BIOCHEMICAL JOURNAL LETTERS

Structural distinction of rat GSH transferase subunit 10

We have previously noted a GSH transferase with high GSH peroxidase activity which is prominent in the liver soluble fraction of pre- and post-natal rats. It has a subunit M_r of 25500, intermediate between Yb and Ya, and appears to be closely related to Alpha class subunit 2 (Meyer *et al.*, 1985; Ketterer *et al.*, 1986; Dalton, 1987; Scott & Kirsch, 1987). It may be separated from subunits 1 and 2 by chromatofocusing on PBE 118 (Dalton, 1987). Since its apparent size and activity are distinct from other well-characterized subunits we have given it the designation subunit 10 (Ketterer *et al.*, 1988). A subunit of identical properties was also obtained from adult rat kidney by Hayes (1988, 1990) and termed Y1. To confirm that subunit 10 (Y1) is a distinct gene product we now report that it has a unique primary structure.

GSH transferases were obtained from newborn rat liver cytosol (kindly provided by Dr. G. Yeoh, University of Western Australia) by affinity chromatography on GSH–agarose and the subunits separated by reverse phase h.p.l.c. (Kispert *et al.*, 1989). Subunit 10 was eluted between subunits 2 and 6 and was not completely separated from subunit 2. The subunit 10 fraction was purified further by diluting with 1 vol. of water and re-applying to h.p.l.c. Then 20 μg of purified subunit 10 was cleaved with CNBr and the fragments separated by microbore reverse phase h.p.l.c. (Kispert *et al.*, 1989). Uncleaved subunit 10 and CNBr peptides were then subjected to gas phase sequencing using an Applied Biosystems 470A Sequencer. The partial sequence of 113 residues obtained showed 90%, 60% and 57% identity with Alpha class subunits 2, 1 and 8 respectively (see Fig. 1). These data confirm our earlier conclusion that subunit 10 is a unique GSH transferase subunit of the Alpha class and is most closely related to subunit 2. Two residues were obtained in approximately equal yield at position 83, suggesting that micro-heterogeneity exists in subunit 10.

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Subunit
          1                   10                  20
 10   P G K P V L H Y F D G R G R M E P I X X L L A A A G V X F
  2   . . . . . . . . . . . . . . . . .     . . . . . . .   .
  1   S . . . . . . . I A . . . . . C .     . . . . . . .   .
  8   E V . . K . Y . . Q . . . . . . V .     . . . T . . .   .

          63                  70                  80
 10   K L V Q T K A I L N A I A T K X N L Y G T/L D
  2   . . . . . R . . . . Y . . . .   . . . .  K  .
  1   . . A . . R . . . . Y . . . .   D . . .  K  .
  8   L . T . . R . . . S Y L . A .   . . . .  K  .

          94          100
 10   Y  A  E G V A D L E
  2   .  .  . . . . . . D
  1   . S/T . . I L . L T
  8   .  .  D . T Q . . M

         105         110
 10    V  L  Y Y P Y
  2    .  .  H . . .
  1    I  I/M Q L V I
  8    I  I  G A . F

         112                 120                 130
 10    P P G E  K  E A S L A K I K D K A R N R Y F P A Y E K V L
  2    . . . .  .  . . . . . . . . . . . . . . . . . F . . . .
  1    . P D Q ./R . . K T . L A . . R T K . . . L . . F . . . .
  8    A . Q .  .  . E . . . L A V K R . K . . . . . V F . . I .

         170                 180
 10    D P I V G D N F P L X K A L Q T R V
  2    . . S A L A . . . .   . . . R . . .
  1    . A S L L T S . . .   . . F K S . I
  8    S A P . L S D . . .   Q . F K . . I
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Fig. 1. Comparison of partial primary sequence of GSH transferase subunit 10 with those of subunits 1, 2 and 8

The partial primary structure of subunit 10 purified by h.p.l.c. was obtained by gas-phase sequencing as described in the text. The sequences of subunits 2 and 8 are from Telakowski-Hopkins *et al.* (1985) and Ålin *et al.* (1989) respectively; those of the variants of subunit 1 are from Pickett *et al.* (1984) and Lai *et al.* (1984). A dot indicates a residue identical with that of subunit 10.

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On the mechanism of induction of chick embryo hepatic δ-aminolaevulinate synthase by translational blockers

Two groups have reported the surprising observation that the addition of cycloheximide (CHX) to the culture medium of chick embryo hepatocytes results in rapid elevations in δ-amino-