Magnesium Ion-Dependent Discriminating System Comprising DNA

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It has been found that small mutations of certain genes are the definitive origin of many heritable disorders and cancers, thanks to striking development of recent molecular biology. Such new findings have highlighted the importance of gene mutation assays based on the difference of DNA base sequences in diagnostic or medical field. Capillary electrophoresis can be a good candidate for an ideal method on such gene analysis, because the methods can be performed with trace amount of samples, high resolution and shorter running time. We describe here an effect of oligonucleotide, which was introduced onto capillary inner surface, on the recognition of an overall sequence of sample DNA fragments as an affinity ligand. This method possesses real potential for the gene mutation analysis.

We studied the affinity capillary electrophoresis using $(dT)_{12}$ as immobilized affinity ligand. $(dT)_{12}$ was immobilized onto the inner surface of silica capillary. If magnesium ion was not added, every oligonucleotide was detected in very similar manner to the case of simple polyacrylamide coated capillary. In contrast, the addition of $MgCl_2$ brought about a dramatic effect in the detection of $(dA)_{12}$ which is complementary partner of the immobilized $(dT)_{12}$. The peak of $(dA)_{12}$ was gradually broadened and finally disappeared with increasing concentration of Mg^{2+}, while peaks on all other mismatched nucleotides were kept sharp in shapes despite the only one base mismatched to $(dT)_{12}$. The peak disappearance of $(dA)_{12}$ in high Mg^{2+} concentration would be due to the enhancement of affinity of $(dA)_{12}$ with the immobilized $(dT)_{12}$. Mg^{2+} is known to stabilize a DNA duplex, making tight complex with anionic phosphates in the DNA strand.

To generalize this affinity capillary electrophoresis, we applied this system to an analysis of K-ras sequence and its one base mutant. Ras protein, which is a family of small G-proten, is very important in a cellular signal transduction for cell proliferation or differentiation.. It is also reported that a point mutation at a certain base on codon 12 in K-ras gene is one of the major origin of cancer. Thus, anti-sense sequence of c-K-ras codon 10-13 (5'-GCCACCAGCTCC-3') was immobilized onto a capillary inner surface. The results were very similar to those of analyses of $(dA)_{12}$ and its one base mutant sequences using the $(dT)_{12}$ immobilized capillary. Detection peak for c-K-ras codon 10-13 (5'-GGAGCTGGC-3'), which is complementary partner of the affinity ligand, again gradually disappeared as increasing Mg^{2+} concentration, while the one base mutant of the sequence (5'-GGAGCTAGTGGC-3') was still detected in the presence of Mg^{2+} at a concentration of 250 μM..

In this system, only the detection peak for the DNA fragment, which has perfect complementary sequence to the immobilized ligand, should disappear with controlling the Mg^{2+} concentration. Thus, an existence of mutation of certain gene would be simply determined by the detection peak existence under an appropriate Mg^{2+} concentration.