Detection of *E. coli* O157:H7 Using Electrochemical-Chemical-Chemical Redox Cycling

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Signal amplification by enzyme labels in enzyme-linked immunosorbent assays (ELISAs) is not sufficient for detecting a low number of bacterial pathogens. An advantageous combination of an enzyme product [for fast electrochemical−chemical−chemical (ECC) redox cycling that involves the product] and an enzyme substrate (for slow side reactions and ECC redox cycling that involve the substrate) has been developed to obtain a low detection limit for *E. coli* O157:H7 in an electrochemical ELISA that employs redox cycling. In our search for an alkaline phosphatase substrate/product couple that is better than the most common couple of 4-aminophenyl phosphate (APP)/4-aminophenol (AP), we compared five couples: APP/AP, hydroquinone diphosphate (HQDP)/hydroquinone (HQ), L-ascorbic acid 2-phosphate/L-ascorbic acid, 4-amino-1-naphthyl phosphate/4-amino-1-naphthol, and 1-naphthyl phosphate/1-naphthol. In particular, we examined signal-to-background ratios in ECC redox cycling using $Ru(NH_3)_6^{3+}$ and tris(2-carboxyethyl) phosphine as an oxidant and a reductant, respectively. The ECC redox cycling that involves HQ is faster than the cycling that involves AP, whereas the side reactions and ECC redox cycling that involve HQDP are negligible compared to the APP case. These results seem to be due to the fact that the formal potential of HQ is lower than that of AP and that the formal potential of HQDP is higher than that of APP. Enzymatic amplification plus ECC redox cycling based on a HQDP/HQ couple allows us to detect E. coli O157:H7 in a wide range of concentrations from 10^3 to 10^8 colony-forming units/mL.

References

[1] Akanda, M. R; Tamilavan, V; Seonhwa, P; Jo. K; Hyun, M; Yang, H, Anal. Chem, **85** (2013) 1631-1636.