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NEW RESULTS ON OESTROGEN GLUCURONYLTRANSFERASE

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SUMMARY

Oestradiol-17β, 3-glucuronyltransferase (oestrogen glucuronyltransferase) from the microsomes of pig intestine homogenate was extracted by sodium dodecyl sulfate. Centrifugation of the sodium dodecyl sulfate treated microsomes at 230,000 $g_{av.}$ yielded a clear supernatant, which when passed through Bio-Gel A-5m column yielded several peaks of enzyme activity. In the presence of 0·05% sodium dodecyl sulfate only two peaks of activity were obtained. The molecular weights were 750,000 and 410,000, respectively. Sucrose density gradient centrifugation in the absence of sodium dodecyl sulfate resulted in several activity peaks and in its presence only two activity peaks were obtained which had molecular weights of 58,000 and 147,000. Activity of enzyme in the presence of sodium dodecyl sulfate was considerably lower. These results show that oestrogen glucuronyltransferase from pig intestine microsomes is active in the aggregated form and that several forms of the same enzyme may be existing.

INTRODUCTION

In previous studies on the oestrogen glucuronyltransferase (acceptor unspecific, EC.2.4.1.17) from the intestine of the pig, it was shown that the enzyme is present in all subcellular fractions and specifically conjugates oestradiol-17β and oestrone[1–3]. The enzyme was also found to be present in the outer membrane of the mitochondria[4]. The oestrogen glucuronyltransferase from the intestine of the pig has been studied as far as the kinetic properties are concerned[1–4]. Recently Vessey and Zakim[5–9] have studied more extensively the glucuronyltransferase from rat, guinea pig and bovine tissues using *p*-nitrophenol and σ-aminophenol as substrates. The present series of investigations were carried out to get more information about the physical properties of the glucuronyltransferase which conjugates the physiological substrates oestrone and oestradiol-17β. The enzyme was extracted from the microsomal fraction of the pig intestine with sodium dodecyl sulfate.

Under carefully controlled conditions such as pH, time, amount of sodium dodecyl sulfate (SDS) and molarity of buffer, it was found that out of the five different reagents used to "solubilize" microsomal activity, SDS was most effective, Fig. 1. The SDS treated microsomes were centrifuged twice for 30 min at 230,000 $g_{av.}$; the specific activity of the enzyme in the clear supernatant increased 10 fold. This high speed supernatant (hereafter referred to as SDS-supernatant) served as starting material for investigating the molecular weight and sedimentation coefficients.

Gel filtration was carried out using Bio-Gel A-5m column (1·5 × 90 cm) equilibrated with 0·10 M Tris–HCl buffer, pH 7·6. The protein and enzyme activity profiles are shown in Fig. 2. The major amount of activity is eluted after the void volume; however, several peaks of activity are obtained probably indicating different states of aggregation of the enzyme. The enzyme activity in the most active fraction was increased 18 fold over that of the untreated microsomes. The molecular weight corresponded to 790,000 Daltons. The other activity peaks corresponded to approximate molecular weights of 570,000, 445,000 and 340,000 Daltons.

In order to find out whether SDS could prevent aggregation and thus increase enzyme, activity gel filtration was carried out by incorporating 0·05% SDS in the column and eluting buffer. The results obtained are presented in Fig. 3. It appears that SDS prevents aggregation to a large extent evidenced by the appearance of only two peaks of activity. The approximate molecular weights were found to be 790,000 and 410,000 Daltons for the faster moving and slower moving components, respectively; the enzyme activity was decreased.

