Sensitive Noncompetitive Measurement of Small Molecules by Open Sandwich Immunoassay

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Proteins having multiple epitopes can be easily measured by sandwich ELISA employing two kinds of antibodies, which permits high sensitivity as well as wide working range of more than three orders of magnitude. On the other hand, so called monovalent antigens with less than 1000 in MW are not susceptible of sandwich assays due to their small size, and have almost always been measured by competitive assays. However, while competitive assay needs only one antibody, due to the principle of ratiometric measurement, optimization of the reaction condition is inevitable in order to attain suitable sensitivity and working range, which are often inferior than those of sandwich assays.

As an alternative immunoassay for small antigens, here we propose a noncompetitive 'Open Sandwich Immunoassay', which is based on the principle of stabilization of antibody variable region Fv upon binding with antigen. By ELISA detecting labeled V_H fragment bound to immobilized V_L in the presence of sample in microplate wells, various small molecules with MW around 200-300 were found to be measured with superior detection limit and working range than those attained with corresponding competitive assays. The results imply a common antigen recognition mode of anti-hapten antibody, and also wide applicability of the assay to the sensitive and handy analysis of low molecular weight substances in the areas such as environmental analysis and clinical diagnostics.