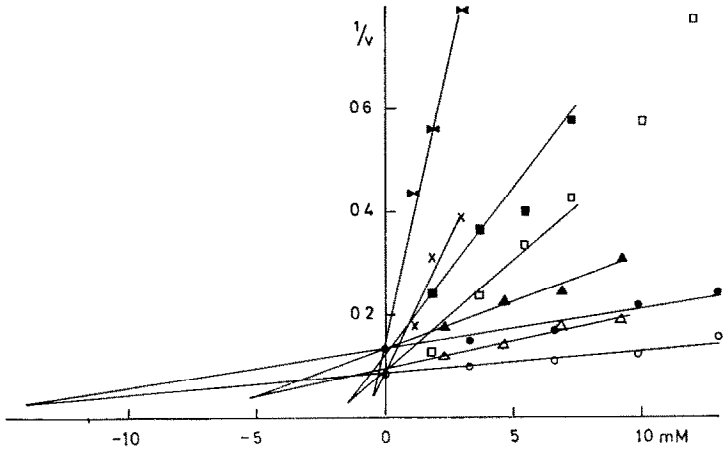

FIG. 1. The inhibition of the conjugation of *p*-nitrophenol by the different concentrations (mM) of methanol (● ○), ethanol (▲ △), propanol (■ □) and butanol (⋈ ×) in the presence of UDPGA and a UDPGA transferase preparation from guinea pig liver determined simultaneously in triplicate and the averages plotted according to Dixon.[13] The dark and light signs: 0·067 mM and 0·13 mM of *p*-nitrophenol, respectively.

RESULTS AND DISCUSSION

Low concentrations of methanol, ethanol, propanol and butanol were found to inhibit competitively the guinea pig liver UDPGA transferase when *p*-nitrophenol was used as substrate (Fig. 1). High concentrations (> 10 mM) of at least propanol caused an uncompetitive inhibition. High alcohol concentrations are generally known to denature enzymes.

The K_i values obtained for each of the alcohols follow the water-olive oil partition coefficient, if the length of the alkyl chain is also taken in consideration (Fig. 2). At least in case of the lower alcohols (C_1–C_4), a simple correlation between partition coefficient (K_p) and inhibitor constant (K_i) seems to be valid and can be formulated as K_p (alcohol) = k · K_i (alcohol) where the constant k = 8. The affinity of the aliphatic alcohol towards the enzyme preparation seems thus to increase with its lipid solubility, indicating either that a nonpolar site is of importance in the active center of the enzyme, or that a nonpolar barrier must be passed before the substrate can reach the active center. Since the enzyme preparation was not solubilized this water repellent site might be a remnant of the smooth surfaced endoplasmic reticulum backbone to which the drug metabolizing enzyme systems are attached in cells.

The correlation observed between water solubility and K_i values agrees with data concerning the excretion of β-D-glucopyranosiduronic acid fractions after the administration of different primary aliphatic alcohols *in vivo*.[1]

The competitive inhibition of a phenol (*p*-nitrophenol) by aliphatic alcohols probably indicates that the same UDPGA transferase enzyme also *in vivo* transfers the D-glucuronic acid moiety to both types of compounds to form β-D-glucopyranosiduronic acids. The conjugation of some phenolic compounds is, however, catalyzed by different transferase enzymes, thus *o*-aminophenol and *p*-nitrophenol are uncompetitive inhibitors.[11] On the other hand competitive inhibition is observed in case of salicylamide and *p*-nitrophenol in the conditions used in this study.[10]

Both methyl and ethyl β-D-glucopyranosiduronic acids have been isolated in crystalline state from rabbit urine.[2] Thin layer chromatography (silica gel) of the different reaction mixtures in the solvent systems ethylacetate–acetic acid–water and methanol–formic acid–water[12] followed by sulphuric acid visualization did not reveal any new

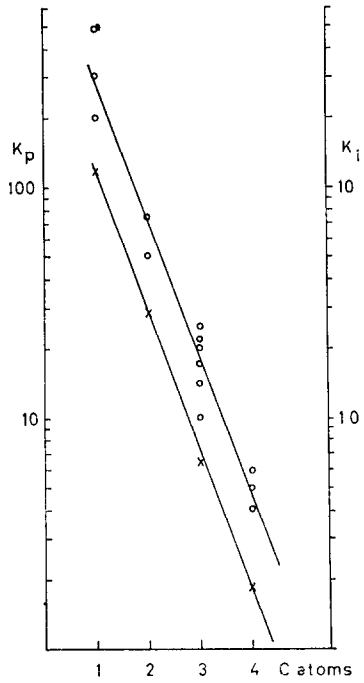

FIG. 2. K_i values of the different water soluble primary aliphatic alcohols (○) from experiments with *p*-nitrophenol and guinea pig liver UDPGA transferase preparations and water–olive oil partition coefficients[14] (×) both plotted against the number of carbon atoms of the alcohols.

spots. The K_i values, however, indicate that the concentrations of the possible new β-D-glucopyranosiduronic acids most probably were below the limit of detection.

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