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Enhancement of anion-exchange chromatography of DNA using compaction agents

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Abstract

The use of adsorptive chromatography for preparative nucleic acid separations is often limited by low capacity. The possibility that the adsorbent surface area sterically accessible to nucleic acid molecules could be increased by reducing their radius of gyration with compaction agents has been investigated. The equilibrium adsorption capacity of Q Sepharose anion-exchange matrix for plasmid DNA at 600 m*M* NaCl was enhanced by up to ca. 40% in the presence of 2.5 m*M* spermine. In addition, compaction agent selectivity has been demonstrated. Spermine, for example, enhances the adsorption of both plasmid and genomic DNA, spermidine enhances binding only of plasmid, and hexamine cobalt enhances only the binding of genomic DNA. Compaction may be generally useful for enhancing adsorptive separations of nucleic acids.

Keywords: Adsorption; Compaction agents; Preparative chromatography; DNA; Spermine; Spermidine; Hexamine cobalt

1. Introduction

The increasing importance of nucleic acids in medicine and biological sciences [1–4] is driving demand for improved methods of separation of DNA from RNA, proteins, and other contaminants. Anion-exchange chromatography is widely used for purification of RNA and DNA, with plasmid DNA remaining adsorbed in high salt (usually 0.65–0.75 *M* NaCl) while proteins and RNA flow through the column. With a proper gradient, it is also possible to separate supercoiled and open circular forms of DNA on an anion-exchange column [5]. The main drawback to this method is the low loading capacity of typical matrices, which typically adsorb ca. 0.5–1.0 mg plasmid DNA per ml in contrast to 10–100 mg per ml for proteins.

We speculated that the chromatographic separation of nucleic acids could be improved using compaction agents. Compaction agents are involved in vivo in regulating cell growth and differentiation and at high concentration can reduce the volume occupied by DNA by 10^4–10^6 [6,7]. Well-characterized compaction agents include spermine, spermidine, hexamine cobalt(III), and manganese chloride.

Compaction agents associate with the backbone of double-stranded DNA by binding in either the major or the minor groove, in direct contact with the negatively charged phosphate oxygens and the partial

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charges on the bases [8,9]. In a seminal study [10], Wilson and Bloomfield showed that to condense phage λ DNA with spermine approximately 90% of the charges on the DNA must be neutralized. The second effect of compaction agents is inter-molecular bridging by the simultaneous binding of sites on two adjacent DNA helices, which stabilizes direct association and helps to promote condensation at low ionic strength [11].

The hypothesis tested here was that compaction of plasmid DNA and the reduction of its radius of gyration would facilitate access to pores and allow an increased amount of plasmid to pack onto the same adsorbent surface area. The reduced effective charge of the plasmid might also allow closer packing on the adsorbent surface because of reduced electrostatic repulsion between plasmid DNA molecules.

2. Experimental

2.1. Plasmid production

The plasmid pBGS19luxwt (5.9 kb), a pUC19 derivative expressing *Vibrio harveyi* luciferase [12], was isolated from *Escherichia coli* strain JM109 cultivated in a 20 l Applikon fermentor [13]. Purification involved alkaline lysis [14], RNAse treatment (200 U/l), Celite (World Minerals) filtration [15], isopropanol precipitation, and anion-exchange chromatography [16].

Anion-exchange chromatography employed a fast protein liquid chromatography (FPLC) system (Amersham Pharmacia Biotech) with a 60 cm×2.5 cm Spectrum column packed with 150 ml Q Sepharose Fast Flow (Amersham Pharmacia Biotech) and equilibrated in 10 column volumes of 10 m*M* Tris–HCl and 1 m*M* EDTA at pH 8.0 (terminating electrolyte, TE) with 570 m*M* NaCl. Loading and elution were carried out at a linear velocity of 90 cm/h. The column was washed with 1 column volume of TE+570 m*M* NaCl followed by 4 column volumes of TE+600 m*M* NaCl. A linear gradient of NaCl (600 to 700 m*M* NaCl) in TE over 4 column volumes was used to elute the plasmid. Absorbance was monitored at 254 nm and appropriate fractions were collected with a final yield of 0.75±0.02 mg plasmid DNA per g dry cell mass.

