PURIFICATION OF UDP-GLUCURONYLTRANSFERASE FROM UNTREATED RAT LIVER

Brian BURCHELL
Department of Biochemistry, The University. Dundee DD1 4HN, Scotland

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1. Introduction

Liver microsomal UDP-glucuronyltransferase (EC 2.4.1.17) catalyses the glucuronidation of many endogenous compounds (such as steroids and bilirubin) and xenobiotics [1]. This pharmacologically important enzyme is a major protein involved in detoxication of potentially toxic compounds and drugs [2]. Recent evidence [3] suggests that glucuronidation of polycyclic hydrocarbons may be of key importance as a determinant of their carcinogenic potential.

Various lines of evidence suggest that there may be more than one UDP-glucuronyltransferase (see [2,4] and [5]). Indeed Bock et al. [6] have proposed that different isoenzymes of UDP-glucuronyltransferase may be induced in response to phenobarbital and 3-methylcholanthrene. However, the existence of more than one enzyme protein cannot be verified until catalytically active UDP-glucuronyltransferase has been purified to homogeneity.

Several attemps have been made to purify UDP-glucuronyltransferase, since the initial work of Isselbacher et al. [7]. Recently, the enzyme has been purified 150–200-fold over the cell free homogenate, from phenobarbitaltreated rat liver [8].

In this laboratory we have previously obtained highly active partially purified UDP-glucuronyltransferase from untreated rat liver by detergent solubilisation and ion-exchange chromatography [5,9]. The present paper describes the additional affinity chromatographic step, which is required to purify UDP-glucuronyltransferase to homogeneity. Purification was over 900-fold. The pure enzyme forms 4.6 μmol glucoronide/min/mg protein with 4-nitrophenol as substrate. Only one polypeptide, mol. wt 57 000 was visible after sodium dodecyl sulphate–gel electrophoresis.

2. Materials and methods

Lubrol 12A9 (a condensate of dodecyl alcohol with approx. 9.5 mol ethylene oxide/mol) was a gift from ICI Organics Division, Manchester, England.

UDP-Hexanolamine agarose (12 μmol UDP-hexanolamine/g agarose) was a kind gift from Professor Robert L. Hill, Department of Biochemistry, Duke University, North Carolina, and had been synthesised by the method of Barker et al. [10].

2.1. *Enzyme assays*

UDP-Glucuronyltransferase activity towards various substrates was assayed by previously described methods: 4-nitrophenol and 2-aminophenol [11] and 1-naphthol [12]. 1-[1-^{14}C]naphthol was purchased from the Radiochemical Centre, Amersham, England. One unit of enzyme activity represents 1 nmol glucuronide formed/min.

Epoxide hydratase activity was assayed by the method of Oesch [13].

2.2. *Purification of UDP-glucuronyltransferase*

Partially-purified UDP-glucuronyltransferase was prepared from the livers of five male (200 g) Wistar rats, by the method previously described [9] and equilibrated in 0.05% Lubrol/25 mM potassium phosphate buffer, pH 7.4 (Buffer A). A sample of this preparation (approx. 4 mg) was applied to a UDP-hexanolamine agarose column (4 × 1.2 cm), previously equilibrated with buffer A. The majority

of the protein was eluted with buffer A. Bound UDP-glucuronyltransferase was eluted from the column with 10 ml 5 mM UDP-glucuronic acid in buffer A.

2.3. *Gel electrophoretic analysis*

This was performed in the presence of 0.1% sodium dodecyl sulphate by the method previously described [9]. The molecular weight of UDP-glucuronyltransferase was estimated by comparison with proteins of known molecular weight as standards. The standards used: bovine serum albumin (mol. wt 68 000), pyruvate kinase (mol. wt 57 000) and ovalbumin (mol. wt 43 500) were purchased from Sigma, London and epoxide hydratase (mol. wt 49 500) purified in this laboratory [14].

3. Results and discussion

3.1. *Purification of UDP-glucuronyltransferase*

When partially-purified UDP-glucuronyltransferase was applied to the UDP-hexanolamine agarose column, approximately 70% of the applied enzyme activity remained bound to the column. UDP-Glucuronyltransferase activity (40%) was specifically eluted from this column with 5 mM UDP-glucuronic acid. This step resulted in a further 40-fold purification of the enzyme. The overall specific activity of the enzyme preparation had been increased from 0.34 units/mg protein to 4657 units/mg protein when 4-nitrophenol was used as substrate (table 1). Thus enzyme has been purified 908-fold over the 10 000 × *g* supernatant. The yield of over the 10 000 × *g* supernatant. The yield of UDP-glucuronyltransferase activity was 3-fold lower after elution from the DEAE–Sephadex column (table 1, Step 7) in comparison to the previously published data [9]. Despite this and the low overall yield (1.75%). UDP-glucuronyltransferase had clearly been purified to homogeneity. The purified enzyme exhibited a half-life of 18 days when stored at 0°C in ice.