To further study the aggregation process and to get more information on the molecular weights of the

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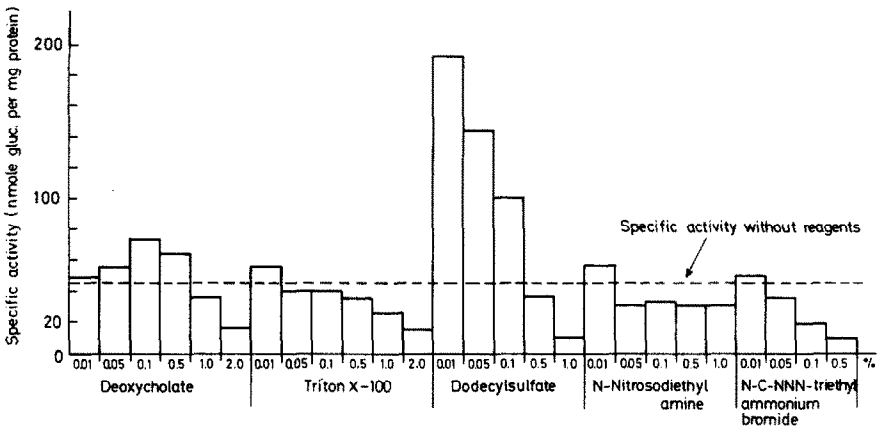

Fig. 1. Effect of different amounts of different reagents on the microsomal oestrogen glucuronyltransferase activity. Twice washed microsomes were mixed with different amounts of the reagents, kept for 30 min at 2 to 4°C and shaken occasionally; the total volume was 1·0 ml. For enzyme assay 53 μg of protein was incubated with (4-^{14}C)-oestradiol-17β (30 nmol) and UDP-glucuronic acid (1·0 μmol) in 1·0 ml of 0·10 M Tris–HCl buffer, pH 7·6 for 30 min at 37°C. The unreacted oestradiol-17β was removed by extraction with water saturated ethyl acetate. To the water phase NaCl was added in a small excess and the radioactive oestradiol-17β 3-glucuronide was extracted with water saturated *n*-butanol. An aliquot was pipetted into a glass counting vial, 12 ml of scintillation fluid[1] was added and counted in a Packard liquid scintillation spectrometer. For further details refer to previous publications[1–3].

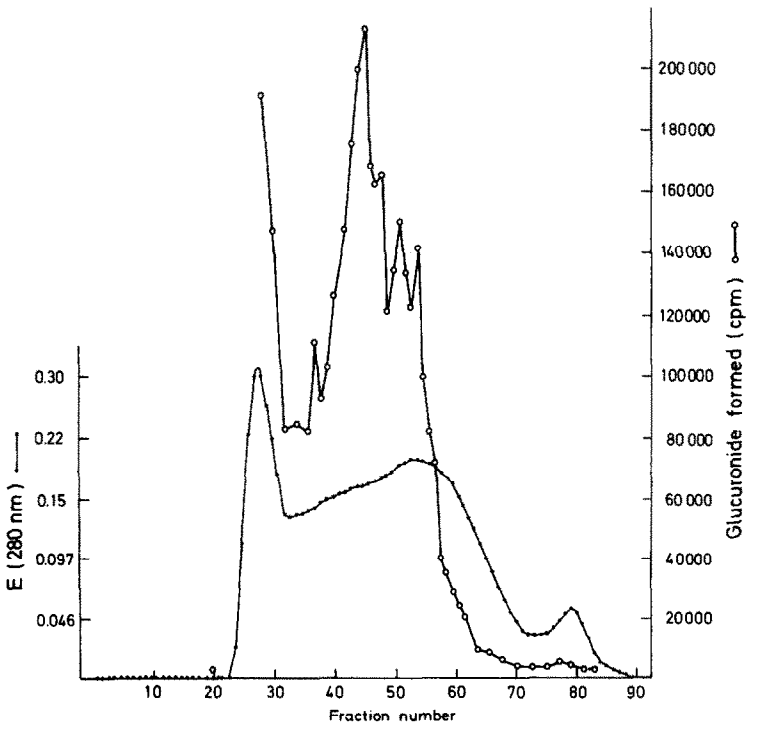

Fig. 2. Bio-Gel A-5m column (1·5 × 90 cm) chromatography of the SDS supernatant (230,000 $g_{av.}$) after treating the twice washed microsomes with 0·05% sodium dodecyl sulfate. The column was equilibrated with 0·1 M Tris–HCl buffer, pH 7·6, containing 0·1 mM EDTA, 2 to 3 ml of the SDS-supernatant (10 to 15 mg protein) was layered over the top of the gel; the elution of protein was monitored by U.V.-cord II and fractions of 2 ml were collected. The enzyme activity was measured as described in the legend for Fig. 1.

