Role of Plasmid DNA Isoforms During Anion Exchange Chromatography

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Introduction

New development in the modern biotechnology increased the need for plasmid DNA (pDNA) with sizes above 10 kbp (large pDNA), but their chromatographic purification is often challenging due to low process yields and column clogging. There are indirect proofs that open circular (OC) pDNA isoform is the main troublemaker due to its physical entrapment within the narrow channels of chromatographic media. Increasing the channel size of chromatographic support should decrease the negative impact and improve the chromatographic performance. The aim of the study was to use novel Convective Interaction Media® (CIM®) monolith chromatographic columns with large, 6 µm channels, for analytical and preparative separation of pDNA. The effect of supercoiled (SC), OC and linear (LIN) pDNA isoforms on chromatographic performance was thoroughly evaluated.

1. Experimental approach

Development of analytical chromatographic method for separation different pDNA isoforms was performed using 15.4 kbp large pDNA (pFix15, containing different ratios of OC, SC and LIN isoforms). Weak anion exchanging analytical column was used (CIMacTM pDNA). Separation of pDNA isoforms in 100 mM TRIS, pH 8.0, was realized in ascending linear gradient of sodium chloride (NaCl), guanidinium chloride (GdnCl), guanidinium thiocyanate (GdnSCN) or combined GdnCl/GdnSCN at 30°C applying different flow rates of mobile phase (0.2, 0.5 or 1.0 mL/min). pDNA isoforms were monitored using UV detection at 260 and 280 nm.

On preparative scale weak anion exchangers CIMmic DEAE 0.1 mL disks , 1 mL CIMmultus™ DEAE columns (2, 3 or 6 µm channel diameter) or commercially available resins with different bead sizes and pore diameters as well as convective membranes (Sartobind® Q Nano) were evaluated. Different amounts of 11.6 and 15.4 kbp large pDNA samples of different isoform composition (dissolved in 50 mM TRIS, 10 mM EDTA, 0.2 M NaCl, pH 7.2) were loaded onto columns at 6 mL/min (1 mL columns) or 1 mL/min (0.1 mL disks), followed by column washing and elution with 50 mM TRIS, 10 mM EDTA, 1 M NaCl, pH 7.2. Elution was performed at 0.4 mL/min for 1 mL columns , while two-step elution at 1 and 0.1 mL/min was used for 0.1 mL disks and commercially . The UV signal at 260 nm and pressure drop over the column were monitored. After elution, a wash step was performed with 0.1 M NaOH solution. All pDNA fractions (loading sample, flow-through fraction, elution and neutralised 0.1 M NaOH wash) were collected and analysed by UV spectroscopy for overall concentration of nucleic acids or HPLC analysis for OC, SC and LIN pDNA isoform.

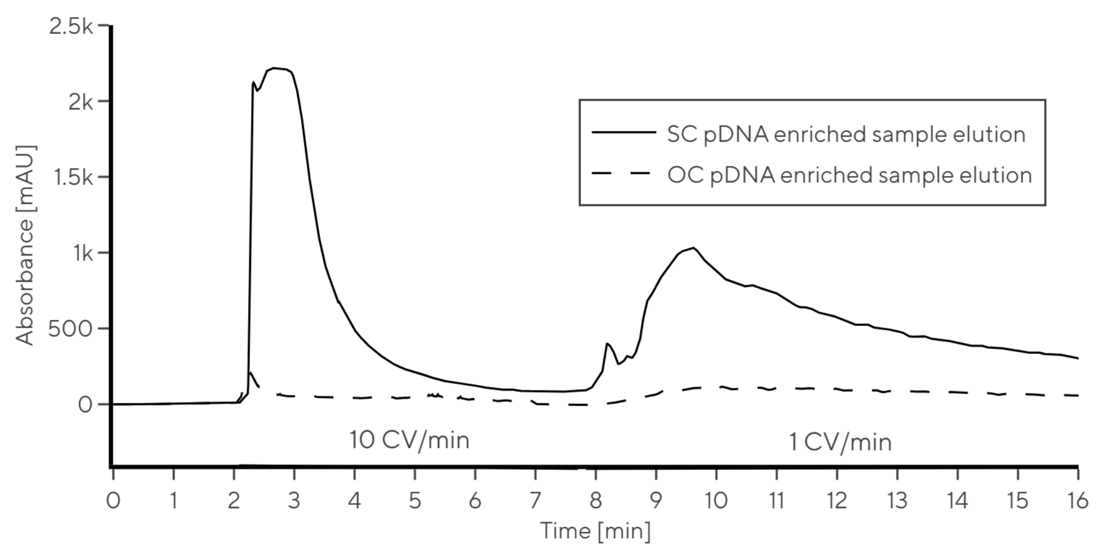

Figure 3: Comparison of elution profiles for SC pDNA-enriched sample (solid line) and OC pDNA-enriched sample (hatched line), 15.4 kbp pDNA was used.

