Differential retention of DNA on Ceramic Hydroxyapatite (CHT™) and Ceramic Fluoroapatite (CFT™)

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Abstract

The well-known affinity of DNA for ceramic hydroxyapatite (CHT) has made it a popular and effective tool for removal and/or purification of DNA. The recent introduction of ceramic fluoroapatite (CFT) raises the question of its relative DNA fractionation properties and their practical ramifications. The present study compares the retention behavior of DNA on both supports, under a range of conditions designed to quantify and explain the mechanism of any differences in their relative performance. Results indicate that retention is dominated by crystal-calcium interactions on both media but the contribution of crystal-phosphates is relatively stronger on CFT. This creates a range of new opportunities for managing the behavior of DNA on the two resins, either for purification of DNA products from other contaminants, or for removing DNA from protein preparations. A practical method development strategy is provided.

Introduction

Purification of biological molecules such as proteins and nucleic acids by hydroxyapatite (Ca10(PO4)6 (OH)2) was first established by Tiselius et. al. in 1956 (1). The calcium phosphate matrix is capable of various chemical interactions with DNA, including charge repulsion by crystal phosphates and metal affinity interactions with crystal calcium (Figure 1)(2-6). Ceramic media overcome the physical fragility of traditional hydroxyapatite and have made the technique suitable for a wide range of analytical and preparative applications. CFT differs from CHT by replacement of the hydroxyl group with fluoride (7).

DNA is of central interest in the field of biotechnology for its own utility as a therapeutic agent and its importance as a contaminant in therapeutic proteins. The ability to "manage" the retention behavior of DNA could provide a valuable tool in both application areas. The present study compares the retention behavior of DNA on CFT and CHT with the intent of providing such a tool.

Results and Discussion

Effect of pH

Figures 2 and 3 compare the retention of DNA on CHT and CFT, at pH 6.0, 7.0, and 8.0 in the absence of NaCl. DNA retention on CHT exhibits progressive retention with increasing pH. CFT shows a single weakly binding peak at pH 6.0, but at pH 7.0 it shows two peaks, the later one showing the same increased retention trend as CHT, but the other binding more weakly than the pH 6.0 peak. At pH 8.0 the entire population elutes in a single peak at the very beginning of the phosphate gradient. This appear to reflect an increase in the charge of DNA phosphates as a result of their protonation above their pK of ~6.2. (8, 9). At low ionic strength, stronger charge is expected to make DNA more rigid. This apparently makes it less able to conform to the surface topography of CFT. We interpret the split behavior at pH 7.0 as reflecting the DNA population being in a transition state. This may be a secondary function of the size distribution of the sample. At pH 8.0, the entire DNA population appears to have achieved a level of rigidity that prevents strong binding.

Effect of NaCl

Figures 4A-C, and 5A-C compare the behavior of DNA on CHT and CFT at pH 6.0, 7.0. and 8.0 as a function of sodium chloride concentration. The same trend is apparent in both data sets. Increasing sodium chloride concentration causes increasing DNA retention. This appears to reflect damping of charge repulsion by DNA-phosphates and crystal-phosphates. As increasing NaCl concentration damps a greater proportion of electrostatic repulsion between sample and stationary phase, the DNA is bound increasingly its metal affinity for the calcium. This interaction can be eluted only with phosphate. Even saturated NaCl will not dissociate it.

Beyond the common response exhibited by both media, it is apparent that CHT binds DNA much more strongly than CFT. We interpret this as an indication that calcium is topographically less accessible on the surface of CFT. Preliminary data with proteins (not shown) support this interpretation.

Sodium chloride generally has the effect of weakening protein retention on CHT™ and CFT™ although to varying degrees. Acidic protein such as albumin bind predominantly by metal affinity of carboxyl clusters for crystal calcium. They are little affected by sodium chloride. Enhancing DNA removal for such proteins can be accomplished by increasing NaCl concentration on CHT. Increasing NaCl on CFT would have the effect of driving the respective solutes toward increasingly similar retention, with the effect of diminishing DNA removal efficiency. Small basic proteins such as lysozyme bind exclusively by cation exchange on crystal phosphates. DNA removal could be enhanced for such proteins by increasing NaCl concentration on CHT: lysozyme will be eluted by the salt, DNA will bind more strongly, maximizing the differential between the two. Large proteins like IgG typically bind by both mechanisms. Experimentation is required to determine which whether DNA removal would be enhanced most by decreasing its retention with NaCl on CFT, or by increasing its retention with NaCl on CHT. Effectivity of DNA removal from proteins that bind well to CFT at pH 8.0 should be excellent due to elution of DNA at ultra-low phosphate concentrations (in the absence of NaCl). In the case of DNA purification, its behavior can be likewise manipulated to provide the best separation from the most challenging contaminants.

