Synergistic Release of DNA from pH Responsive Polyion Complexes (PICs)

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ABSTRACT

Polyion complexes based on an outer layer of cationic polyhydroxylamine (PT) were prepared on carboxylated microparticles (900nm) pretreated with polyamine (A) and a middle layer of Polyacrylic Acid (PA). A synergistic effect between PT and PA layers provides greater binding with release of DNA (11.5 µg/mg, 0.36se) on pH change compared to PT adsorbed without a supporting polyanion (7.95 µg/mg, 0.38se).

Keywords: DNA, release, polyion complex, microparticle, pH

1 INTRODUCTION

Historically polyion complexes (PIC) formed in solution between polyions of opposite charge, polycations and polyanions, were considered to be largely stoichiometric in structure [1]. More recent evidence suggests that non-stoichiometry occurs within PICs according to formulation conditions [2]. The formation of PIC multilayers or nanolayers on substrates is now receiving attention [3]. Our development of a polycationic polyhydroxylamine with a pKa below 9 allows the synthesis of alternating polyion layers on a magnetic bead substrate . These beads have the capability to bind polyanionic DNA at low pH (4) and elution at a moderate pH(8.5), with obvious utility in isolation and purification of DNA in aqueous media.

2 EXPERIMENTAL METHODS

Eight bead types were prepared in 1 mg lots of 4 replicates by a layer-by-layer deposition of polyion solution onto a carboxylated polystyrene micro-particle core (nominal 0.9 µm), with thorough washing by pH 4 10mM Potassium Acetate, 7mM Potassium Chloride buffer of each layer. These were: 1. Control bead [C], 2. Control treated with 1% w/w polydiallyldimethylammonium chloride (Alcofix^{TM}) [C/A], 3. C/A treated with 1% 2w/w Na Polyacrylic Acid 240k (PA) [C/A/PA], 4. C/A/PA treated with Polyhydroxylamine 1% w/w [C/A/PA/PT]. Four further bead types were prepared: 5. a carboxylated bead treated withPT [C/PT], 6. or with PA [C/PA], 7 followed by PT [C/PA/PT] and 8 is type 5 followed with PA [C/PT/PA].

The beads were then characterized by:

2.1 Zeta Potential

electrophoretic mobility in pH corrected K Acetate 10mM / 7mM KCl buffer (HCl/NaOH) using a Malvern Zetasizer 3000HSa. Zeta potential given in mV (se) at pH4.0 , 2.00 mS.

2.2 Dye Binding and Elution

Nominally cationic Congo Red (CR 1ml 100µM binding to anionic sites), and anionic Neutral Red (NR 1ml 100µM, binding to cationic sites), were independently assessed for binding to beads at pH4 (1 hour) by spectrophotometer at 495nm and 525nm respectively. Elutions were performed at pH8.5 (200µl, 11mM Tris HCl) [EB1] and subsequently at pH12 (200µl, 10mM NaOH) [EB2]. Eluted dye yields are calculated as µM/mg beads (se). NR acidified to adjust for metachromasy.

2.3 DNA Binding and Elution

Genomic DNA from Calf Thymus (Sigma D-1501) was bound to 1mg of bead samples at pH 4 (50µg/ 1ml, 10mM Kacetate buffer), 10 minute incubation.

200µl Elution at pH8 and pH12 as dye binding above. DNA spectrophotometric OD at 260nm. Yield of DNA as µg/mg beads (se). Agarose Gel Electrophoresis was carried out oneluted samples; stained with Ethidium Bromide (EtBr), 20µl moderate pH (8) and high pH (12) samples 4µl glycerol OG loading dye.

3 RESULTS AND DISCUSSION

Changes in zeta potential with each laminate layer are consistent with the polyion charge assigned to that laminate ([C] close to its iep) (Figure.1).

