A novel regulation system of gene expression responding to protease signal

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ABSTRACT
A cationic polymer, in which a cleavage site with Caspase 3 (CPCC) was introduced, was synthesized. Then an interaction between the polymer and a DNA was investigated with gel electrophoresis in the presence or absence of Caspase 3. When the CPCC polymer was added to the DNA solution, the migration of the DNA was suppressed with the increase of the addition of the polymer in the gel electrophoresis. However, this delay was cancelled with the addition of Caspase 3. These results suggested that this synthesized polymer interacted with the DNA, and the side chain cleavage of this polymer by Caspase 3 reduced the interaction between the polymer and the DNA. Such system would be useful for the design of an artificial gene regulation system responding to cellular apoptotic signal.

In eukaryotic cells, DNA is encapsulated into nucleus forming nucleosome with cationic histone. This interaction causes inhibition of the access of general transcriptional factors to suppress the gene transcription mainly through electrostatic interaction. When the gene transcription is activated, the lysine residues on the histone is acetylated by histone acetyltransferase (HAT), so that electrostatic interaction is weaken due to the cancellation of the cationic charges on the histone to allow the access of general transcription factors to the DNA strand¹². In this paper, we attempted to design a new gene regulation system responding to intracellular Caspase signal mimicking the function of this DNA-histone construction. For the design of such system, we adopted Caspase 3 signal as a regulation of gene function instead of histone acetyltransferase. Caspase 3 is one of the cysteine protease and is a key molecule of cellular apoptotic signal³. This enzyme recognizes DEVD sequence and cleavage the peptide bond at the aspartic acid in carboxy-terminal side⁴.

Thus we designed a new cationic polymer possessing cleaving sites for Caspase 3 (CPCC). This graft-type polymer has pendant-peptide moieties constructing with cleavage sequences (DEVD) for Caspase 3 and cationic sequences (KKKKKK). Since these branched peptide should have +2 charge in physiological pH, the polymer would be able to interact with DNA by electrostatic interaction to suppress the gene transcription. Cleavage of

![Figure 1. Schematic illustration of transcriptional regulation by CPCC](https://academic.oup.com/nass/article-abstract/1/1/149/1070313/149100313?display=b NEVER USE THIS URL AGAIN)
branched peptide on CPCC polymer chain will causes dramatically change of the polymer net charge, because the charge of the pendant peptide changed from +2 to −4. As a result, CPCC will become repulsive rather than attractive against DNA during the reaction with Caspase 3. This may dissociate the DNA from CPCC polymer to active transcription (Figure 1).

Figure 2 shows synthetic scheme of CPCC. N-Methacryloyl-alanine was easily obtained by the reaction of alanine with methacryloyl chloride. Then peptide monomer was synthesized on automatic peptide synthesizer (Applied Biosystems 431 A) using a Fmoc-Lys Alko Resin as a solid support and Fmoc strategy for amino acid assembly. Finally, the methacryloyl-peptide and acrylamide were copolymerized using ammoniumpersulfate (APS) and \(N,N,N',N'\)-tetramethylethylenediamine (TMEDA) as a redox initiator couple to obtain CPCC polymer (37%). The peptide content was estimated by the result of elemental analysis to 6.18 mol%.

Using this polymer, the interaction between CPCC and DNA (1234 bp, Linear DNA) was investigated in the presence or absence of Caspase 3. When the CPCC polymer was added to the DNA solution with the charge ratio (CPCC cation / DNA anion ratio) of 1, the migration of the DNA was suppressed a little (Lane 3). On the other hand, the migration was dramatically decreased when the charge ratio was 5 (Lane 4). These results indicate that CPCC interacts with DNA. Then, Caspase 3 was added to the CPCC-DNA complex solution. After incubation at 37 °C for 90 min, the migration of DNA in the CPCC-DNA complex with the charge ratio of 1 was recovered to the same position for the free DNA (Lane 5, 6). In the case of the complex with the charge ratio of 5, DNA-migration was also recovered to the nearly same position for the free DNA after the Caspase 3 treatment (Lane 7). These results suggested that the pendant peptide of CPCC was recognized by Caspase 3 and was cleaved causing the dissociation of the DNA from the CPCC-DNA complex.

We described here a synthesis and characterization of CPCC. This polymer was interacted with DNA probably with an electrostatic interaction. Moreover, CPCC-DNA interaction was cancelled with Caspase 3 signal. We expected that this polymer was potentially useful for a gene regulation system. Now we applying this CPCC-DNA complex to gene expression system. The result will be reported in due course.

REFERENCES