Materials and Methods

CFT Type 1 40 µm and CHT Type 1 40 µm were obtained from Bio-Rad Laboratories (Hercules, Ca). Experiments were conducted on a MT2 column obtained from Bio-Rad Laboratories (Hercules, Ca). The column dimensions were 0.7 x 5.2 cm. The column was dry packed using a density of 0.60 and 0.81 for CHT and CFT respectively. Each column was wetted in ten column volumes of 20% ethanol before equilibration. All chromatography experiments were automated and executed using the Biologic System and software (Bio-Rad Laboratories, Hercules, Ca). Sheared salmon sperm DNA was manufactured from Epperdorf AG (Hamburg, Germany, distributed in North America by Brinkman Instruments, Inc). It was further purified across CFT to give a DNA concentration of 30 ug/mL (50 ug/mL per A260 unit).

DNA retention was evaluated on CFT and CHT at pH 6.0, 7.0, and 8.0. The equilibration buffer was 10mM NaPO4. The elution buffer was 0.8M NaPO4. All experiments were run with the same configuration:

1. Equilibrate with 10 column volumes (CV) equilibration buffer.
2. Inject 100 microliters sample.
3. Wash 5 CV.
4. Elute with a 10 CV, linear gradient to 100% elution buffer.

Following baseline runs in these buffers each sample was rerun in the same buffers plus 0.25M NaCl, then in the same buffers plus 0.5M NaCl, and finally in the same buffers plus 1.0M NaCl. All experiments were monitored at UV 260 nm.

Conclusions

DNA binds both CHT and CFT by metal affinity of DNA phosphates for crystal calcium. Electrostatic repulsion between crystal and DNA phosphates reduces the net binding energy. The latter effect is damped with the addition of NaCl, causing DNA retention to increase. Conformational changes (increased rigidity) of DNA at low ionic strength and alkaline pH also alter retention of DNA on CFT, but not CHT. This apparently reflects reduced accessibility of crystal calcium on CFT, which is manifested as weaker retention of DNA. The combination of these effects provides process developers with a flexible new set of tools for enhancing removal of DNA from protein preparations, or for removing proteins from DNA.

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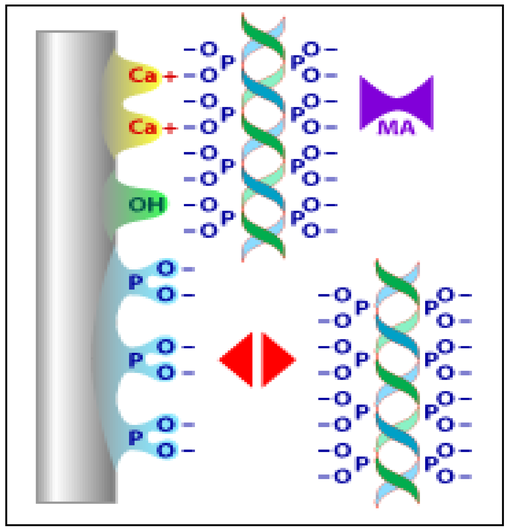

Figure 1: Interaction of DNA with hydroxyapatite. Red arrows indicate electrostatic repulsion. Purple "staple" indicates metal affinity.

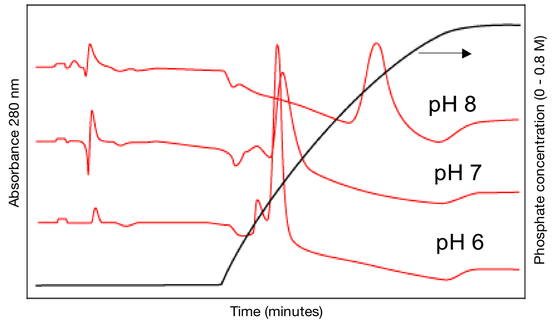

Figure 2: Effect of pH on DNA elution from CHT.