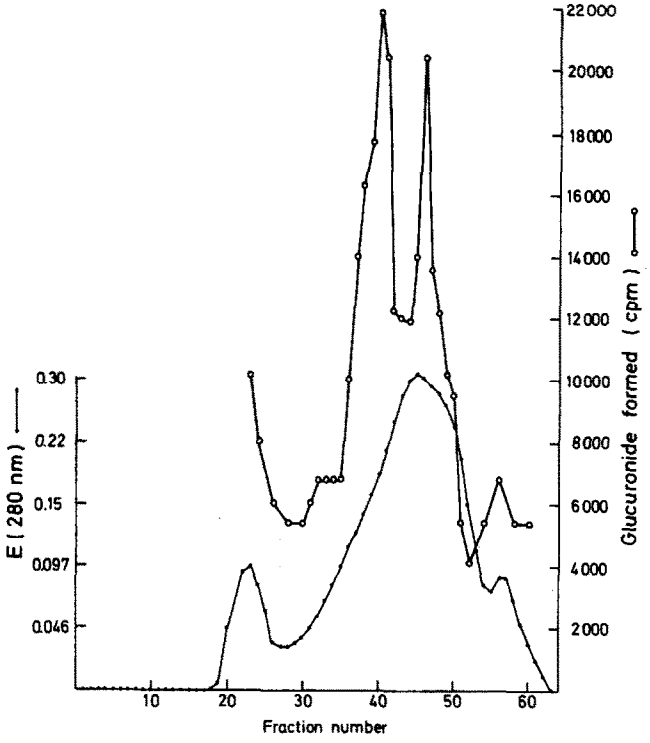

Fig. 3. Bio-Gel A-5m column (1·5 × 94 cm) chromatography in the presence of 0·05% sodium dodecyl sulfate of the SDS-supernatant (230,000 $g_{av.}$) after treating the twice washed microsomes with 0·05% sodium dodecyl sulfate. 3·5 ml of the high speed supernatant (20 mg protein) was layered over the top of the gel. For details refer to the legend for Fig. 2.

