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Nucleic acid affinity of clustered-charge anion exchange adsorbents: Effects of ionic strength and ligand density

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ABSTRACT

In previous work we demonstrated the improved protein-binding capacity and selectivity of ion-exchange adsorbents displaying a "clustered" rather than random, distribution of surface charges. For example, anion-exchange adsorbents displaying 5 mM of positive charge in the form of 1 mM penta-argininamide show much higher affinity and capacity for alpha-lactalbumin than do adsorbents displaying the same 5 mM total charge in the form of single dispersed argininamide charges. We also found that clustered adsorbents selectively favor proteins with inherent charge clustering. In the present work, "clustered" penta-argininamide adsorbents showed DNA binding capacity comparable to that of conventional dispersed adsorbents with 10–100-fold higher ligand density. We also observed that at moderate ionic strength the DNA affinity of all adsorbents tested *increased* with salt while RNA affinity decreased, so that selectivity for DNA over RNA was enhanced as salt concentration increased.

1. Introduction

Ion-exchange chromatography is widely used for the separation of biomolecules [1,2] due to the strength and reversibility of ion-exchange adsorption, and the possibility of tuning selectivity and resolution for the biomolecule of interest [3–7]. The recent increased need for nucleic acids in biological sciences and in medicine (e.g., for plasmid DNA vaccines [8–12]), has called for improved techniques for nucleic acid purification. Thus, there is an on-going interest in improving the affinity and selectivity of ion-exchange adsorbents for nucleic acids.

Traditional ion-exchange adsorbents display a random charge distribution which creates a heterogeneous landscape of adsorption sites [13] and fortuitous charge clustering and geometric matching between adsorbates and adsorbent play an important role in adsorption. For the ion-exchange capture of small ions, a distance-of-charge-separation approach showed that the close proximity of the positive charges on the nitrogen atoms of a polyamine ligand improved its selectivity for divalent (SO_4^{2-}) over monovalent (Cl^-, NO_3^-) ions compared to isolated distributed tertiary and quaternary amine functionalities [13–16]. In our previous work, a higher affinity and capacity for negatively charged proteins was observed for an adsorbent displaying uniform-size clusters of positive charges, than for an adsorbent with the same total charge density displayed as dispersed charges [7]. It was also shown that proteins with highly charged patches were particularly favored in both binding capacity and selectivity on the clustered-charge adsorbent.

In the present work, we tested the capacity and selectivity of clustered-charge anion exchangers for nucleic acids, which display high charge densities compatible with multivalent interactions with clustered adsorbents. It was shown that clustered-charge adsorbents of relatively low ligand density can have a higher DNA affinity than the conventional adsorbents of much higher ligand density. Clustered charge adsorbents also showed enhanced selectivity for DNA over RNA, which can be useful for practical purposes.

2. Experimental

2.1. Materials

AminoLink coupling resin, AminoLink reductant and Micro BCA protein assay kits were from Pierce (Rockford, IL). Q Sepharose Fast Flow and DEAE Sepharose were from GE Healthcare (Piscataway, NJ). Penta-argininamide was custom synthesized by Biomatik (Wilmington, DE); the amide was introduced to avoid the formation of a zwitterionic adsorbent ligand also displaying a negatively charged C-terminal carboxylate. Salmon sperm DNA was from Stratagene (La Jolla, CA). DEAE Plasmid *Plus* resin was from Qiagen (Valencia, CA). Baker's yeast RNA and all other reagents were from Sigma Aldrich (St. Louis, MO).

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2.2. Adsorbent preparation

Four mL of well-suspended AminoLink coupling resin was placed in a 10 mL disposable polypropylene filter column from Pierce (Cat no 29924) and washed with 10 mL of coupling buffer (0.1 M sodium phosphate buffer, 0.15 M NaCl, pH 7.2) to remove preservatives, then mixed with 2 mL of penta-argininamide solution (3, 6, 12 or 30 mM) on a Cole-Parmer Roto-torque gyratory rotator at room temperature for at least 4 h, and then at 4 °C for 4 days. The supernatant was then drained, and the adsorbent washed with 10 mL of coupling buffer to remove unbound peptide. The adsorbent was then mixed with 2 mL of 66 mM sodium borohydride in 25% ethanol/75% PBS, followed by 30 min rotation to deactivate any remaining aldehyde groups, and washed with 15 mL of 1 M NaCl and 5 mL of storage buffer (10 mM Tris–HCl, pH 8.0), drained and resuspended in 0.67 adsorbent volume of binding buffer (10 mM Tris–HCl, 10 mM NaCl, pH 8.0).

The concentration of peptide ligand on the adsorbent was determined by bicinchoninic acid assay (Micro BCA Protein Assay, Pierce) of the residual supernatant peptide and the modified adsorbent itself. Peptide adsorbents with a final ligand density of 1.4, 2.4, 4.8 and 24 mM penta-argininamide were prepared and stored at 4 °C for later use.

Q Sepharose Fast Flow, Qiagen DEAE Plasmid Plus resin and GE Healthcare DEAE Sepharose were vortexed and placed in a 10 mL disposable polypropylene filter column from Pierce (Rockford, IL). Each adsorbent (4 mL slurry as supplied by the manufacturer) was washed with 10 mL of binding buffer (10 mM Tris–HCl, 10 mM NaCl, pH 8.0) to remove preservatives, drained and then resuspended by adding 0.67 packed adsorbent volume of binding buffer. The ligand density of Qiagen DEAE Plasmid Plus resin was determined to be 125 ± 5 mM by titrating the resin with 5.5 M NaOH using a Metrohm Titrino model 794 titrator, while those of GE Healthcare DEAE and Q Sepharose Fast Flow were supplied by the manufacturer.

