Antibody Engineering-based Approach for Hapten Immunometric Assays with High Sensitivity

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Immunoassays are essential in clinical chemistry to determine trace amounts of bioactive molecules. Small molecules (haptens) such as synthetic drugs, steroids and oligopeptides are now exclusively measured by competitive immunoassays. However, the subfemtomole range measurement of haptens has been very difficult, as the sensitivity of the competitive immunoassays is essentially limited by the affinity of the anti-hapten antibody that hardly reaches the range of 10^{11} (M^{-1}) as the affinity constant (K_a). Although the sandwich immunoassay, that is a typical immunometric (noncompetitive) assay format, enables even subattomole range measurements of macromolecules, this procedure cannot directly be applied to haptens due to their low molecular mass.

To overcome such limitations, we established an immunometric assay format that can detect attomole-range hapten molecules based on antibody engineering technology. 11-Deoxycortisol (11-DC; M_r 346.5), a corticosteroid serving a diagnostic index for pituitary-adrenal function, was selected as a model target hapten. A fusion of a single-chain Fv fragment (scFv) specific for 11-DC and alkaline phosphatase (ALP) was generated for use as an enzyme-labeled antibody. After binding reaction of 11-DC and fixed amounts of the fusion protein (scFv-ALP), the unbound scFv-ALP was removed by the aid of two anti-idiotype monoclonal antibodies recognizing the scFv paratope or framework. Fluorometric measurement of ALP activity from the immune complexes of scFv-ALP and 11-DC afforded an extremely low detection limit (20 amol/assay) and practical specificity. We will also show our recent study on the isolation of specific scFvs to inclusion complexes from a mutant scFv library of cyclodextrins and some haptens that will be useful for constructing high throughput hapten immunometric assays.