Biochemical Pharmacology, Vol. 24, pp. 1291–1292. Pergamon Press, 1975. Printed in Great Britain.

# DETERMINATION OF UDP-GLUCURONYLTRANSFERASE USING UDP-[$^{14}$C]GLUCURONIC ACID

J. M. ZIEGLER, B. P. LISBOA*, A. M. BATT and G. SIEST

Laboratoire de Biochimie, Faculté des Sciences Pharmaceutiques et Biologiques, 7, rue Albert Lebrun, B.P. 403, 54001 Nancy Cedex, France; Centre de Médecine Préventive, 2, avenue du Doyen Jacques Parisot, 54500 Vandoeuvre les Nancy, France; and *Universitäts Krankenhaus Eppendorf, Frauenklinik, Martinistrasse 52, 2 Hamburg 20, Germany

(*Received* 21 *October* 1974; *accepted* 16 *January* 1975)

**Abstract**—A method for the evaluation of UDPGT activity is proposed using [$^{14}$C]UDPGA. Free UDPGA, glucuronide and aglycone were separated on an Amberlite XAD2 column. This method is suitable for every substrate able to be bound to the resin such as phenolic compounds

The activity of UDP-glucuronate glucuronyl transferase (UDPGT; EC 2.4.1.17) is usually determined by the conjugation of coloured or fluorescent aglycones such as *p*-nitrophenol (PNP) or 4-methyl umbelliferone. Due to their spectrophotometric properties, they are widely used and the consumption of substrate is measured without any separation of the conjugate.

However these methods have certain disadvantages, since the number of such substrates is limited. Some methods have been described using either labelled aglycones [1–4] or $^{14}$C-labelled UDP-glucuronic acid ([$^{14}$C]UDPGA) [4–6]. The first methods however, need numerous different substrates to be labelled and for most of them the separation of the glucuronide is obtained only by organic solvants extraction.

In the present paper we describe a simple method for evaluating conjugation of any type of aromatic aglycones. This method has been demonstrated to be suitable to compare the ability of 13 phenolic molecules to be conjugated [7]. The radioactivity of UDP-α-D-glucuronic acid ([U-$^{14}$C]D-glucuronic) is transferred to the glucuronide. The excess of UDPGA is separated from the glucuronide and aglycone by column chromatography (Amberlite XAD2, Serva) as described for urinary steroids by Bradlow [8]. The radioactivity of the glucuronide is counted, in eluted fractions, by liquid scintillation.

## EXPERIMENTAL AND RESULTS

The method is described in the present paper with *p*-nitrophenol as aglycone.

*Enzyme source.* Microsomal fractions of liver cells (male Sprague–Dawley rats) are used as enzyme source as previously described [9]. Microsomal suspensions are used as soon as prepared.

*Incubation medium.* The incubation method is derived from the Frei method [10] with the following final concentrations in the reaction mixture: Tris–(hydroxymethyl)-aminomethane, HCl buffer (pH = 7·4) 50 mM; UDPGA (Boehringer) 1 mM; [$^{14}$C]UDPGA (NEN) (>200 mCi/m-mole) 0·08 μCi; *p*-nitrophenol (Merck) 0·14 mM. Enzyme source: 0·35 mg total microsomal proteins (determined by Lowry method [11] with bovine albumine as standard). Final volume = 0·1 ml.

Prior to use, ethanol must be first removed from commercial [$^{14}$C]UDPGA by vacuum distillation. The reaction tubes were incubated at 37° for 30 min. Incubation was stopped by adding 200 μl of 30 mM sulfotungstic acid. The precipitated proteins were separated by centrifugation. Control tubes were kept in ice and sulfotungstic acid was added before PNP.

*Separation of glucuronide.* The supernatant of the incubation mixture was transfered to a 10 × 1 cm Amberlite XAD2 column (Serva, Heidelberg) 300–1000 μm. Amberlite was equilibrated in phosphate buffer 66·6 mM, pH = 7, ionic strength = 0·4. Thirty ml of this buffer was used to elute excess UDPGA and [$^{14}$C]UDPGA. The PNP-glucuronide (PNPG)

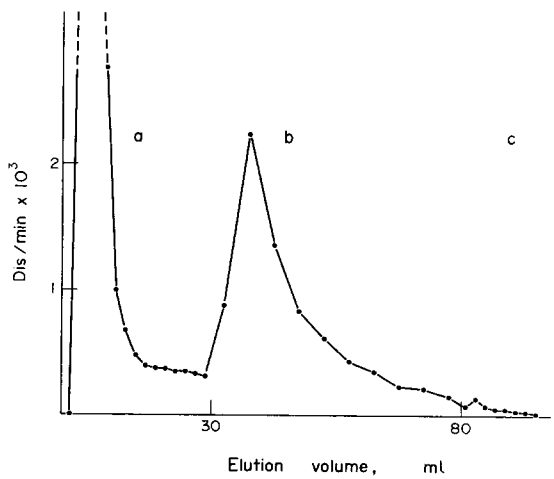


Fig. 1. Separation of [$^{14}$C]UDPGA from [$^{14}$C]PNPG obtained by enzymatic reaction, (a) Elution of excess [$^{14}$C]UDPGA by 30 ml phosphate buffer 66·6 mM, pH = 7. (b) Elution of [$^{14}$C]PNPG by 50 ml distilled water. (c) Final methanolic elution of PNP (as determined by spectrometric measurement, which does not appear on the figure).

was eluted using distilled water (50 ml) and finally the excess aglycone was washed out by methanol (40 ml) (Fig. 1). Elution by distilled water may be omitted if aglycone must not be separated (Fig. 2).

