Paradoxical Action of Solvents on Bacterial and Liver β-Glucuronidases

Abstract. Dichloromethane and chloroform have been found to inhibit the action of liver β-glucuronidase on phenolphthalein glucuronic acid at concentrations which produce a considerable enhancement of bacterial β-glucuronidase. Other solvents, including *n*-butanol, benzene, and toluene, have very little effect on the liver enzyme but nevertheless potentiate the bacterial enzyme.

Increased information concerning conjugation with glucuronic acid has aroused considerable interest in the problem of hydrolysis of conjugated steroids in blood and urine. Commercial preparations of highly active β-glucuronidase from mammalian liver and bacteria are available for the treatment of biological materials to liberate conjugated steroids. Such enzymes usually yield more consistent results than acid hydrolysis does. Combination of hydrolysis with prior and subsequent extraction with appropriate solvents permits determination of free and conjugated materials.

In this laboratory, recent studies on the hydrolysis and extraction of adrenal cortical steroids from plasma yielded variable recoveries and even an occasional apparent absence of conjugated forms when liver β-glucuronidase was employed. A recent bulletin issued by the Sigma Chemical Company (*1*) called attention to a remarkable potentiation of bacterial β-glucuronidase activity by chloroform. Since dichloromethane was regularly employed in our procedure, it was thought worth while to study the effects of this and other solvents on both types of β-glucuronidase.

In the present study (*2*), we used the method of Talalay, Fishman, and Huggins (*3*) with phenolphthalein glucuronic acid as substrate for the assay of glucuronidase activity. The enzymes employed were liver β-glucuronidase (Ketodase, Warner-Chilcott, *4*) and bacterial β-glucuronidase (Sigma). For measuring the effect of various solvents on bacterial glucuronidase activity, the digestion medium consisted of 4 ml of 0.075*M* phosphate buffer at *p*H 6.8, 0.5 ml of 0.01*M* phenolphthalein glucuronide, 0.1 ml of solvent, and 0.5 ml of the enzyme preparation containing approximately 25 Fishman units. For controls, the solvent was replaced by an equal volume of buffer. After digestion for 1 hour at 37°C, the reaction was stopped by the addition of 5 ml of 0.40*M* glycine buffer at *p*H 10.45. The effect of solvents on liver β-glucuronidase activity was evaluated in the same manner as the effect on the bacterial enzyme, except that 0.1*M* acetate buffer (*p*H 4.5) was used as part of the digestion medium rather than phosphate buffer. Blanks containing the buffer and the enzyme were included; the substrate solution was added after termination of the digestion period and addition of the glycine buffer. The amount of phenolphthalein liberated by the enzyme reaction was calculated from the optical density of standards at 540 mμ.

Table 1 shows the results obtained with each solvent, referred to the control activity (about 25 units) as 100 percent. There is a marked difference in response of the two enzyme preparations since most of the added compounds, except methanol and hexane, potentiated the bacterial but not the liver enzyme. Dichloromethane and chloroform were moderately potent inhibitors of the liver enzyme, dropping activity to 28 and 71 percent of controls, respectively, while more than doubling that of the bacterial preparation.

Although methyl and ethyl alcohols had little effect on the bacterial β-glucuronidase, the most marked enhancement of all was seen with propyl, butyl, and amyl alcohols, the last amounting to 817 percent. Three chlorinated hydrocarbons—dichloromethane, chloroform and ethylene dichloride—doubled or tripled the activity of bacterial β-glucuronidase. Carbon tetrachloride, benzene, and toluene nearly doubled it.

Our results emphasize the difference between two available β-glucuronidase preparations when studied under comparable conditions. Both have been employed in the hydrolysis of conjugated steroids for endocrine assay although it has previously been noted that the bacterial enzyme is less reliable than the liver in the presence of plasma proteins. The remarkable enhancement produced by butanol may make the former more useful under such circumstances. On the other hand, the 72-percent inhibition of liver β-glucuronidase by dichloromethane eliminates the use of this solvent for extraction prior to hydrolysis, and attention must be paid to the possibility that other organic compounds commonly used may depress the liver enzyme enough to interfere with its use.

The theoretical implications of these results are also important. The work of Bernfeld, Jacobson, and Bernfeld (*5*) showed that inactivation of liver β-glucuronidase by dilution or by addition of such macromolecular polyanions as polymethacrylic acid could be reversed by albumin and DNA. The possibility that the bacterial β-glucuronidase as obtained is made up largely of dissociated or inhibited forms which are reactivated in our experiments has not been substantiated in trials with albumin and DNA. The varied chemical nature of our "activators" (aromatic hydrocarbons, chlorinated aliphatic hydrocarbons, and aliphatic alcohols) also makes this explanation seem unlikely. Fishman and Green (*6*) reported transferase activity associated with glucuronidase when certain alcohols were present during the incubation. Since we measured phenolphthalein liberated rather than free glucuronic acid, transfer of the glucuronic acid moiety to butanol might have occurred. The fact that the greatest enhancement was obtained with the highest alcohol tried (amyl) is also suggestive of esterification. It remains to be seen whether the enhanced glucuronidase activity is as great with a conjugated steroid as it is with phenolphthalein glucuronic acid.

In the case of the nonalcoholic hydrocarbons, ester formation cannot explain the potentiation. Since the 0.1-ml portions used here did not always go completely into solution, a small solvent second phase was often present. Visual inspection did not support any preferential extraction of liberated phenolphthalein. There remains the possibility of extraction of an inhibitor from the bacterial β-glucuronidase.

Table 1. Effect of various solvents on activity of liver and bacterial β-glucuronidases. Each substance was added in 0.1 ml quantity; results are shown in terms of percentage of control enzyme activity (25 ± 2 Fishman units).

Solvent	Source of enzyme	
	Liver	Bacteria
Dichloromethane	28	249
Chloroform	71	271
Carbon tetrachloride	105	188
Ethylene dichloride	83	269
n-Hexane	79	107
Benzene	83	198
Toluene	92	180
Methyl alcohol	99	116
Ethyl alcohol	83	129
n-Propyl alcohol	100	258
n-Butyl alcohol	92	585
n-Amyl alcohol	99	817

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References and Notes

1. Sigma Chemical Co., "Urgent bulletin re bacterial β-glucuronidase" (St. Louis, Mo., Nov. 1958).
2. This work was supported in part by a grant from the National Institute for Arthritis and Metabolic Diseases.
3. P. Talalay, W. H. Fishman, C. Huggins, *J. Biol. Chem.* 166, 757 (1946).
4. Mammalian liver β-glucuronidase (Ketodase) was generously supplied by Raphael Cohn, of Warner-Chilcott Laboratories, Morris Plains, N.J.
5. P. Bernfeld, S. Jacobson, H. C. Bernfeld, *Arch. Biochem. Biophys.* 69, 198 (1957).
6. W. H. Fishman and S. Green, *J. Biol. Chem.* 225, 435 (1957).

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