論文の内容の要旨

Effect of repeating aminoethylene units in the side chain of N-substituted polyaspartamides on pDNA and mRNA delivery

(ポリアスパルタミド側鎖に導入した繰り返しアミノエチレン構造の pDNA 及び mRNA デリバリー特性に及ぼす効果)

氏名　内田　寛邦

Polyion complexes formed from polycations and negatively charged nucleic acids, such as plasmid DNA (pDNA) and messenger RNA (mRNA), have been recognized as promising carriers for gene-related molecular therapy. The design of the complexes, termed as polyplexes, is still a critical issue to achieve efficient and safe introduction of nucleic acid compounds into target cells. Efficient endosomal escape plays a key role to obtain efficient transgene expressions. There is a previous report that a polyaspartamide derivative with two repeating aminoethylene units in the side chain, poly{*N*-[*N*'-(2-aminoethyl)-2-aminoethyl]aspartamide} (PAsp(DET)), showed high transfection efficiency with low cytotoxicity. The detailed investigations revealed that the capacity of pH-sensitive membrane destabilization contributed to the efficient endosomal escape of polyplexes formed from PAsp(DET). These studies motivated the author to further investigate the relationship between the protonation behavior and biological properties of the N-substituted polyaspartamides possessing the different number of repeating aminoethylene units in the side chain.

A series of the N-substituted polyaspartamides possessing the different number of the repeating aminoethylene units in the side chain were synthesized by the aminolysis reaction of poly(β-benzyl-L-aspartate) (PBLA) with ethylenediamine (EDA), diethylenetriamine (DET), triethylenetetramine (TET), and tetraethylenepentamine (TEP) to obtain poly[*N*-(2-aminoethyl)aspartamide] (PAsp(EDA)), PAsp(DET), poly(*N*-{*N*'-[*N*''-(2-aminoethyl)-2-aminoethyl]-2-aminoethyl}aspartamide) (PAsp(TET)), and poly[*N*-(*N*'-{*N*''-[*N*'''-(2-aminoethyl)-2-aminoethyl]-2-aminoethyl}-2-aminoethyl)aspartamide] (PAsp(TEP)), respectively. The quantitative aminolysis of the side chain was confirmed by NMR. The obtained polymers were characterized by the potentiometric titration to estimate their protonated states. The pronation degree and p*K*a of the N-substituted polyaspartamides were calculated for each polymer. The detailed protonated structures were estimated by a computer simulation (the SPARC on-line calculator http://ibmlc2.chem.uga.edu/sparc/) of low molecular model compounds. The protonation degrees

calculated by the computer simulation of model compounds were correlated with those by the potentiometric titration of polyaspartamides, suggesting that the calculated protonated structures of model compounds were reasonable to explain the protonated states of the repeating aminoethylene units in the side chain of N-substituted polyaspartamides.

Those polyaspartamides were applied to the pDNA delivery against a human hepatoma (Huh-7) cells. As a result, a distinctive odd-even effect of repeating number of aminoethylene units was observed on the endosomal escape and the transfection efficiency of the polyplexes constructed from these polyaspartamides, as the polyaspartamides possessing the even number of repeating aminoethylene units (PA-E) allowed more efficient endosomal escape and transfection efficiency of the polyplexes than those possessing the odd number of repeating aminoethylene units (PA-O). This odd-even effect was correlated with the membrane destabilization activity of the polyaspartamides. Indeed, PA-Es showed the stronger cellular membrane destabilization activity selectively at acidic endosomal pH than PA-Os. Eventually, it was concluded that the strong membrane destabilization is assumed to be induced by the formation of di-protonated structure in 1,2-diaminoethane units (-NH_2^+-CH_2-CH_2-NH_2^+-) in PA-E side chains at acidic pH.

Next, the N-substituted polyaspartamides were applied to mRNA delivery to Huh-7 cells. The efficiencies for protein expressions from mRNA polyplexes varied depending on the incubation time of the polyplexes in the culture medium. At the early time-point within several hours, PA-E polyplexes showed higher transfection efficiencies than PA-O polyplexes, which corresponding to the order of the expressions from pDNA polyplexes. In contrast, after one or more days of transfection, polyplexes formed from PAsp(TET) showed the highest transfection efficiency, followed by those from PAsp(TEP), PAsp(EDA), and PAsp(DET). The endosomal escaping efficiencies of mRNA polyplexes showed similar tendencies to those of the pDNA polyplexes; PA-E polyplexes appeared to disperse more efficiently in entire cytoplasmic region than the PA-O polyplexes from the observation by the confocal laser scanning microscopy. These results suggest that, in the case of mRNA, the endosomal escaping ability of mRNA polyplexes played a critical role to achieve protein expressions at an early time point. At the longer time point, the resistance of mRNA polyplex against RNase is likely to be correlated with the tendency of the protein expression of mRNA polyplexes. Indeed, PA-O polyplexes showed the higher resistance against RNase than PA-E polyplexes. In the cytoplasm, the higher resistance of PA-O polyplexes to RNase contributes to their longer survival compared to PA-E polyplexes at the longer incubation, leading to the highest protein expressions of PAsp(TET) polyplexes.

In conclusion, the present study demonstrated that subtle difference in the number of repeating

aminoethylene units in the N-substituted polyaspartamide side chain induced the large difference in the biological performance of polyplexes. In pDNA delivery, polyplexes from PA-Es (PAsp(DET) and PAsp(TEP)) possessing the strong membrane destabilization ability in the endosome showed the higher transfection efficiency than those from PA-Os (PAsp(EDA) and PAsp(TET)). On the other hand, in the mRNA delivery, the polyplex stability is a critical factor for the persistent expression because of the instability of mRNA relative to pDNA. In fact, PAsp(TET) polyplexes possessing the lower endosome escape ability and higher stability against RNase than PAsp(DET) and PAsp(TEP) polyplexes showed the highest transfection efficiency at longer incubation time. These findings are useful for the development of the new carrier systems for the safe and efficient nucleic acids delivery.