Synthesis of Alkylated Poly(1-vinylimidazole) for a New pH-Sensitive DNA Carrier

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ABSTRACT

The poly(1-vinylimidazole) (PVIm) with several alkyl groups has been synthesized as a new pH-sensitive DNA carrier. The resulting alkylated PVIm (PVIm-R) was water-soluble in spite of the hydrophobic alkyl groups and the deprotonation of the imidazole groups at physiological pH. Hemolysis assay showed the PVIm-R enhanced membrane disruptive ability at endosomal pH, owing to the protonation of the imidazole groups with a pKₐ value around 6.0. Agarose gel retardation assay proved that the quaternary alkylated imidazole groups worked as DNA binding groups. The resulting PVIm-R/DNA binary complex showed significant gene expression.

INTRODUCTION

Synthetic polycations are known to be perspective agents for the non-viral gene delivery. In the process of the gene delivery, polycation/pDNA complexes have to overcome many barriers. To increase transfection efficiency, it is necessary for polycation to enhance endosomal escape.

We have already synthesized the poly(1-vinylimidazole) with several aminoethyl groups, that is, aminated poly(1-vinylimidazole) (PVIm-NH₂), to make complexes with DNA. However, PVIm-NH₂/DNA complexes were unstable because of low content (2.5 mol%) of introduced aminoethyl groups. Therefore, another polycation such as lactosylated poly(1-lysine) (PLL-Lac) was added to the PVIm-NH₂/DNA complex, resulting in the ternary complex formation. The resulting ternary complex has led to the significant gene expression in human hepatoma HepG2 cells.

In this study, to improve properties of PVIm-NH₂, we have synthesized the poly(1-vinylimidazole) with several alkyl (R) groups, that is, alkylated poly(1-vinylimidazole) (PVIm-R), to enhance interaction with DNA. The chemical structure of the PVIm-R is shown in Fig.1. The PVIm backbone is the water-soluble homopolymer possessing many imidazole groups with a pKₐ around 6.0. Namely, the PVIm possesses a large capacity for H⁺ buffering at endosomal pH. To compare the effect of alkyl chain length, we have chosen butyl (Bu) and octyl (Oc) groups as alkyl groups. Furthermore, the content of alkyl groups is controllable. The resulting butylated PVIm (PVIm-Bu) and octylated PVIm (PVIm-Oc) are expected to enhance endosome membrane fusion by the amphiphilic effect of both hydrophobic alkyl groups and water-soluble PVIm backbone.

![Fig.1. Design concept and chemical structure of alkylated poly(1-vinylimidazole) (PVIm-R). (x=3, PVIm-Bu; x=7, PVIm-Oc)](https://academic.oup.com/nass/article-abstract/52/1/677/1108820)

RESULTS AND DISCUSSION

PVIm was reacted with 1-bromobutane or 1-bromooctane for one day. The GFC profile of the resulting polymer indicated that unreacted 1-bromobutane or 1-bromooctane reagents were removed by the purification process, including dialysis. The number-average molecular weight of the isolated polymer determined by GFC was about 8.8×10⁴. ¹H NMR spectrum of the resulting polymer showed the characteristic signals of both PVIm-Bu and PVIm-Oc [δ 1.8-2.2 (methylene), 2.3-3.7 (methine), 6.4-7.2 (imidazole) ppm] and butyl or octyl [δ 0.7-0.9 ppm (terminal methyl)] moieties. From the signal ratio, the content of butylated imidazole and octylated one is estimated to be 18 mol% and 23 mol%, respectively. Thus, we have synthesized the alkylated poly(1-vinylimidazole).

It should be noted that the aqueous solution of both PVIm-Bu and PVIm-Oc as well as that of PVIm exhibited no significant turbidity above pH 6.0. Both PVIm-Bu and PVIm-Oc was therefore water soluble in spite of hydrophobic alkyl groups and the deprotonation of the imidazole groups.

To examine the interaction of the PVIm-Bu with real cell membranes, the effects on the hemolytic activity were
investigated. The PVIm-Bu caused negligible hemolysis at pH 7.4, while the hemolytic activity significantly increased at pH 6.0. These results suggest that the membrane disruptive ability of the PVIm-Bu increased at endosomal pH. It is therefore expected that the PVIm-Bu enhance the ability to escape from acidic endosomal vesicles.

Then, we examined whether the PVIm-Bu formed the polyion complex with DNA by agarose gel electrophoresis, as shown in Fig. 2. DNA was mixed with various amounts of PVIm-Bu at imidazole/phosphate ratios from 0 to 60. Almost no free DNA was observed at the imidazole/phosphate ratio of 5, which almost corresponded to the alkyl/phosphate ratio of 1. These results suggest that the PVIm-R formed the complex at the stoichiometric charge ratio DNA to introduced alkyl groups; namely, the quaternary imidazole groups worked as DNA binding groups. At pH 6.0, the free DNA observed at pH 7.4 completely disappeared at the alkyl/phosphate ratio of 0.5. This is due to the protonation of PVIm backbone.

Gene carrier should form stable complex with DNA for DNA delivery. To examine the complex stability, as shown in Fig. 3, we attempted to release DNA from the polyion complex by competitive exchange with polyanions. As the concentration of the dextran sulfate increased, the DNA increasingly migrated in the case of the PVIm-NH₂/DNA complex. On the other hands, in the PVIm-Bu/DNA complex, no DNA migrated even in the presence of the higher concentration (5mM) of the dextran sulfate. These results suggest that the PVIm-NH₂/DNA complex released the DNA by exposure to polyanions and that the PVIm-Bu/DNA complex stably retained the DNA.

We finally examined the gene expression mediated by the PVIm-R/DNA complex. As shown Fig.4, the PVIm-R/DNA complex mediated remarkable gene expression, which was higher than that mediated by the control poly(ethyleneimine) (PEI)/DNA complex. It is worth noting that the PVIm-Bu/DNA complex showed gene expression values 100 times higher than that of the PVIm-NH₂/DNA complexes.

CONCLUSION

We have synthesized the new pH-sensitive polycation PVIm-R. The resulting PVIm-Bu/DNA complex has enhanced the gene expression, which is presumably mediated by the amphiphilic effect of both hydrophobic alkyl groups and water-soluble pH-sensitive PVIm backbone.

REFERENCES


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