Plasmid DNA Adsorption Behavior on CHT™ Ceramic Hydroxyapatite Type II Support

Cheryl Aberin and Samuel Franklin, Bio-Rad Laboratories, Inc., Hercules, CA 94547 USA

Introduction

Plasmid DNA adsorbs to the surface of most commercially available chromatographic supports. These chromatographic supports were designed and developed for protein purification and thus have relatively small pore dimensions. The small pores lead to low adsorption capacity of plasmid DNA on the support; in fact, binding capacities for plasmid DNA average 50 times less than those for protein (Ferreira et al. 2000). There is thus a need to optimize the adsorption capacity for plasmid DNA on most of the chromatographic supports currently available. Previously we demonstrated that chromatography on CHT ceramic hydroxyapatite Type II yielded plasmid of near pharmaceutical grade in a single step (Aberin and Franklin 2002). Here we report on the effect of size and type of plasmid DNA, as well as the nature of the buffer system, on the dynamic binding capacity (DBC) and recovery on CHT Type II support.

Methods and Results

CHT Type II support (20 µm particle size) was used to screen plasmid DNA to determine adsorption behavior. The three different types of pure plasmid DNA were: pS3 (10 kb), pS5 (9.48 kb), and pUC19 (2.6 kb) obtained from an outside vendor and loaded to saturation onto a 1 ml CHT column (Econo-Pac® prepacked CHT-II cartridge, Bio-Rad Laboratories, Inc.) DNA concentration was determined spectrophotometrically at 260 nm. Table 1 shows the adsorption behavior of the different types of plasmid DNA loaded onto a CHT column using 10 mM sodium phosphate, pH 7.0, 1 mM EDTA as the binding buffer. The plasmid DNA was eluted with 0.4 M sodium phosphate, pH 7.0, 1 mM EDTA. Fractions collected were analyzed spectrophotometrically at 260 nm to determine DNA concentration. The linear flow rate used to bind and elute the samples was 311 cm/hr. Both DBC and yield varied with plasmid type.

We next evaluated the effect of different buffer systems on the adsorption behavior of different plasmids on CHT. Buffers used for screening were 0.01 M sodium phosphate, pH 7.0 and 7.5; 0.02 M borate, pH 7.5; and these buffers containing different additives (NaCl and $CaCl_2$). Plasmid was eluted with 0.4 M sodium phosphate, pH 7.0.

Table 1. Adsorption capacity of different plasmids on CHT Type II support.

	Plasmid		
	pS3 (10 kb)	pS5 (9.48 kb)	pUC19 (2.6 kb)
DNA loaded, µg*	2,148	1,390	2,950
Unbound, µg	1,735	1,197	2,574
Total bound, µg	413	193	376
Bound/eluted	227	151	213
% Yield	55	78	57
DBC, µg/ml	227	151	213

*Samples were loaded in pH 7.0 phosphate buffer.

The yield and DBC were again similar for pS3 and pUC19 in phosphate buffer (Table 2). These values increased for pUC19 but decreased for pS5 in borate.

Table 2. Effect of buffer on plasmid DNA adsorption capacity on CHT Type II support.

	Plasmid in Phosphate Buffer, pH 7.5			Plasmid in Borate Buffer, pH 7.5	
	pUC19	pS3	pS5	pUC19	pS5
DNA loaded, µg	2,950	2,148	1,390	2,800	1,370
Unbound, µg	2,574	1,735	1,197	2,300	1,219
Total bound, µg	376	413	193	500	151
Bound/eluted	213	227	151	358	83
% Yield	57	55	78	72	55
DBC, µg/ml	213	227	151	358	83

Addition of 0.3 M NaCl to the phosphate and borate buffers significantly increased the DBC for pS5 (Table 3) but did not improve the yield.

Table 3. Effect of 0.3 M NaCl on pS5 adsorption capacity on CHT in pH 7.5 phosphate and borate buffers.

	Phosphate Buffer		Borate Buffer	
	Control	0.3 M NaCl	Control	0.3 M NaCl
DNA loaded, µg	1,390	1,380	1,370	1,310
Unbound, µg	1,197	803	1,219	968
Total bound, µg	193	577	151	342
Bound/eluted	151	398	83	274
% Yield	78	69	55	80
DBC, µg/ml	151	398	83	274

Table 4. Effect of different additives on pS5 adsorption capacity.

