Bioimprinting as a receptor for detection of kwakhurin

Abstract

Bioimprinting was performed against ovalbumin (OVA) to confer its binding cavities for kwakhurin (Kwa), an isoflavonoid, produced solely by Pueraria candollei var. mirifica (P. candollei). The characterization of bioimprinted-OVA (biOVA), evaluated by an enzyme-linked immunosorbent assay (ELISA), revealed that it functioned as a specific receptor for Kwa. Using biOVA, two systems, i.e., an indirect competitive ELISA (icELISA) and the even simpler and more rapid competitive enzyme-linked bioimprinted-protein assay (cELBIA), were developed as novel techniques for the quantitative analysis of Kwa in P. candollei and its related products. The two analysis methods were found to have limits of detection (LOD) of 4.0 and 2.5 μg/mL, respectively. The high reliability of the developed icELISA and cELBIA using biOVA was also demonstrated by various validation analyses. Subsequently, bioimprinting was performed using eight other proteins to investigate them as candidate scaffolds for the generation of binding cavities for Kwa. Interestingly, two bioimprinted-IgG monoclonal antibodies (biMAbs) recognized Kwa, but their original binding affinity to hapten was lost. That is, the MAbs obtained a new binding ability to Kwa in exchange for their original binding affinity, raising the possibility that biMAb could be alternatively used as a probe for the quantitative analysis of Kwa as well as biOVA. This is the first report of small molecules recognition by MAbs used as proteins for bioimprinting.