INCREASED CIRCULATORY LIFETIME OF THE ANTILEUKEMIC ENZYME L-ASPARAGINASE

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The narrow substrate specificity and high catalytic efficiency of enzymes makes them especially suitable as drugs. The enzyme L-asparaginase has been shown to induce remission in humans from asparagine dependent leukemias and its lack of cytotoxicity to bone marrow stem cells has contributed to its successful use in combination therapy (Cooney & Rosenbluth (1975)). Unfortunately rapid removal of L-asparaginase following intravenous injection and its immunogenicity reduce its effectiveness as a tumour inhibitor (Holcenberg & Roberts (1977)). Therefore an L-asparaginase derivative showing a markedly increased plasma half life would be more efficacious. This paper reports the synthesis and preliminary investigations on the circulatory properties of an L-asparaginase-dextran conjugate.

L-asparaginase (M.W. 130,000 daltons) from Erwinia carotovora has been covalently and irreversibly bound to an activated soluble dextran of molecular weight 250,000 daltons. The enzyme retained 60% of its initial activity and Sepharose 4B chromatography showed the resulting enzyme conjugate to be a homogeneous product of molecular weight approximately 1.5×10^6 daltons, corresponding to five dextran strands bound to a single enzyme molecule. The dextran strands bound to the "non-essential" lysine groups of the enzyme and titration of the number before and after conjugation indicated that between eight and ten were lost suggesting at least two per subunit were derivativized by dextran.

Two series of rabbits were intravenously dosed (300 IU Kg^{-1}) with either the native enzyme or conjugate. Blood samples taken from the marginal ear vein allowed the estimation of serum enzyme levels following injections. Both the enzyme and conjugate were rapidly distributed within ten minutes as would be expected for a high molecular weight species confined to the extracellular space. Following distribution both were eliminated by a single first order process. The conjugate exhibited a markedly increasing circulatory half life, 20-40 hrs., compared with the native enzyme, 6-10 hrs.

The mechanisms by which foreign proteins are eliminated in non-immune animals are poorly understood. The increased circulatory lifetime imparted by covalent attachment of dextran may be a general phenomenon and the binding of carbohydrate may serve to sterically protect the foreign protein from recognition by elimination processes such as the reticuloendothelial system (Sheerwood et al (1977) Marshall et al (1977)). Support for this suggestion is the lower immunogenicity that we have shown for the conjugate compared with the native enzyme (Wileman (1979)). The results suggest that the major limitations to the use of L-asparaginase as a drug, its rapid inactivation IN VIVO and the immunological consequences of repeated injections may be overcome by its immobilization with soluble dextran.

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