	[NaCl]				[$CaCl_2$]			
	Control	0.3 M	0.6 M	1.0 M	1 mM	3 mM	5.5 mM	10 mM
DNA loaded, µg*	1,370	1,310	1,320	1,310	1,480	1,400	1,350	1,280
Unbound DNA, µg	1,219	968	701	698	1,301	814	779	542
Total bound DNA, µg	151	342	619	612	179	586	571	738
Bound/eluted	83	274	540	518	136	357	485	651
% Yield	55	80	87	85	76	61	85	88
DBC, µg/ml	83	274	540	518	136	357	485	651

*Samples were loaded in 20 mM borate, pH 7.5 containing 1 mM EDTA.

Plasmid pS5 adsorption capacity on the CHT Type II support was found to increase with sodium chloride up to 0.6 M in borate buffer and then remained constant (Table 4). However, with calcium chloride, the adsorption capacity continued to increase with concentration. At 5.5 mM and higher, the effluent was turbid due to precipitation of calcium phosphate. Adding EDTA to the effluent cleared the solution.

Discussion

The adsorption behavior and recovery of plasmid DNA on CHT varied with the size and type of the plasmid as well as with the buffer systems and additives used. This is consistent with observations that size, base composition, and secondary structure affect plasmid binding to ion exchange supports (Ferreira et al. 2000). Plasmids prepared by different lysis procedures may be nicked or denatured to various degrees, also affecting binding capacities and recoveries (Ferreira et al. 2000).

CHT adsorption capacity for plasmid DNA was enhanced using sodium chloride. DNA swells at low ionic strength. In the presence of sodium chloride, the plasmid DNA is more tightly constricted, which may allow further penetration into the pores of the CHT, resulting in increased binding. The effects of an increase in calcium chloride concentration may be due to bridging of the plasmids to each other or to the phosphate residues of the CHT.

Use of the buffer systems described here, or further modifications thereof, may significantly increase the DBC, recovery — or both — of many other types of plasmid on CHT and other chromatography media.

References

Aberin CS and Franklin SG (2002). Plasmid purification using CHT ceramic hydroxyapatite support. Bio-Rad bulletin 2731.

Ferreira GN et al. (2000). Downstream processing of plasmid DNA for gene therapy and DNA vaccine applications. Trends Biotechnol 18, 380–388.

Information in this tech note was current as of the date of writing (2003) and not necessarily the date this version (Rev A, 2009) was published.

BIO-RAD

Bio-Rad Laboratories, Inc.

Life Science Group

Web site *www.bio-rad.com* ***USA*** *800 4BIORAD* ***Australia*** *61 02 9914 2800* ***Austria*** *01 877 89 01* ***Belgium*** *09 385 55 11* ***Brazil*** *55 21 3237 9400* ***Canada*** *905 364 3435* ***China*** *86 21 6426 0808* ***Czech Republic*** *420 241 430 532* ***Denmark*** *44 52 10 00* ***Finland*** *09 804 22 00* ***France*** *01 47 95 69 65* ***Germany*** *089 318 84 0* ***Greece*** *30 210 777 4396* ***Hong Kong*** *852 2789 3300* ***Hungary*** *36 1 455 8800* ***India*** *91 124 4029300* ***Israel*** *03 963 6050* ***Italy*** *39 02 216091* ***Japan*** *03 6361 7000* ***Korea*** *82 2 3473 4460* ***Mexico*** *52 555 488 7670* ***The Netherlands*** *0318 540666* ***New Zealand*** *0508 805 500* ***Norway*** *23 38 41 30* ***Poland*** *48 22 331 99 99* ***Portugal*** *351 21 472 7700* ***Russia*** *7 495 721 14 04* ***Singapore*** *65 6415 3188* ***South Africa*** *27 861 246 723* ***Spain*** *34 91 590 5200* ***Sweden*** *08 555 12700* ***Switzerland*** *061 717 95 55* ***Taiwan*** *886 2 2578 7189* ***United Kingdom*** *020 8328 2000*