Physico-chemical Structure of Multilayer Polyion Complexes Comprising PolyHydroxylAmine and Genomic DNA

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ABSTRACT

Alternating multilayers of DNA and polyhydroxylamine [PT] were produced through sequential adsorption onto cationic submicron beads [C]. DNA elution on two enclosed layers and a surface layer of DNA, provided 10µg DNA/mg bead/ per layer and total yield upto 54µg/mg bead. Multilayer polyion complex (MPIC) architecture was probed by dye elution. Cationic sites were lost and gained by DNA and PT addition respectively. Comparison of elution of bound CR anionic dye between bead types reveals cationic site blocking on anionic DNA adsorption of 34 nM/mg bead, and addition on cationic PT adsorption of 14-18nM/mg beads, indicating evidence for a form of layer-by-layer construction.

Keywords: Nanolayer, DNA, polyion, layer-by-layer, microparticle, MPIC.

1 INTRODUCTION

Some progress in the development of DNA vectors for possible gene therapy is based on polycation complexation with anionic DNA [1] and the adoption of the layer-by-layer approach to build polyelectrolyte multilaminate formulations [2,3]. Here is reported an investigation of the synthesis of multilayer polyion (polylectrolyte) complexes (MPIC) that use alternating layers of DNA and a novel polycation to produce two layers of enclosed DNA and a third outer layer by final DNA adsorption to all types. The approach uses polyhydroxylamine (PT) as the polycation, with the advantage that with its pKa below 9, it can reversibly bind polyanions by modest changes in pH that lie within physiological limits. Cationic and Anionic dye binding can be used to probe the multilaminate nanoscale structure.

2 MATERIALS AND METHODS

2.1 Multilayer Bead Preparation

Cationic hydroxlamine derivatized magnetic polystyrene microbeads (0.8 µm) were used in 1 mg batches and 4 replicates to prepare five treatments:

A. Control, **B**. DNA, treatment with Calf Thymus DNA (Sigma D-1501 Lot11K7025) at 50µg in 1ml 10mM Potassium Acetate. **C**: DNA/PT Treatment with DNA as in B with post-treatment with 25mg/ml polyhydroxylamine (PT) at pH4 10mM Potassium Acetate. **D**: DNA/PT/DNA as with C with a second treatment with DNA at 50µg/ml. **E**: DNA/PT/DNA/PT treatment as in D with a second treatment with polyhydroxylamine (PT). All treatments were washed twice with 1ml of 10mM Potassium Acetate.

2.2 DNA Binding and Elution

Each of the treatment groups A-E were incubated in 1ml 50µg/ml DNA in 10mM Potassium Acetate. After 2 washing steps with 10mM Potassium Acetate, DNA bound was eluted in 200µl pH8.5 Tris HCl. The yield of DNA was measured from OD at 260nm.

2.3 Dye Binding and Elution

One microgram of each bead type from 2.1. was contacted with 1ml of 0.1mM Congo Red (CR) or Neutral Red (NR) in 10mM Potassium Acetate at pH4.0. The beads were incubated for an hour and washed twice with 1ml 10mM Potassium Acetate buffer. Dye was eluted at pH8.5 with 200µl of 10mM Tris HCl.. NR was corrected to low pH. Dye release yields were calculated as nM/mg beads from spectrophotometry at 495nm for CR and 525nm for NR. A second elution of NR at pH 2 was made after pH8.5.

3 RESULTS AND DISCUSSION

3.1 DNA Release

DNA Release is summarized in Figure 1. DNA elution is increased 50% by layering PT onto an initial layer of DNA, while two layers depth of PT with three layers of DNA elutes 54.5µg/mg of DNA, at pH8, compared to 17.4µg/mg on the core polyhydroxyl-amine bead.