Epoxide hydratase had been found to be the major contaminant protein of the previous partially-purified preparation (see ref. [9]). The present UDP-glucuronyltransferase preparation was therefore assayed for

Table 1
Purification of rat liver UDP-glucuronyltransferase activity towards 4-nitrophenol as substrate

	Total protein (mg)	Specific activity (units/mg protein)	Relative purification	Total activity (Units)	Yield (%)
1. 10 000 × *g* Supernatant	6849	(0.34)	1	–	–
2. Microsomal fraction	1705	(1.33)	3.9	–	–
3. Lubrol-soluble supernatant	1418	20.0	3.9	28 363	100
4. 25–60% Satn $(NH_4)_2SO_4$ precipitate	650	24.0	4.7	15 600	55
5. DEAE-Cellulose eluate/ 6. CM-Cellulose eluate	118	35.7	6.9	4213	15.3
7. DEAE–Sephadex eluate	10.6	115.5	22.5	1224	4.5
8. UDP-Hexanolamine agarose/UDP-Glucuronic acid eluate	0.102	4657	908	477	1.75

[a]After solubilisation (Step 3), UDP-glucuronyltransferase was activated 15-fold by the detergent. The activation at this stage was taken into account to give a true estimation of UDP-glucuronyltransferase purification (see Burchell [9]).

Figures in parentheses represent enzyme activity in the absence of detergent.

One unit activity represents 1 nmol 4-nitrophenylglucuronide formed/min. Preparation up to Step 7 is as previously described [7]. Other methods as in text.

epoxide hydratase activity. None was detected, showing that the major contaminant had been removed during the affinity chromatographic step.

The ammonium sulphate precipitate and the final UDP-glucuronyltransferase preparation were tested for activity towards other substrates. Preliminary results show that high activity towards 1-naphthol (4.3×10^4 units/mg protein) and 2-aminophenol (60.2 units/mg protein) could be detected. The ratio of activities of 4-nitrophenol to 1-naphthol and 2-aminophenol remained approximately the same in the crude extract and in the purified enzyme preparation. Thus enzyme activity towards these three substrates appears to be co-purified. This result infers that the same protein is responsible for the glucuronidation of these three substrates.

3.2. *Gel electrophoresis of purified UDP-glucuronyltransferase*

Polyacrylamide gel electrophoresis in the presence of 0.1% sodium dodecyl sulphate was used to assess the purity of the UDP-glucuronyltransferase preparation. This gel electrophoresis system separated the polypeptides of the ammonium sulphate fraction into more than 30 distinct staining bands.

Figure 1 is a photograph of the stained polypeptides visible after gel electrophoresis. Gel A shows that 3 polypeptides are present in the DEAE–Sephadex eluate (Step 7) which was applied to the UDP-hexanolamine agarose column. The 3 polypeptides possess mol. wt 57 000, 49 500 and approx. 15 000, as previously reported [9]. In comparison only one staining band of mol. wt 57 000 daltons was observed on Gel B, which shows the protein eluted by UDP-glucuronic acid from the UDP-hexanolamine agarose column. Assuming that this polypeptide-staining band is UDP-glucuronyltransferase, then the molecular weight of the enzyme, or its subunit, is 57 000 daltons.

This molecular weight value is also consistent with the results of Gorski [8], in his 200-fold purified preparations of UDP-glucuronyltransferase from phenobarbital-treated rat liver, he observed a major polypeptide of 59 000 daltons, although a second protein 'artefact' was also present.

Therefore, this method can be used to obtain pure preparations of UDP-glucuronyltransferase. The availability of the pure enzyme opens up new avenues

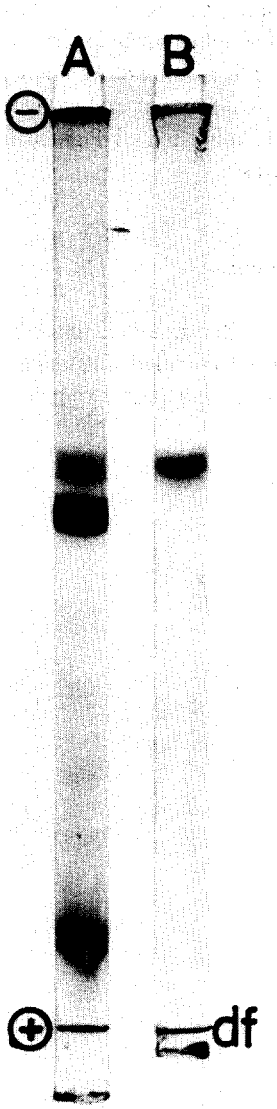

Fig.1. Sodium dodecyl sulphate–gel electrophoretograms of purified UDP-glucuronyltransferase. Disc electrophoresis was performed with 7.5% cylindrical gels (9.5 × 0.6 cm) in the presence of 0.1% sodium dodecyl sulphate (see Materials and methods section). Gel A, DEAE–Sephadex eluate (8 μg protein) specific activity 115 units/mg protein. Gel B, the protein eluted by UDP-glucuronic acid from the UDP-hexanolamine agarose column (1 μg protein) specific activity 4657 units/mg protein. Gels were stained with 0.25% Coomassie Blue for 60 min and destained with acetic acid/methanol/water (7:5:43, by vol.). The direction of migration is from the top to the bottom. (df) Dye front.

for investigation of glucuronide formation in the liver. Assay of the pure enzyme for activity towards other substrates such as bilirubin and steroids should speedily provide information concerning the multiplicity of UDP-glucuronyltransferase and an insight into the biochemistry of inherited UDP-glucuronyltransferase deficiency diseases.

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