The first elution at pH 8.5 resulted in DNA release from C/AF/PA/PT [6µg/mg], that is significantly greater than the quaternary amine C/AF beads [Figures.2 & 5]. C/AF/PA beads show low DNA elution at both pH conditions. A greater fraction of DNA bound to an outermost PT layer is eluted at moderate pH 8.5 than at pH12. Both zwitterionic core bead and Polyhydroxylamine coated beads show cationic character [Figure .3].

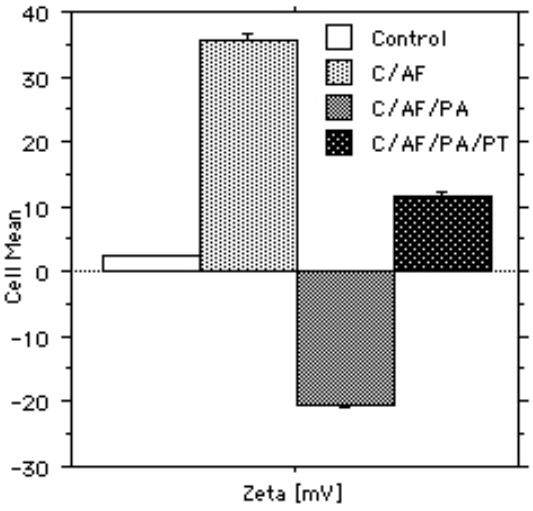

Figure 1: Zeta potential of Beads 1-4 at pH 4.00.

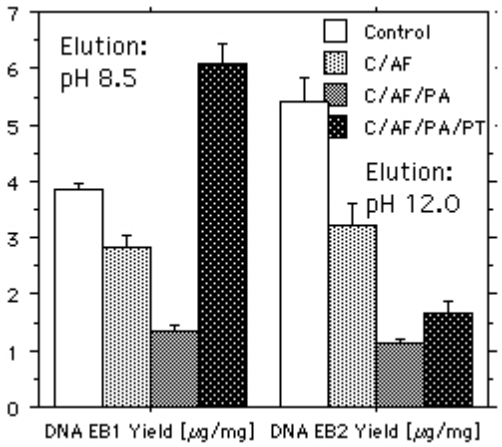

Figure 2 DNA binding and elution from beads 1-4. First elution at pH8.5 on left, second at pH 12.0 on right.

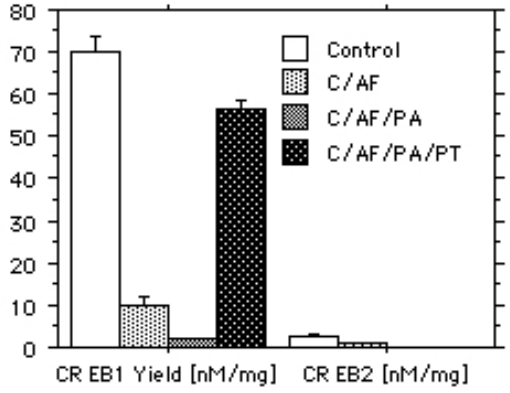

Figure 3 Anionic CR elutions from beads 1-4.

Beads with PT present show greatest DNA and CR elution at the lower pH (8.5). Figure 5 confirms the OD260nm elution results with agarose gel electrophoresis. Cationic NR (uptake and thus) release by PT beads mirrors that of a PA surface [Figure 4.], suggesting that the PA layer is largely available for dye binding even as C/AF/PA/PT type PIC. Greater DNA release from a PA/PT type layering is confirmed with the second experiment using bead types 1,5-8 compared with simple adsorption to the control surface as C/PT [Figure 6].