Bead Type	DNA Elution [µg/mg]	Change n-(n+1)
A Core Bead	17.4 (0.3)	- µg
B. C/DNA	18.3 (0.4)	0.9
C. C/DNA/PT	28.5 (0.6)	10.2
D.C/DNA/PT/DNA	44.2 (1.3)	15.7
E. C/DNA/PT/DNA/PT	54.5 (1.1)	10.3

Table 1: DNA eluted from beads at pH8.5. as total elution and adjacent layer difference as µg DNA/mg beads.

Since all treatments had final DNA exposure, the difference in elution of bound DNA eluted between consecutive layers can be calculated as a change due to PT treatment or second exposure to DNA of the outer-most layer. Between B&A, two treatments with DNA provides an extra 1µg of eluted DNA, suggesting that the elutable DNA binding sites are nearly 95% saturated by a single incubation, compared with a 50% increase in elution for a C/DNA/PT/DNA system by a second incubation (D-E). Each PT layer (Types C & E) provides a further 10 µg/mg binding capacity of genomicDNA in this case (Table 1).

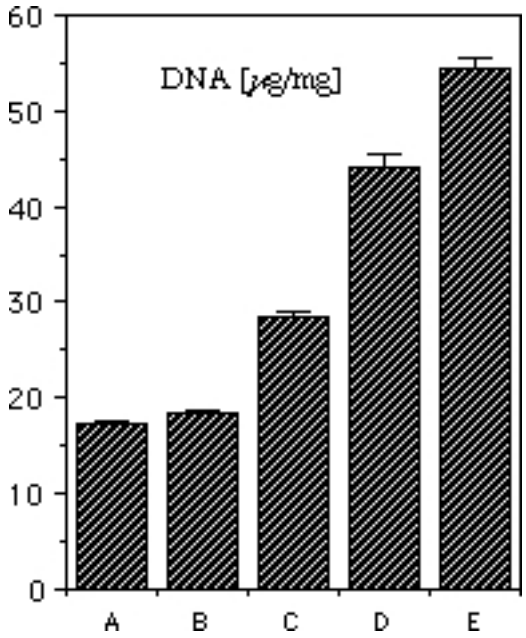

Figure 1 DNA eluted from Types A–E at pH8.5. A:Core Bead B: C/DNA, C: C/DNA/PT, D: C/DNA/PT/DNA, E: C/DNA/PT/DNA/PT

3.2 Anionic Dye Release from Cationic Sites

Congo Red release at pH8.5 (CR is nominally anionic on binding at pH4.) is summarized in Figure 2

Bead Types A,C and E show greatest release corresponding to anionic CR binding to cationic PT surfaces. Changes in binding between layers are calculated in Table 2. by subtraction of bound amounts between adjacent layers

DNA binding to bead Types B & D resulted in similar reductions in CR elution of 34 nM/mg compared to A and C without an outer DNA layer (Table 2), whilst PT adsorption to C & E increases cationic binding sites for anionic CR by 13-18 nM/mg beads.

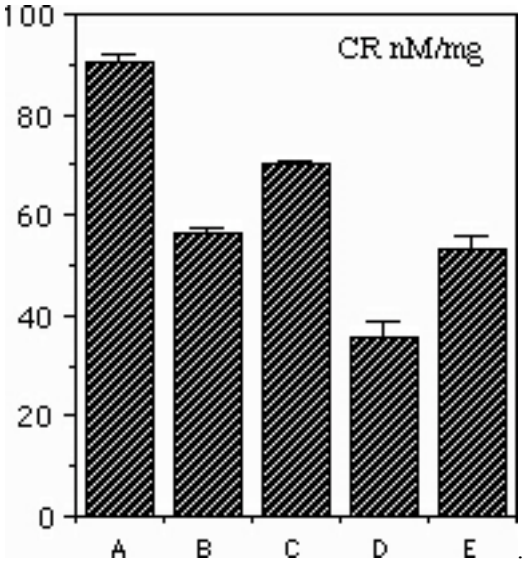

Figure 2 Congo Red dye release from types A-E at pH8.5.