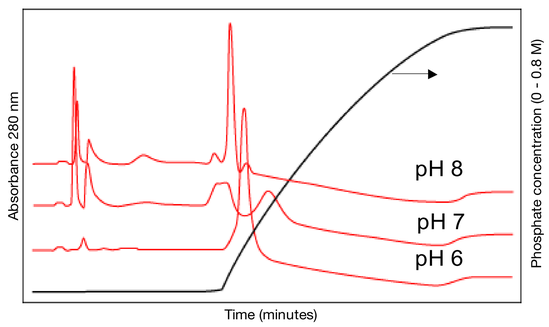

Figure 3: Effect of pH on DNA elution from CFT.

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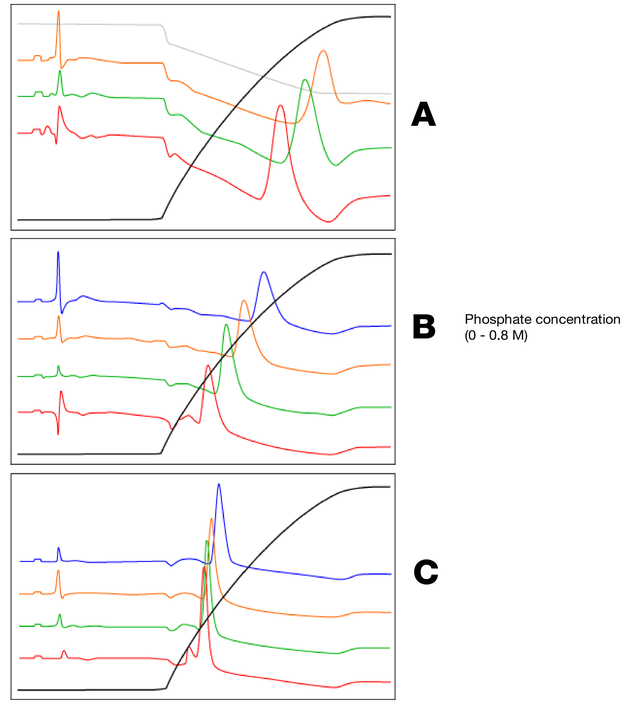

Figure 4: Effect of sodium chloride on elution of DNA from CHT. **A**: pH 8, **B**: pH 7, **C**: pH 6. **Red**: 0 M NaCl; **Green**: 0.25 M NaCl; **Orange**: 0.50 M NaCl; **Blue**: 1.0 M NaCl.

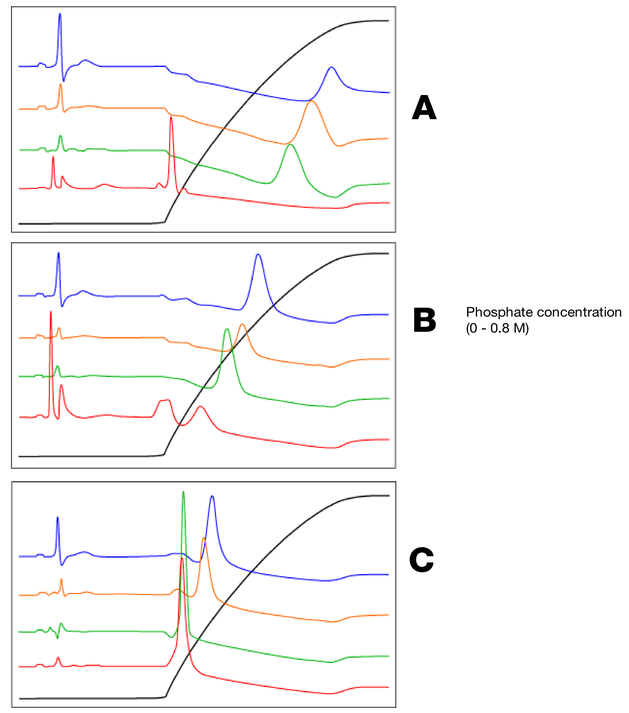

Figure 5: Effect of sodium chloride on elution of DNA from CFT. **A**: pH 8, **B**: pH 7, **C**: pH 6. **Red**: 0 M NaCl; **Green**: 0.25 M NaCl; **Orange**: 0.50 M NaCl; **Blue**: 1.0 M NaCl.