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Preparation and characterization of an IgG-dehydroepiandrosterone conjugate

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Dehydroepiandrosterone (DHEA) (MW 288.43Da) is the major secretory steroid product of the adrenal gland and is now marketed as a drug[1]. DHEA is highly insoluble in water and its administration enterally, and especially parenterally, is difficult to achieve. Here we have solubilised DHEA (used as a model drug) by conjugating it to IgG immunoglobulin. DHEA-IgG conjugates could serve as a mean to target drugs to specific tissues expressing appropriate antigens.

Immunoglobulin G (IgG) type antibodies (MW 150 kDa) consist principally of two identical heavy chains (55 kDa) and two light chains (25 kDa) which are held together by disulphide bonds. IgG possesses a number of functional groups suitable for conjugation. The most accessible sites on IgG for drug attachment are the ϵ-amino groups of lysine residues (approximately 80 residues per IgG molecule).

Schiff base formation and reductive amination were used to conjugate a ketone-containing molecule (DHEA) with an amine-containing molecule (IgG) resulting in a zero-length cross-link where no additional spacer atoms are introduced between the molecules. The keto group at carbon 17 of DHEA can react with the ϵ-amino groups of lysine residues on IgG to form Schiff bases. This is enhanced at alkaline pH values (pH 7-10). A reducing agent such as sodium cyanoborohydride was used to convert specifically the Schiff base bond into an alkylamine linkage[2].

[^{3}H] DHEA was solubilised in DMF prior to interaction with IgG in 0.75 M K_2HPO_4(pH 9) (buffer)[3]. The approximate ratio of solvent used to solubilise both IgG and DHEA is 1 ml of DMF in 5 ml of buffer . The percentage of [^{3}H] DHEA bound to IgG following the reaction was determined by precipitating the protein with trichloroacetic acid (TCA)(20% final concentration). IgG and DHEA at molar ratio of 1:50, 1:100, and 1:200 were used in the coupling reaction in the presence of $NaCNBH_3$. A molar ratio of 1:100 of IgG:DHEA yielded solubilised product with the highest percentage of conjugation (62%) in the solvent mixture after incubation for 24 h. Only 6.0% of [^{3}H] DHEA was conjugated to IgG in the absence of $NaCNBH_3$. indicating that $NaCNBH_3$ is necessary to push the reaction forward to form stable linkage.

The extent of DHEA conjugation to IgG was also measured by capillary zone electrophoresis (CE) using a mobile phase of 50mM sodium borate (buffered to pH 9.4) containing 30% (v/v) acetronitrile and 6M urea[4]. The separation was achieved at 30 kV and monitored UV absorbance at 210 nm. The migrating time of nature IgG was found to differ from IgG conjugate that of the latter's charge status resulting from the attachment of the drug. Thus, migration time were 5.6 (native IgG) and 5.9 (conjugated) mins. Working in progress to use such conjugate in drug targeting in vivo.

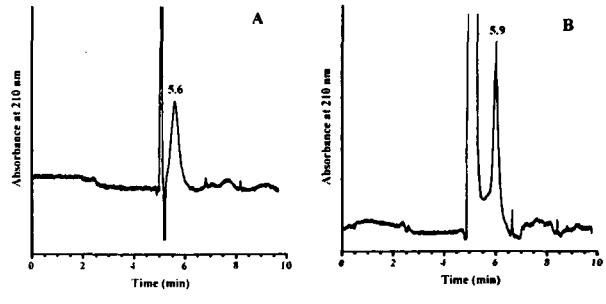

Figure 1. Capillary zone electrophoresis of IgG and IgG-DHEA conjugate

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