Biochemical and Immunological Comparison
of Placental Glutathione S-transferase
From Human and Awassi Sheep.

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ABSTRACT

Glutathione S-transferase from human and sheep placentae were isolated and their biochemical and immunological properties were compared. The specific activity for human enzyme in the crude extract was 0.091 (umoles/min/mg protein), while the specific activity of the sheep enzyme was 0.034 (umoles/min/mg protein). Affinity chromatography on glutathione linked agarose was used to purify the enzyme. The purification procedure resulted in a homogeneous enzymes from both sources with more than 82% yield of the applied activity and more than 230 purification fold in case of human enzyme and 61% yield and 490 purification fold for the sheep enzyme. The specific activities for the purified transferase were 21 and 16 (umoles/min/mg protein) for human and sheep enzyme respectively.

Both purified enzymes displayed single protein band on nondenaturated gel electrophoresis while the two enzymes contained two different types of subunits as revealed by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. The human placental transferase had 20,749-Mr and 21,928-Mr subunits while the sheep transferase had 21,928-Mr and 23,120 Mr subunits.

Chromatofocusing fractionation of the two enzymes on polybuffer exchanger-94 showed that, the human placental transferase was a mixed of three acidic isozymes designated A-1, A-2 and A-3 and showed pI values of 6.0, 5.7 and 5.5 respectively. The isozyme A-1 accounted for 48% of applied activity while A-2 and A-3 had 18% and 33% of the transferase activity respectively. In contrast to human placenta enzyme the sheep placenta transferase was resolved under the same conditions into five enzymatic peaks designated as C-1 through C-5 with pI values of 8.0, 7.7, 7.4, 7.3 and 7.1 respectively, and accounted for about 21%, 30%, 20%, 18% and 10% of the applied transferase activity.

Substrate specificity study indicated that, the human and sheep placental transferases preferred 1-chloro-2,4-dinitrobenzene as a second substrate and both enzyme used other substrates but at a lower rate. The two enzymes exhibited similar stability profiles at different storage conditions, such as, room temperature (20°C),
4°C and -20°C. The effects of some synthetic organic azides on human transferase activity were investigated, both α - β diazido-o-xylene and α - β diazido-p-xylene effectivity inhibited the conjugation of 1-choro-2,4-dinitrobenzene with glutathione. This inhibition was reversible and occurred at lower concentrations compared to other selected organic azides.

In addition to the biochemical similarities between human and sheep placentae transferases, the two enzymes exhibited immunological similarities. Double immunodiffusion analysis showed a cross-reactivity between antibody raised against the human placental enzyme and crude extracts prepared from both sheep and goat placentae. Similarly the results of immunotitration and immunoblotting clearly showed that the human and Awassi sheep placentae enzymes are immunologically similar.

The human placenta transferase differs from the sheep enzyme in some aspects. The human enzyme has acidic isozymes while the sheep enzyme has a basic isozymes, moreover slight difference in the molecular weight of the subunits between the two enzymes was also observed. These differences may be due to the difference in the genetic material coded for each enzyme, and reflected on the amino acid composition of the two enzymes.