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Bead Type	CR Elution nM/mg (se)	Change Δ n-(n+1)
A Core Bead	90.4 (1.4)	nM/mg
B. C/DNA	56.2 (1.4)	-34.4
C. C/DNA/PT	70.1 (0.8)	13.9
D.C/DNA/PT/DNA	35.7 (3.2)	-34.4
E. C/DNA/PT/DNA/PT	53.3 (2.6)	17.6

Table 2: Elution at pH8.5 of bound CR from Bead A-E and differences in elution between layers.

3.3 Cationic Dye Release from Anionic Sites

The pH dependant release of bound Neutral Red (NR), a nominally cationic dye binding to anionic sites, appears more complex. Neutral Red release at pH8.5 is summarized in Fig3 andTable3 along with second elution of NR at pH2. NR is nominally cationic on binding at pH4 . Cationic NR shows reversal of CR behaviour at pH8.5 but at a lower absolute (max. 8nM/mg bead). NR elution at higher pH8.5 may represent NR bound to anionic DNA released from the PIC, whilst second elution at pH2 may be release of dye from weak acidic groups as they are protonated. If so the NR is largely released from DNA. Elution of NR at pH2 is likely from protonated DNA not initially released by first elution.

Total NR binding for a DNA layer (B&D) is thus 11nM/mg and to 3.5nM/mg respectively. This could indicate that a greater fraction of the negative sites of the DNA binding to a PT layer with type D relative to type B**.** This also shows that DNA as a polyanion in the PIC can act to bind and carry positively charged material for low pH release.

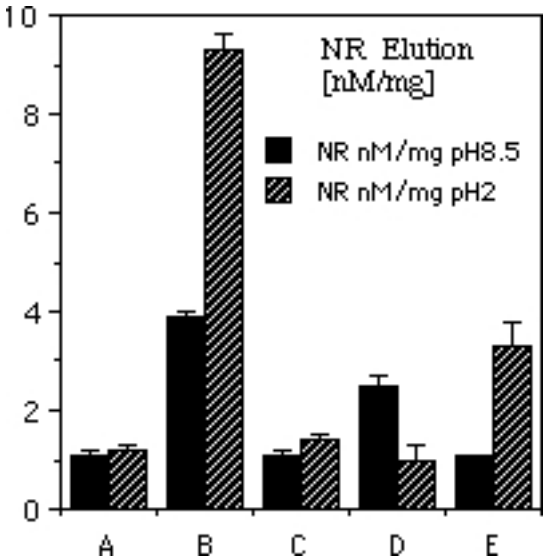

Figure 3. Neutral Red release from types A-E at pH8.5 then 2

Type	NR E1 [nM/mg]	Δ n-(n+1)	NR E2 pH2	Δ n-(n+1)
A	1.1(0.1)	nM/mg	1.2(0.1)	-
B	3.9(0.1)	+2.8	9.3(0.3)	+8.1
C	1.1 (0.1	-2.8	1.4(0.3)	-7.9
D	2.5(0.2)	+1.4	1.0(0.1)	-0.4
E	1.1(0.0)	-1.4	3.3(0.5)	+2.3

Table 3 Neutral Red dye elution at first pH8.5 and then low pH 2 from bead types A-E.

4 CONCLUSION

Multilayered Polyion Complexes (MPICs) were successfully formulated with alternating layers of polyhydroxylamine (PT) and mammalian genomic DNA, on a cationic magnetic microbead support,. In this paper MPICs enclosed upto two layers of DNA and a final third surface layer. The polycation PT allows elution of DNA below pH9, and yields 10-15 µg/mg bead, of DNA per layer, and upto 54µg/mg bead. Comparison of elution of bound CR anionic dye between bead types reveals cationic site blocking on DNA adsorption of 34 nM/mg bead, and addition on PT adsorption of 14-18nM/mg beads, indicating a form of layer-by-layer construction.

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