2. Results – Analytical quantification of 15.4 kbp large pDNA isoforms using CIMac pDNA columns

- OC pDNA is not detected on standard CIMac pDNA (1.4 µm channels) at all, regardless the flow rate used – see Figure 1A.
- Elution recovery of OC pDNA from CIMac pDNA column with 6 µm channel diameter in ascending NaCl gradient was significantly improved especially at flow rates below 0.5 mL/min, but the resolution between the isoforms was inferior. The replacement of NaCl with GdnCl improved the resolution between OC and SC isoforms considerably (Figure 1B).

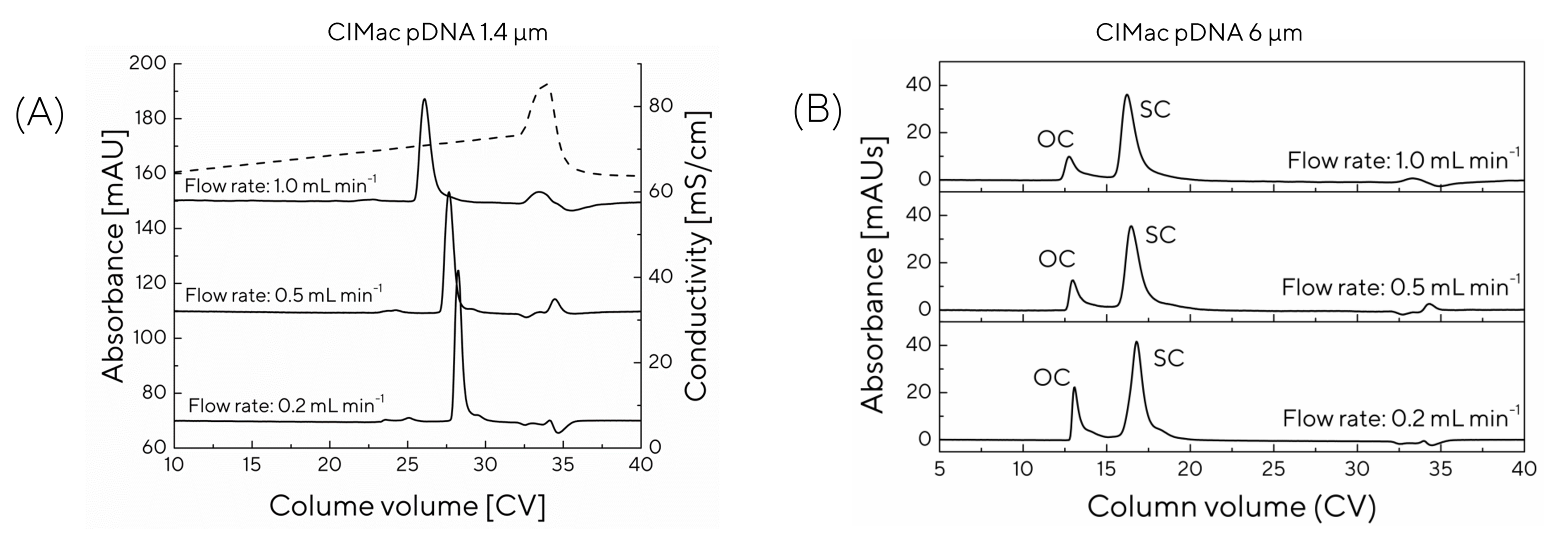

Figure 1: Elution profile of pFix15 on CIMac pDNA columns in GdnCl gradients at different flow rates. (A) Isoform separation using column with 1.4 µm channel size. (B) Isoform separation using column with 6 µm channel size.

- Extreme broadening of the LIN pDNA in GdnCl gradient disabled the quantification of samples containing all three isoforms (Figure 2A).
- Substituting chloride with thiocyanate anion decreased the peak width of LIN pDNA, but it shifted its retention time towards OC pDNA (no separation between OC and LIN pDNA).
- Combining GdnCl and GdnSCN into a mixed elution was a trade-off, enabling separation of all 3 isoforms (Figure 2B).