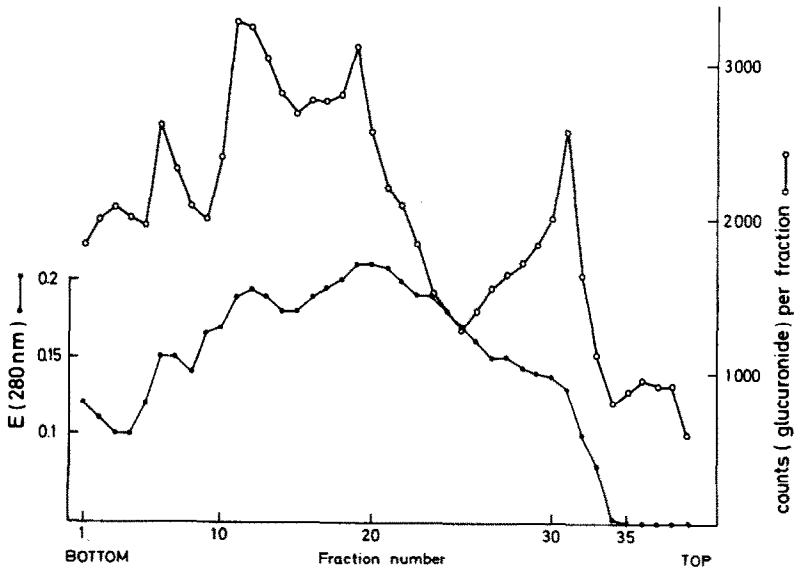

Fig. 4. Centrifugation of the SDS-supernatant through a 5 to 20% sucrose density gradient. 100 μl (800 μg protein) was layered on top of the gradient and the tubes centrifuged in a Beckman L2-65B ultracentrifuge for 13 to 14·5 h using a SW 41 rotor at 180,000 $g_{av.}$ The tubes were pierced at the bottom with a hollow needle and 20 drops fractions were collected. One set of fractions was used to determine enzyme activity and the other for absorbance measurement at 280 nm.

different protein fractions, sucrose density gradient centrifugation was carried out in the absence and in the presence of SDS[10]. The sucrose gradient ranged from 5 to 20% in 0·05 M Tris–HCl buffer, pH 7·6. The SDS-supernatant equivalent to 800 to 1000 μg protein was layered over the gradient and centrifuged in a Beckman L2-65B ultracentrifuge for 14·5 h at 180,000 $g_{av.}$ using a SW 41 rotor. The tubes were pierced from the bottom and 15 to 25 drops were collected and enzyme activity was determined; a second tube was used to measure absorbance at 280 nm. As can be seen from Fig. 4 several peaks of activity are obtained which compare well with the pattern obtained after gel filtration without SDS. The appearance of several peaks of activity is indicative of the formation of aggregates. However, quite in contrast to the activity in the fractions after gel chromatography, the activity of the fractions obtained after sucrose density gradient centrifugation is significantly lower.

The next sucrose density gradient centrifugation was carried out in the presence of 0·05% SDS in order to prevent aggregation and to study the influence on enzyme activity. Figure 5 shows that here again SDS prevents the formation of aggregates and that the number of activity peaks is reduced to two. However, enzyme activity does not appear to be affected, the most active fraction possessing a specific activity which is 3 fold higher than that of the untreated microsomes. The slower moving component, peak I, has a sedimentation value of approximately 4 S while the faster moving component, peak II, has a sedimentation value of 7·5 S.

In Fig. 6 the molecular weights (ordinate) of four standard proteins are plotted against the experimentally determined sedimentation values (abscissa). It may be seen that a linear relationship was obtained. Plotting the sedimentation values of peak I and peak II on the straight line, peak I corresponds to a molecular weight of 58,000 and peak II of 147,000 Daltons.

Polyacrylamide gel electrophoresis using 0·05% SDS [11, 12] of the supernatant obtained after treating the microsomes with SDS, yielded two major bands and several minor bands. Staining and destaining was done as described in ref. 11 and 12. One of the major bands had a mobility in the region between bovine serum albumin and ovalbumin, while the second had the mobility of the dimer of bovine serum albumin.

The phospholipid to protein ratio of the microsomes and the most active fraction from the Bio-Gel column did not vary significantly; the calculated ratio was 1:1 on weight basis.

CONCLUSIONS

1. Sodium dodecyl sulfate extracts oestrogen glucuronyltransferase from the membranes of the microsomes and this enzyme activity is non-sedimentable.

2. Gel filtration of the "solubilized" enzyme in the absence of dodecyl sulfate leads to different states of aggregation. All aggregates possess enzyme activity. In the presence of sodium dodecyl sulfate, aggregation is partially prevented but with concomitant decrease in enzyme activity.

