

## Protein bioconjugates: the novel delivery system for L-asparaginase

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**Background and Aims:** One of the primary drugs used in the treatment of Childhood acute lymphoblastic leukemia is L-asparaginase. Most human tissues can self-synthesize L-asparagine while leukemic cells are unable to synthesize L-asparagine. Lipid conjugation strategy can present a good delivery system with high efficacy for L-asparaginase. L-asparaginase conjugation with lipid can increase the half life and increase the stability of L-asparaginase to proteolytic enzymes and finally can enhance the enzyme efficacy in longer time.

**Methods:** The amino groups of L-asparaginase were reacted with the carboxylic group of different lipids (behenic, lauric or palmitic acid) according to carbodiimide chemistry. Initial reactions were carried out (at RT, for 24 h) at lipid:protein molar ratios of 6:1, 12.5:1, 25:1, 50:1, 100:1 and 200:1. Impurities and non reacted lipids were removed by dialysis membrane (12-14 kDa MW cut-off) against distilled water at RT for 24 h. The degree of conjugation and enzyme activity was quantified using modified ninhydrin calorimetric method and Nesslerization, respectively. The protein content was determined using Bradford-method with bovine serum albumin and L-asparaginase as the standards. Molecular weight of the products was confirmed via SDS-PAGE. Micelle formation of the bioconjugate alone or in combination with surfactant (s) was evaluated according to the CMC, size and zeta potential.

**Results:** The ratios of 12.5-100:1 of three lipid bioconjugates had the accepted level of activity, conjugation degree and protein content. The lipid bioconjugates had broad molecular weight bands, higher than the native enzyme. The bioconjugates had negative charge in nanometer scale while the smaller ones belong to the higher chain length of the lipid.

**Conclusions:** These bioconjugates could be a good candidate for the delivery of L-asparaginase. Recovery of the descended activity and decreasing the size of the micelle is achieved by the presence of surfactant, especially with poloxamer and lecithin.

**Keywords:** L-asparaginase; Bioconjugation; Lipid-Protein drug delivery