

A NOVEL VECTOR OF 2-DIETHYLAMINOETHYL(DEAE)-DEXTRAN-MMA GRAFT COPOLYMER FOR NON-VIRAL GENE DELIVERY

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Background: Non-viral gene delivery vectors may have significant advantages comparison with their viral counterparts. In this study, we tried to achieve a development of non-viral DEAE-dextran-MMA graft copolymer (DDMC) expression system for a high transfection or a low cytotoxicity of mammalian cells to be possible to autoclave.

Methods: DDMC was obtained by graft-polymerizing MMA onto DEAE-dextran, in water using ceric ammonium nitrate(CAN) to obtain a stable latex of DDMC, which is very effective as a non-viral gene delivery vector. Characterizations of a reaction complex between DDMC and DNA were carried out by precipitation time, infrared absorption spectrum, and measurements of DNase degradation by measuring the absorbance for TB isolated from DNA in the water with a spectrophotometer. For transfection of HEK293cell by DDMC/DNA, A pCAGGS/LacZ, which expresses β -galactosidase at eukaryotic cells, was inserted under CAG promoter of a plasmid, pCAGGS.

Results: From the viewpoint of thermodynamics for the complex reaction between DNA and DDMC, the Gibbs free energy change at complex reaction $\Delta G[\text{Jg}^{-1}]$ can be written as $\Delta G = \Delta H - T\Delta S$ where $\Delta H[\text{Jg}^{-1}]$ is the enthalpy change and $\Delta S[\text{Jg}^{-1}\text{K}^{-1}]$ is the entropy change at the complex reaction by DNA/ DDMC. The Gibbs free energy change $\Delta G[\text{Jg}^{-1}]$ at complex reaction should be minus for its large plus entropy change(ΔS) by a hydrophobic force from hydrophobic domains of poly(MMA) in DDMC, because the enthalpy change $\Delta H[\text{Jg}^{-1}]$ is very small. It became to easy for DDMC to form Poly-ion complex(PIC) between DNA and DDMC by a hydrophobic force of graft poly(MMA) depending on its large plus entropy change(ΔS). DDMC has been confirmed having a very strong DNase protection facility by DNase degradation test. Transfection activity was determined using the β -Galactosidase Assay and a higher value of 336 times or more was confirmed for DDMC samples than one of the starting DEAE-dextran hydrochloride. The resulted DDMC having amphiphilic domain, to form a polymer micelle, should become a stable latex with a hydrophilic-hydrophobic micro-separated-domain. The complex by DDMC and plasmid DNA may be formed on the spherical structure of the amphiphilic micro-separated-domain of DDMC and have a good affinity to cell membrane. The IR absorption spectrum shift to a high energy direction at around 3450cm^{-1} owing to the complexes between DNA and DDMC may mean to form more compact structures not only by a coulomb force between the phosphoric acid of DNA and the diethyl-amino-ethyl(DEAE) group of DEAE-dextran copolymer but also by a force from multi-inter-molecule hydrogen bond in backbone polymer DEAE-dextran and a hydrophobic force from graft poly(MMA) in DDMC.

Conclusions: DDMC has reasonably a very high DNase protection facility, a low cytotoxicity, and a high transfection efficiency. The high efficiency of DDMC autoclaved can make it a valuable tool for gene delivery in vivo.