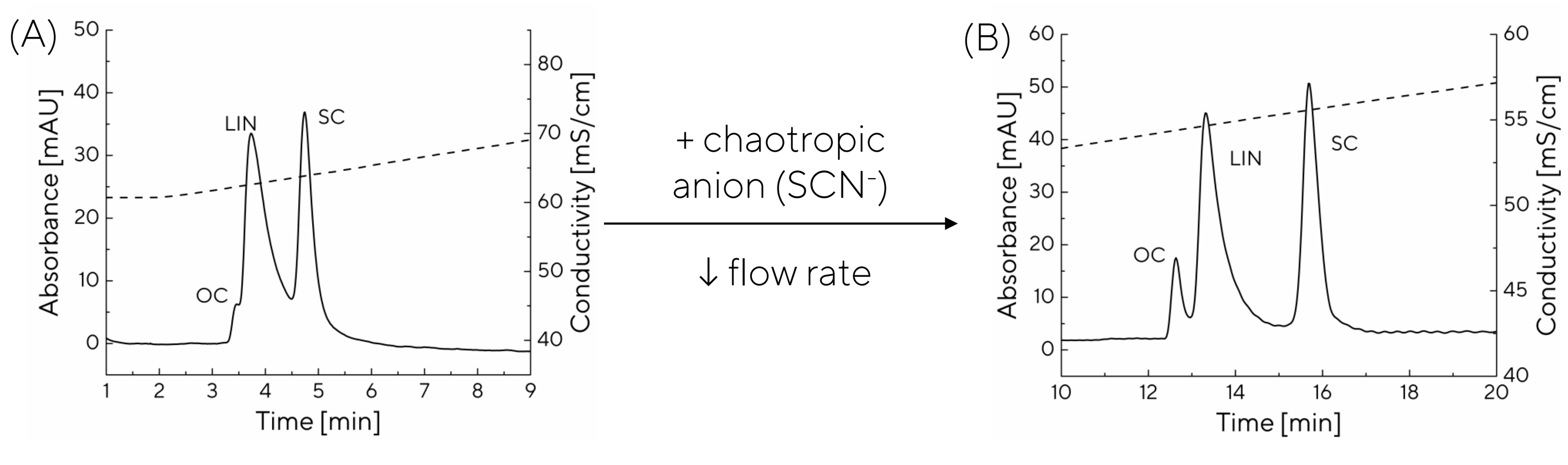

Figure 2: Elution profile of pFix15 on CIMac pDNA columns in GdnCl gradients. (A) Elution profile of pFix15 spiked with LIN pFix15 isoform at 1.0 ml/min. (B) Elution profile of pFix15 spiked with LIN pFix15 isoform at 0.5 ml/min and added chaotropic anion (SCN−).

3. Results – Preparative chromatography - elution of SC- or OC-enriched pDNA (> 10 kbp) from CIMmic DEAE 2 µm

Figure 3 shows elution profiles from column with 2 µm channel diameter, fully saturated with 15.4 kbp large pDNA. Expected elution behaviour and recovery were achieved with SC-enriched sample (99% SC composition), see solid line in Figure 3. The backpressure at the end of the run returned to the initial value.
The pDNA elution was negligible, when eluting OC pDNA-enriched sample (55% OC composition) from 2 µm CIMmic DEAE column (dotted line in Figure 3). Washing the column with 0.1 M NaOH afterwards irreversibly blocked the column . With this we have confirmed the OC pDNA being the main culprit for issues when using anion-exchanging monoliths for capturing large pDNA samples.

4. Results – elution recovery for 11.6 kbp pDNA on different chromatographic media during preparative loading

The difference between porous particles and convection media was demonstrated with preparative loading of pDNA (11.6 kbp) onto 1 mL columns. Convective entrapment of OC isoform was confirmed on both types of stationary phase, when convective channel diameter was below 3 µm. Porous beads in addition showed a reduced recovery of supercoiled pDNA, caused by diffusional entrapment within the internal porous structure of the beads. Use of convective AEX monoliths or membranes with channel diameter > 3.5 µm has been shown to increase yields and prevent irreversible pressure build-up and column clogging.

Table 1: Evaluation of OC pDNA reversible entrapment and overall as well as OC and SC pDNA recoveries for porous beads and convection-based stationary phases. 0.5 mg of 11.6 kbp large pDNA with OC pDNA percentage of 22% was loaded on 1 mL columns.

Column	Convective channel size (µm)	Pore size (nm)	Overall pDNA recovery (%)	SC pDNA recovery (%)	OC pDNA recovery (%)
CIMmultus™ DEAE	2	2000	91	92	88
CIMmultus™ DEAE	6	6000	98	98	98
SOURCE ™ 15Q	2.25	2–100	70	70	57
POROS™ 50 D	7.5	100–800	15	15	4
Fractogel® EMD	6–13.5	80	82	84	88
Sartobind® Q	3–5	3000–5000	99	100	99

5. Conclusions

- OC, SC and LIN pDNA isoforms of up to 16 kbp large plasmids could be analysed using CIMac pDNA column with 6 µm channel diameter.
- Addition of stronger chaotropic salts in the elution buffer (guanidinium and thiocyanate) improves the resolution between pDNA isoforms.
- OC pDNA was confirmed to be responsible for blocking the columns and low recoveries in preparative anion-exchanging chromatography of large pDNA.
- We have shown the applicability of anion exchanging convective monoliths or membranes with > 4 µm channels diameter for efficient pDNA capture at least up to 16 kbp in size.

References

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