

Identification of a Binding Motif of Epigallocatechin Gallate Using a Phage Display Random Peptide Library

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Epigallocatechin gallate (EGCG) is known as a component of green tea. It has been shown that EGCG has various activities including anticancer and antiviral activities. However its cellular target molecules and mechanism of action still remain obscure. To identify the cellular targets of EGCG, I sought the binding motif of EGCG using a phage display random peptide library (PD-RPL). PD-RPL is widely used for mapping of epitopes of monoclonal and polyclonal antibody, identifying of peptide ligands, and mapping substrate sites for proteases and kinases. Appropriate length of peptide with random sequence is expressed as a fusion protein with N-terminal end of pIII (minor coat protein) of M13 filamentous phage. Several rounds of panning of PD-RPL against target molecule result in increase of phage titer, that means the amplification of target molecule binding peptide-expressing phages. Screening of phage clones with binding activity followed by the sequencing of random peptide-coding region can easily elucidate the binding motif of target molecule. Searching of database for proteins having the identical or similar sequence of binding motif may help identification of target molecules.

In this study, EGCG derivative kindly provided by Professor Toshiyuki Kan at University of Shizuoka was used as a target of heptapeptide PD-RPL. EGCG derivative-Affigel-10 (Bio-Rad) matrix was prepared by coupling of primary amine of EGCG derivative and *N*-hydroxysuccinimidyl ester of Affigel-10. Coupling was monitored by UV absorption derived from aromatic structure of EGCG. Decreased UV absorption measured after the completion of reaction implicates the successful conjugation of EGCG-derivative and Affigel-10. Panning of heptapeptide PD-RPL against EGCG-Affigen-10 is now in progress.