

論文内容の要旨

論文題目 Thermodynamic research on the DNA condensation induced by the PEG-PLL block copolymer (ブロック共重合体 P E G - P L L によって誘起される DNA の凝縮に関する熱力学的研究)

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(本文) Biological systems typically confront a formidable problem of DNA condensation: the necessity, for example, of fitting approximately 1.5 m of human diploid DNA into a 20- μ m-diameter cell and into a still smaller nucleus throughout most of the cell cycle. In eukaryotes, nucleosomes partially solve this problem; each nucleosome compacts the DNA associated with it 6- or 7-fold. Human DNA, however, must be compacted approximately 75,000-fold; this obviously involves more than nucleosome formation. DNA also exists in other compact structures such as viruses and prokaryotic nucleoids that lack nucleosomes. In the T2 or T4 bacteriophage, 60 μ m of DNA must be compacted 600-fold to fit into a virion whose largest dimension is 0.10 μ m; and in *E. coli*, 1.2 mm of DNA fits into a cell no more than 2 μ m long.

The various biological phenomena in cell such as proliferation, transcription are thought to be performed in the specific structure of DNA condensed in nucleus. Human body is composed of enormous numbers of cells which are all originated from only one cell through cell division and differentiation. Although the process and mechanism of differentiation is not clearly understood, it is thought to be realized by the combination of specific kind of genes to be selected to function as a specific function of operation system in computer is realized by using only several partial programs not all in it, as supported by observation of chromosomal DNA in different kinds of cells. By what method the selection of specific kind of genes is performed is not obvious. The elucidation of the mechanism may be realized by the structural investigation of DNA condensed in cell. When a system in human body is damaged, cells in the system are proliferated and the function of the system is restored, which is thought to be realized by response of those cells to any signal from the damaged system, in more details, several specific proteins are related to the process and interact with any specific genes in the chromosome to make them function although the mechanisms of restoration are not yet fully clarified. Therefore the elucidation not only the structure of DNA condensed in nucleus of living cell but also its relationship to their biological functions is important for elucidation of biological mechanism of human body. And it is also important for developing a drug for a treatment human of various diseases including a genetic disorder. In these respects, thermal analysis leading to structural analysis of DNA may be important for the clarification of biological process and therapy of human diseases together with electronic scattering, atomic force scattering or x-ray scattering.

In the respect of gene therapy, also, thermal analysis can be useful. A reason that gene vector is required for gene therapy is to protect the gene for therapy from various nucleases in human body. Presumably DNA is protected by gene vector to block the access of those nucleases to DNA. Although DNA can be protected by gene vector quite effectively, it yet may be not sufficient for practical use. To elucidate the complex structure, that is, the relative binding position of gene vector to DNA, can be lead to clarify the mechanism of protection of DNA from nucleases. If it is clarified, it leads to develop a more effectively protecting gene vector from nuclease.

To clarify the structure of the condensed DNA many theoretical and experimental studies have been done. Thus as a force inducing the condensation to

elongated DNA, several kind of forces have been suggested, including fluctuation of counterions, charge ordering (or charge reorganization), release of low molecular counterions, release of water molecules, delocalization of counterion. But they are not still controversial. If the thermodynamic parameters can be obtained using calorimetry, their theoretical condensation mechanisms may be evaluated. However, formation of precipitates followed by DNA condensation makes difficult the evaluation of DNA condensation. To make the evaluation possible a new condensing agent suppressing the condensed DNA not to form precipitates is required.

Our group has synthesized condensing agents, for example, poly(ethylene glycol)-block-polylysine (PEG-PLL), able to suppress formation of precipitation to condensed DNA which was developed by connect covalently an end of a linear hydrophilic poly(ethylene glycol) (PEG) to an end of a cationic polylysine (PLL). Considering the fact that DNA condensed by PEG-PLL, it is indicated that PEG chain surrounds the condensed DNA by cationic PLL segment, that is, a micelle structure. Using these block copolymers, DNA condensation without being interfering by forming precipitates can be investigated.