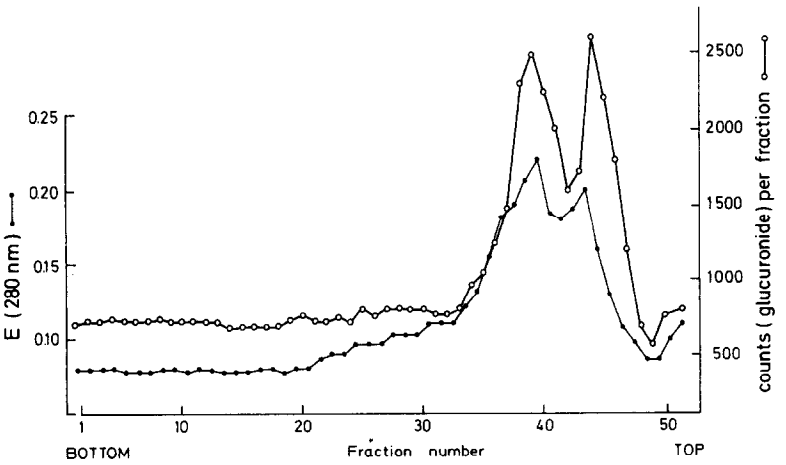

Fig. 5. Centrifugation of the SDS-supernatant through a 5 to 20% sucrose density gradient containing sodium dodecyl sulfate. 100 μl (1000 μg protein) was layered on the top of the gradient and the tubes centrifuged in a Beckman L2-65B ultracentrifuge for 14·5 h using a SW 41 rotor at 180,000 $g_{av.}$. The details are mentioned in the legend for Fig. 4.

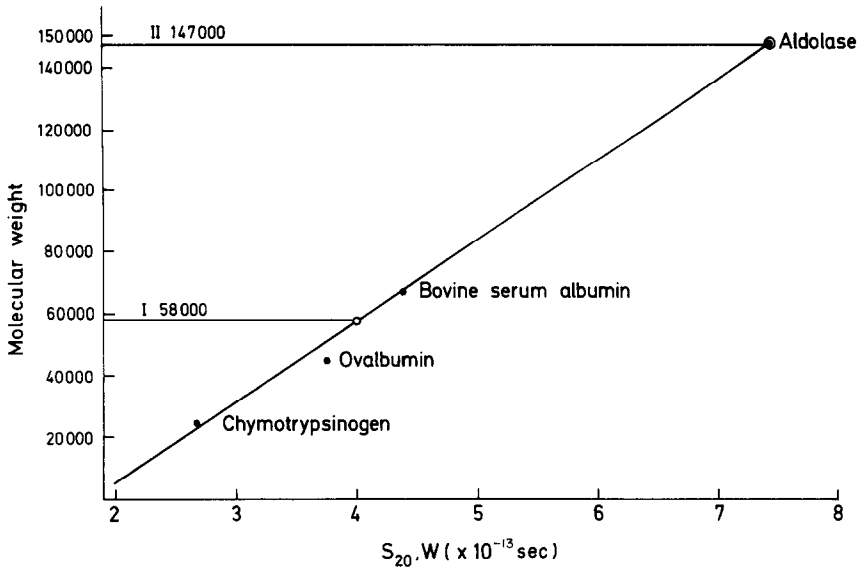

Fig. 6. Centrifugation of four standard proteins through a 5 to 20% sucrose density gradient containing 0·05% sodium dodecyl sulfate; other details were the same as described in the legend for Fig. 5. The distance from the meniscus to the centre of the protein peak was measured and the corresponding molecular weight of the respective protein was plotted on the ordinate. The sedimentation coefficients on the abscissa are values calculated according to McEwens[13].

3. Sucrose density gradient centrifugation in the presence of SDS yields a component with a molecular weight of approx. 58,000 and a second component of approx. 147,000 Daltons. The value for the former component on gel electrophoresis was found to be approx. 50,000 Daltons.

4. As evidenced by the molecular weight estimation on Bio-Gel A-5m columns SDS could form complexes with protein or lipid–protein structures and thus increase the apparent molecular weight.

5. Thus it is likely that oestrogen glucuronyltransferase from the intestines of the pig exists in the microsomal membranes in different forms or states of combination with lipids and protein or as isoenzymes. Maintenance of these complex structures is essential for enzymic activity and should give some information about the association of the oestrogen glucuronyltransferase with the microsomal membrane.

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