2.3. Adsorption isotherm measurement

Each adsorbent (25 μl suspension; 15 μl settled adsorbent volume) was aliquoted into 1.5 mL Eppendorf tubes and incubated with different volumes of nucleic acid stock solution (10–320 μl of 25 μg/mL DNA stock solution in binding buffer or 20 μg/mL RNA stock solution in binding buffer) plus binding buffer to bring the total volume to 1.0 mL plus 15 μl of settled adsorbent. Adsorption was then allowed to equilibrate on a gyratory rotator at room temperature for 1 h, a time found in control experiments to be sufficient for equilibration. After a 10-min centrifugation at 16,000 × *g* in an Eppendorf model 5415D microcentrifuge, the nucleic acid concentration in the supernatant was quantified by 260 nm absorbance using a Beckman-Coulter DU 530 spectrophotometer. The adsorbent pellets were resuspended in 1 mL of binding buffer and again centrifuged (this supernatant contained a negligible quantity of nucleic acid); bound nucleic acids were eluted with 1 mL of elution buffer (binding buffer + 1 M NaCl) and analyzed spectrophotometrically for determination of bound nucleic acid content and calculation of total mass recovery.

2.4. Data analysis

The mass balances (nucleic acid remaining in supernatant + nucleic acid eluted from adsorbent, divided by nucleic acid originally added) for all adsorption data closed in the range of 83–109%. Adsorption isotherms were fit to the Langmuir isotherm (Eq. (1)) using Igor Pro (WaveMetrics, Lake Oswego, OR; version 6.05) which uses the Levenberg–Marquardt algorithm to search for parameters which minimize χ^2 values, as described previously [7].

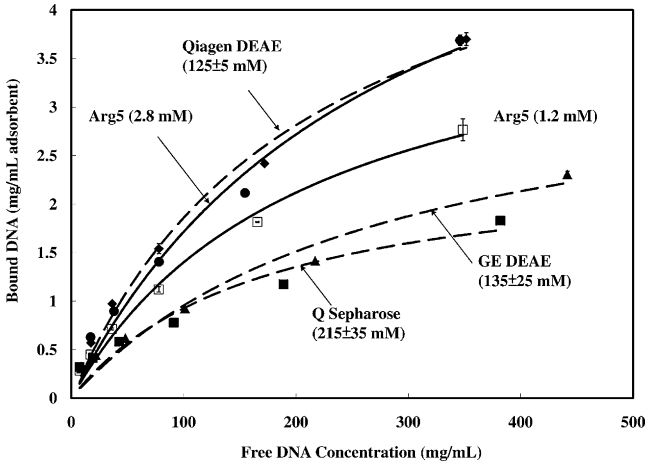

Fig. 1. Adsorption isotherms of salmon sperm DNA with Langmuir fits on Qiagen DEAE resin (♦), GE DEAE Sepharose (▲), penta-argininamide adsorbents (Arg_5: 1.2 mM ligand density (□); 2.8 mM ligand density (●)), and Q Sepharose (■) at 25 °C in 10 mM Tris, 10 mM NaCl at pH 8.0. Error bars correspond to mean ± 1 SD.

$$y = \frac{Q_{max} \times X}{K_d + X} \quad (1)$$

where *y* is the bound nucleic acid concentration, *X* is the free nucleic acid concentration, Q_{max} is the maximum binding capacity, and K_d is the dissociation constant.

3. Results and discussion

3.1. Adsorption isotherms

The performance of the penta-argininamide adsorbent was compared to conventional adsorbents. As shown in Fig. 1, 1.2 mM penta-argininamide adsorbent gave a higher DNA binding capacity (Q_{max} = 4.4 ± 0.5 mg/mL adsorbent) and stronger initial binding affinity (Q_{max}/K_d = 20.6 mL/mL adsorbent) than both Q Sepharose Fast Flow (Q_{max} = 2.5 ± 0.5 mg/mL adsorbent; Q_{max}/K_d = 14.7 mL/mL adsorbent) and GE DEAE Sepharose (Q_{max} = 3.6 ± 0.8 mg/mL adsorbent; Q_{max}/K_d = 13.0 mL/mL adsorbent). The binding capacity (Q_{max} = 6.6 ± 1.2 mg/mL adsorbent) and initial binding affinity (Q_{max}/K_d = 23.2 mL/mL adsorbent) of an increased-ligand-density penta-argininamide (2.8 mM) adsorbent also compared well to those of Qiagen DEAE resin (Q_{max} = 5.8 ± 0.6 mg/mL adsorbent; Q_{max}/K_d = 27.4 mL/mL adsorbent; Table 1). As shown in Fig. 2, extremely strong RNA initial binding affinity and binding capacity were observed on Q Sepharose Fast Flow adsorbent while the 1.2 mM penta-argininamide and the other two conventional adsorbents (Qiagen DEAE resin and GE DEAE Sepharose) exhibited lower RNA binding capacity (Table 1). It is worth noting that the salt-induced reversibility of the nucleic acids' binding to the penta-argininamide adsorbents underscores the contribution of electrostatic interactions [7] compared to possible formation of hydrogen bonds. While arginine is known to form specific hydrogen bonds via its guanidine group with nucleic acids (e.g., HIV 1 TAR hairpins or supercoiled plasmid DNA) displaying characteristic structural motifs [17–24], this mechanism is less favored with the linear nucleic acids used in the present study.

3.2. Salt and ligand density effects of DNA binding

The effect of salt concentration on nucleic acid binding was investigated with single-point adsorption measurements with an initial DNA loading of 40 μg/mL in the solution on the penta-