Actually these condensing agents were developed for efficient gene vector for gene therapy. For a gene vector to be useful for gene therapy, first of all, the problem of formation of precipitates has to be overcome. Although, to overcome the problem, many researches have been performed by many researchers also, our result is one of the most successful developments for gene therapy. These block copolymers are supported by several experimental results as a promising gene vector.

Here was investigated the effect of the secondary condensation on the ITC curve by comparing PLL and PEG-PLL binding to DNA using ITC. And also the transmittance was measured under the same condition with the ITC experiment. Two distinctive endothermic binding stage of PLL and PEG-PLL to DNA were observed, one attributed to the binding of them without DNA condensation, the other that with DNA condensation. Their ITC curves were not so much different with each other at low salt concentration (10 mM), but they showed great difference between them with increase in salt concentration. That is, the heat accompanied by the binding of PLL to DNA decreases to zero at the lower molar ratio of lysine to nucleotide, while in case of PEG-PLL the such molar ratio did not decrease so much as PLL. Because this result seems to mean that the amount of PLL bound to DNA decrease remarkably with increase in salt concentration, the amount of free PLL in the solution was investigated by titrating by the poly(ethylene glycol)- poly(aspartic acid) (PEG-P(Asp)) the free PLL in the supernatant obtained after centrifugation. Based on that result, it is cleared that the amount of PLL bound to DNA did not decrease even if high salt concentration. These results indicate that the difference between PLL and PEG-PLL binding to DNA is attributed to the interference of the heat accompanied by the secondary condensation to the heat accompanied by binding of PLL to DNA. The dependence of the molar ratio at which the endothermic heat disappears on salt concentration is also consistent with the result of the transmittance.

The binding of a variety of poly(ethylene glycol)-block-polylysine (PEG-PLL) to DNA in various salt concentration was investigated thermodynamically using isothermal titration calorimetry (ITC). Thermodynamic parameters of two distinctive endothermic binding processes were obtained by curve fitting of each titration data; the first parameters attributed to the binding process of PEG-PLL to DNA without DNA condensation, and the second to the process with DNA condensation. The titration result exhibited that PEG-PLL having high degree of polymerization (DP) of PLL is more effective to form stable complex. Based on the thermodynamic parameters, it became clear that the binding of PEG-PLL to DNA is accompanied not only by the large increase in entropy, which is thought to be attributed to the release of low molecular

counterions or water molecules bound to in the vicinity of DNA and PEG-PLL, but also by the large decrease of free energy which is thought to be attributed to the electrostatic interaction of PEG-PLL with DNA.

And also we discussed about the structure of condensed DNA induced by PEG-PLL based on the thermodynamic parameter obtained here. These thermodynamic observations may lead to the elucidation of mechanism of PEG-PLL protecting DNA from the enzymes such as endonuclease in serum for efficient gene delivery or the mechanism of reproduction and transcriptional regulation.

Actually for thermodynamic parameters to be obtained from the ITC curve, it should be fitted to any arbitrary bind model. However the conventional fitting models may not be suitable for the polycation binding to DNA followed by conformational change in DNA. Thus originally new fitting model was suggested. Data shown here was obtained using the model.

PLL binding to DNA was accompanied not only by increase in entropy but also decrease in free energy, based on the thermodynamic parameters. Increase in entropy may be attributed to release of low molecular counterion, release of water or delocalization of counterions. Decrease in free energy may be attributed to the electrostatic interaction between DNA phosphates and PLL. Similarly with the case of the PLL binding to DNA, DNA condensation was indicated that it was accompanied not only by release of counterions or release of water or delocalization of counterions but also by electrostatic interaction between them, considering the thermodynamic parameters which shows not only large increase in entropy but also large decrease in free energy.

In future these data and the method of thermal analysis may be effective not only for evaluating the structure of genomic DNA condensed in living cell and its relation on the function but also for developing more stable gene vector against nuclease by clarifying the mechanism to protect DNA from nuclease.