The whole elution was obtained with a solvent flow rate of 60 ml/hr. Two-ml fractions of phosphate buffer, 5 ml distilled water and 2 ml methanol were collected. One half of each fraction was added to 10 ml Instagel® (Packard).

The radioactivity was measured as described above with a standard error smaller than 2·5 per cent (Packard Tri Carb 3380/544 liquid scintillation counter). The second half of the fraction was used for spectrophotometric determination (Zeiss Ikon DMR 21 Spectrophotometer).

The following compounds were tested on the column: UDPGA, [$^{14}$C]UDPGA, PNPG (Sigma), checking the separation by counting and by u.v. spectrophotometric analysis at 261 nm (UDPGA) and 302 nm (PNPG). Chosen elution volumes led to a complete separation of products with a 95 per cent yield.

Identification of radioactive glucuronide produced by the reaction and eluted by distilled water was checked by isotopic dilution. Collected water fractions containing the glucuronide were evaporated by lyophilization. A second chromatography was performed after adding unlabelled PNPG to the medium. The radioactivity and extinction of each fraction were measured. The graph of the ratio radioactivity

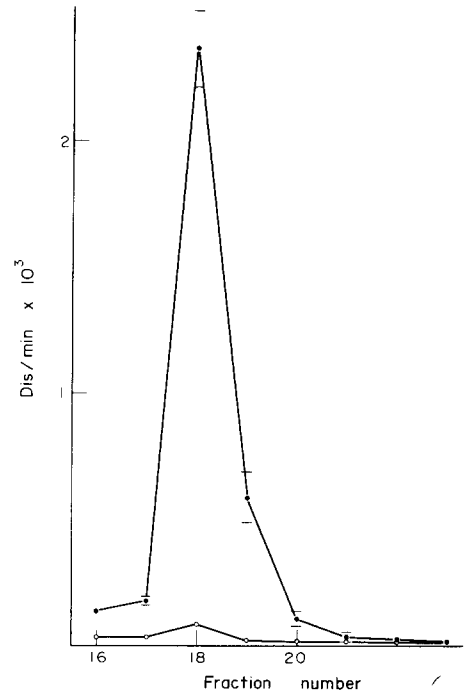


Fig. 2. Elution of [$^{14}$C]PNPG by methanol applied immediately after phosphate buffer. Key—(●) represents mean values ± S.E. from 6 chromatographic elutions; (○) represents elution of control samples where the enzymatic source was added after stopping the reaction.

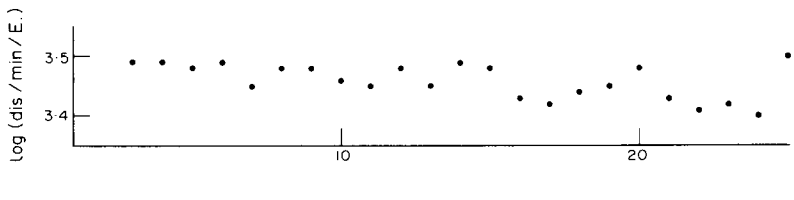


Fig. 3. Identification of the eluted compound (in distilled water) through isotopic dilution.

extinction vs elution time represents a confirmation of identity of both products (Fig. 3).

This method enabled us to compare the conjugability of different phenolic compounds. It is sensitive and reliable. This method provided two major advantages: (1) only one labelled substrate is required whatever the aglycone is; (2) Amberlite properties permit the separation and determination of most substances conjugated *in vivo* or *in vitro*.

*Acknowledgements*—This study was partly supported by a grant from Deutscher Akademischer Austauschdienst —Bonn—Bad Godesberg. The technical assistance of Miss Ch. Lesne is gratefully acknowledged.

## REFERENCES

1. M. Menken, P. V. D. Barrett and N. I. Berlin, *Clin. chim. Acta* **14,** 777 (1966).
2. J. Marniemi and O. Hänninen, *FEBS Lett.* **32,** 273 (1973).
3. W. Lehnert, J. Limberg and W. Kunzer, *Z. klin. Chem. klin. Biochem.* **1,** 23 (1974).
4. A. Aitio, *Xenobiotica* **3,** 13 (1973).
5. M. Pogell and L. F. Leloir *J. biol. Chem.* **236,** 293 (1961).
6. K. P. Wong, *Biochemistry* **125,** 27 (1971).
7. J. M. Ziegler, Thèse de Spécialité, Nancy (1973).
8. H. L. Bradlow, *Steroids* **11,** 265 (1968).
9. J. M. Ziegler, A. M. Batt and G. Siest, *C. R. Soc. Biol.* **167,** 685 (1973).
10. J. Frei, *Enzym. Biol. Clin.* **11,** 385 (1970).
11. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193,** 265 (1951).