COVALENT BINDING OF DEXTRAN TO THE ANTI-LEUKEMIC ENZYME L-ASPARAGINASE REDUCES THE ENZYME ANTIGEN REACTIVITY

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L-asparaginase from Erwinia carotovora is used routinely in the treatment of acute lymphoblastic leukemia. Unfortunately the immunogenicity of asparaginase has proved a major limitation to its use as a tumor inhibitor (Dellinger & Miale 1976). This paper reports investigations on the antigen reactivity of a soluble dextran:asparaginase conjugate.

Erwinia carotovora asparaginase was covalently bound to activated soluble dextrans of molecular weight 10,000 (T10) and 70,000 (T70) daltons, as described previously (Elliott et al 1981). Sepharose 4B gel filtration showed the resulting conjugates to be homogenous products of molecular weight 300,000 and 1.4×10^6 daltons respectively. The relative avidity of the conjugates was determined by comparison with the native enzyme using quantitative immunoprecipitation against asparaginase specific antiserum raised in rabbits. The total enzyme activities remaining following incubation were also determined.

Enzyme activity measurements showed all three to lose catalytic activity when mixed with the antiserum. The residual activities observed in antibody excess were, however, markedly different. The T70 conjugate retained 50% of its activity, a value greater than that observed for the T10 conjugate (20%) and the native enzyme (5%) (Fig. 1). The native enzyme and T10 conjugate readily precipitated when incubated with the antiserum. Protein analysis of the precipitates formed in antibody excess suggested that a maximum of 22 molecules of IgG could bind to a single molecule of asparaginase while similar calculations indicated the binding of only seven IgG to the T10 conjugate. The T70 conjugate failed to precipitate when mixed with the antiserum.

The reduced susceptibility to inactivation and inhibition of immunoprecipitation observed for the conjugates suggests that the attached dextran reduces the interaction between the antibody and the enzyme and also inhibits immune lattice formation. Furthermore, these results suggest that the degree of enzyme protection may be increased by increasing the molecular weight of the dextran attached. The above evidence for reduced antigen reactivity may be of a clinical significance since hypersensitivity reactions particularly life threatening anaphylaxis indicates the cessation of asparaginase therapy in up to 10% of patients (Oettgen et al 1970); Dellinger & Miale 1976). It is possible, therefore, that these conjugates may have therapeutic application.

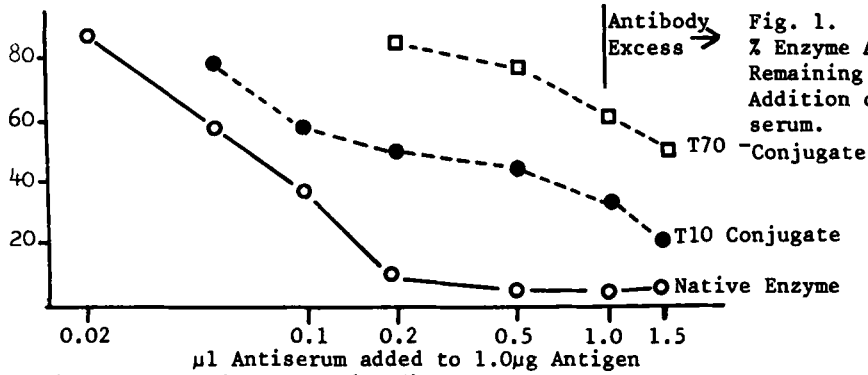

Fig. 1. % Enzyme Activity Remaining After Addition of Antiserum.

Dellinger C.T., Miale T.D. (1976). Cancer 38 : 1843-1846.
Elliott P.N.C. et al (1981) Br. J. Pharmac. (Proceedings in press).
Oettgen H.F. et al (1970) Cancer 25 : 253 - 278.

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