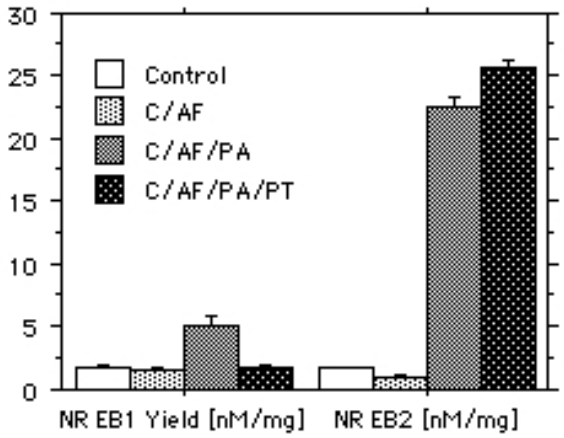

Figure 4 Cationic NR elutions; first and second elutions from beads 1-4

A possible hypothesis is that an electrosteric surface on a PIC would tend to favour shallow penetration of DNA (or other counter polyion) before contact and binding with the most distal chain fractions within the electrosteric profile. This would effectively reduce deeper access into the profile, promoting non-stoichiometric binding and subsequent development of an electrosteric layer by the depositing polyion.

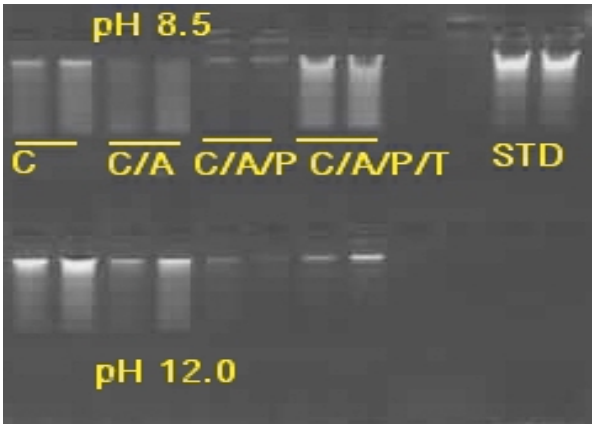

Figure 5: Agarose Gel: DNAct Elution at pH 8.5 and 12 for Control Carboxy Bead [C], with AF first layer [C/A], with PA second layer [C/A/P] and PT third layer [C/A/P/T]. STD = 50µg/ml Standard.

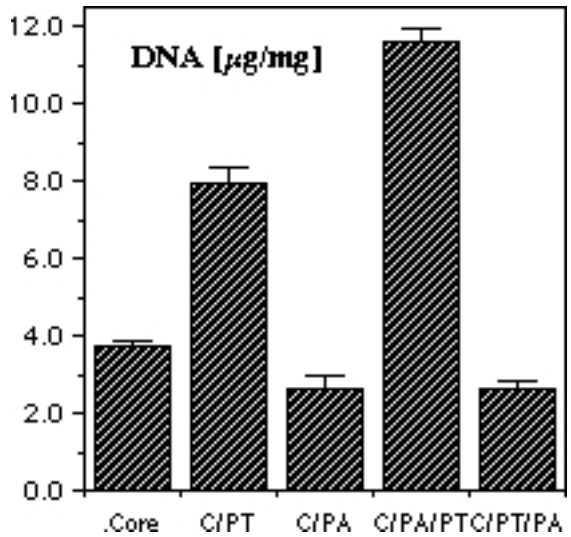

Figure.6: Total DNA elution from bead types 1,5- 8.
Types: 1 Control Bead, 5 [C/PT], 6 [C/PA], 7 [C/PA/PT], 8[C/PA/PT/PA].

4 CONCLUSION

pH dependant DNA binding and elution from polyhydroxylamine [PT] containing Polyion Complexes (PICs) is described where the PICs were synthesized by sequential adsorption on sub-micron particles. Significantly greater binding and subsequent release of DNA is achieved by PT, as a PIC, than as a simply adsorbed polycation (Fig 6.). Synergism exists between anionic PA, as a supporting layer, and cationic PT providing a significant increase in elution of bound (11.6 (0.4se) vs 7.9 (0.4se) µgDNA/mg beads) . Polyanion and polycation layer presence is confirmed by both zeta potential and dye binding results. NR dye recovery from PA with PT present (C/AF/PA/PT) provides evidence that the electrosteric architecture of each polyion layer is largely conserved between adjacent polyion layers.

REFERENCES

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