Selective induction of glutathione *S*-transferase D in rat testis by phenobarbital

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(Received 5 January 1984/Accepted 22 February 1984)

Testis cytosol is shown to contain the Yb₂Yb₂-homodimer glutathione *S*-transferase D in addition to the previously described glutathione *S*-transferases A (Yb₁Yb₁) and C (Yb₁Yb₂). Treatment of rats with phenobarbital induces the level of glutathione *S*-transferase D in testis with no increase in the activities of glutathione *S*-transferases A and C. This result indicates a specific induction of the Yb₂ subunit in testis, in contrast with the situation in rat liver, where phenobarbital specifically induces the Yb₁ subunit.

Glutathione *S*-transferase (GST) exists in multiple forms in rat liver (Mannervik & Jensson, 1982; Hayes, 1983) and other tissues (Scully & Mantle, 1981; Mannervik *et al.*, 1983). Three forms, GSTs A, D (GST-C₂ in the nomenclature of Mannervik & Jensson, 1982) and C, have been shown, in an elegant kinetic analysis, to correspond to the two homodimers and corresponding heterodimer of two Yb-subunits of *M_r* 23 500 (Mannervik & Jensson, 1982). A corresponding structural analysis of GSTs A, C and D has confirmed the existence of two structurally related Yb-subunits, termed 'Yb₁' and 'Yb₂' forming the homodimers GST-A and GST-D respectively (Hayes, 1983). These results are in agreement with predictions made by Tu *et al.* (1982) on the basis of bimodal hybridization of Yb-coding mRNA to GST-coding DNA under conditions of variable stringency. GSTs A, C and D are all present in rat liver, where phenobarbital specifically induces the Yb₁-subunit of the homodimer GST-Am (Hayes *et al.*, 1979; Mannervik *et al.*, 1983). We have previously demonstrated the presence of GSTs A and C in rat testis (Scully & Mantle, 1981) and in the present study we report the presence of GST-D and show that, in contrast with the situation in liver, in testis it is the Yb₂-subunit that is induced by phenobarbital.

Materials and methods

Fractionation of multiple forms of glutathione S-transferase

Preparation of rat testis cytosol, passage through DEAE-cellulose and chromatography on CM-cellulose at pH 6.7 was as described previously (Scully & Mantle, 1981), except that the DEAE-cellulose was equilibrated in 10 mM-Tris/HCl, pH 8. The fraction that did not bind to CM-cellulose at pH 6.7 was titrated to pH 6 with 1 M-acetic acid and loaded on to CM-cellulose equilibrated with 10 mM-sodium phosphate, pH 6. GST-D was eluted with a 0–100 mM-KCl gradient.

Phenobarbital treatment

Groups of five male rats weighing 200 g were administered phenobarbital (40 mg/kg) intraperitoneally in 0.9% NaCl for 5 days before decapitation on day 6. Control groups received an equivalent volume of saline.

Enzyme assays

The activity of the various forms of GST towards DCNB and *trans*-PBO was determined as described by Habig *et al.* (1974).

Results and discussion

Figs. 1(a) and 1(b) show the results of ion-exchange chromatography of rat testis cytosol on CM-cellulose equilibrated at pH 6 and 6.7 respectively. The single GST peak (active with both CDNB and DCNB) observed during chromatography at pH 6 (Fig. 1a) is composed of Yb-subunits (results not shown) and is eluted at the same conductivity (within experimental variation)

Abbreviations used: GST(s), glutathione *S*-transferase(s); CDNB, 1-chloro-2,4-dinitrobenzene; DCNB, 1,2-dichloro-4-nitrobenzene; *trans*-PBO, *trans*-4-phenyl-3-buten-2-one.

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Fig. 1. Effect of phenobarbital administration on the multiple forms of GST. CM-cellulose chromatography was conducted at pH 6 (a, c) and pH 6.7 (b, d) as described in the text for control rats (a, b) and rats receiving phenobarbital (c, d).

as GST-D from rat liver (Sheehan & Mantle, 1984). The two peaks of GST active towards DCNB that are fractionated at pH 6.7 (Fig. 1b) are GSTs C and A (in order of elution) as described previously (Scully & Mantle, 1981). Both GST-D (Fig. 1a) and GST-C (Fig. 1b) are particularly active with *trans*-PBO, in contrast with GST-A (results not shown). This result is in agreement with the data of Mannervik & Jensson (1982) for the corresponding forms from rat liver. Figs. 1(c) and 1(d) show the effect of phenobarbital administration on the activity of the three YbYb-dimers of rat testis (as measured with DCNB as substrate). There is a 3-fold induction of GST-D (the Yb₂Yb₂-homodimer) with no effect on the activity of GST-A or GST-C. In a separate experiment we obtained a 2.5-fold induction of GST-D with no effect on the activity of GST-A or GST-C. This result is in contrast with the situation in rat liver, where (with regard to Yb-subunits) it is the Yb₁-subunit that is specifically induced by phenobarbital (Hayes *et al.*, 1979; D. Sheehan & T. J. Mantle, unpublished work).

We are grateful to the Cancer Research Advancement Board for financial support.

References

- Habig, W. H., Pabst, M. J. & Jakoby, W. B. (1974) *J. Biol. Chem.* **249**, 7130-7139
- Hayes, J. D. (1983) *Biochem. J.* **213**, 625-633
- Hayes, J. D., Strange, R. C. & Percy-Robb, I. W. (1979) *Biochem. J.* **181**, 699-708
- Mannervik, B. & Jensson, H. (1982) *J. Biol. Chem.* **257**, 9909-9912
- Mannervik, B., Guthenberg, C., Jensson, H., Warholm, M. & Alin, P. (1983) in *Functions of Glutathione: Biochemical, Physiological, Toxicological and Clinical Aspects* (Larsson, A., ed.), pp. 75-88, Raven Press, New York
- Scully, N. C. & Mantle, T. J. (1981) *Biochem. J.* **193**, 367-370
- Sheehan, D. & Mantle, T. J. (1984) *Biochem. J.* **218**, 893-897
- Tu, C.-P. D., Weiss, M. J. & Reddy, C. C. (1982) *Biochem. Biophys. Res. Commun.* **108**, 461-467