## S32-4 Immunoassay utilizing open sandwitch principle and application for preparation of antibodies

◯Masayuki KAWAKAMI[1], Hiroshi UEDA[2], Masaki IHARA[2]

[1]Fuji Film Life Sci. Res. Lab., [2]Univ. Tokyo Grad. Sch. Eng.

While proteins can usually be measured with sandwich immunoassays, small molecules with less than 1000 in molecular weight are not considered amenable to the assay, because of the dimension of the molecule. As an alternative, we are attempting noncompetitive detection of small molecules by open sandwich immunoassay (OS-IA) utilizing the antigen-induced enhancement of antibody $V_H/V_L$ interaction.

Taking fragments of human osteocalcin (BGP) as model peptides, OS immunoassay was performed using the cloned $V_H$ and $V_L$ fragments recognizing BGP. OS-IA in both microplate and microfluidics showed superior detection limits as well as wider working ranges than those of competitive assay, which fulfilled the need for clinical diagnosis. We also established a sensitive electrochemical OS-IA using a field effect transistor.

To enlarge the scope of these assays, we further developed rapid preparation methods of antibody fragments ($V_H$, $V_L$) suitable for OS-IA, based on the phage display technology. In addition to Split-Fv system that was already developed by Ueda et al, we established new screening methods that enable rapid identification of antibody fragments by enzymatic conversion of scFv or Fab, which retain high affinity to antigen, to the suitable fragments for OS-IA. Using these fragments, the interaction between $V_H$ and $V_L$ could be readily evaluated. This simple approach with a short preparation time may prove useful to obtain many antibodies suitable for OS -IA.