2.2. Isotherm measurement

The methods used here are largely derived from our previous studies of anion-exchange adsorption of cytochrome b_5 [17–19]. Equilibrium adsorption isotherms were measured, in duplicate, using 0.6-ml microcentrifuge tubes (Fisher). The order of reagent addition was as follows: 10 m*M* Tris–HCl (Sigma) pH 8.0 (all subsequent additions were in this buffer), 2 *M* NaCl, compaction agent, 50% (v,v) slurry of Q Sepharose Fast Flow (Amersham Pharmacia Biotech) adsorbent, and finally the nucleic acid.

After vortexing, tubes were rotated end-over-end in a Roto-Torque Heavy Duty Rotator (Cole-Palmer Instrument Co.) for 1.5 h, a time found in control experiments to be sufficient for equilibration. After equilibration, the tubes were centrifuged in an Eppendorf microcentrifuge for 2 min and the supernatant removed for determination of nucleic acid concentration by absorbance measurement (using $\varepsilon_{260}=0.02$ ml/μg nucleic acid). Adsorbed nucleic acids were then eluted with 2 *M* NaCl and rotated end-over-end for 2 h. The tubes were then centrifuged and absorbance measurements were taken of the resulting supernatant.

3. Results

3.1. Isotherms with compaction agents

Initial testing of adsorption conditions was done with salmon sperm DNA [~2 kilobase pairs (kb), Sigma] at 75 m*M* NaCl concentration. As shown in Fig. 1, the addition of 1 m*M* spermidine (Sigma), at this ionic strength increased binding capacity approximately twofold. An identical experiment with total bakers yeast RNA (Sigma), in contrast, showed no enhancement of binding (results not shown).

As most anion-exchange separations of nucleic acid take place at significant ionic strength, we investigated the effects of compaction agents at high salt concentrations. The binding of salmon sperm DNA and pBGS19luxwt plasmid DNA to Q Sepharose was measured at sodium chloride concentrations

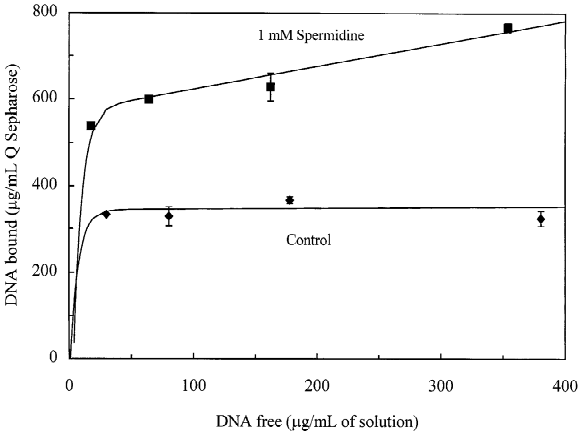

Fig. 1. Salmon sperm DNA equilibrium adsorption isotherm on Q Sepharose in 10 m*M* Tris–HCl, pH 8.0 with 75 m*M* NaCl, with and without 1 m*M* spermidine.

of 100, 300, and 600 m*M*, DNA concentrations of up to 150 μg/ml, and in the presence of no compaction agent, 2.5 m*M* spermidine, 2.5 m*M* spermine or 2.5 m*M* hexamine cobalt (Sigma).

Table 1 summarizes the results for all isotherms. Fig. 2 shows the salmon sperm DNA adsorption isotherms and Fig. 3 the plasmid DNA adsorption isotherms in 600 m*M* NaCl. At 600 m*M* NaCl, spermine and hexamine cobalt clearly enhance the salmon sperm DNA adsorption, but spermidine does not produce any significant increase in linear salmon sperm DNA binding at any ionic strength.

While both salmon sperm and plasmid DNA adsorption can be enhanced by compaction agents, the effects on plasmid differ significantly from those for salmon sperm DNA. Most importantly, spermidine enhances plasmid adsorption at 300 and 600 m*M* NaCl, while not affecting adsorption of linear salmon sperm DNA. This difference in behavior between circular plasmid and linear DNA is potentially very useful, as linear genomic DNA is an important contaminant in plasmid preparations.

At 100 m*M* NaCl spermine and hexamine cobalt induce precipitation of DNA. This effect was previously known [20] and we have extended this work, taking advantage of the previously unknown selectivity between plasmid DNA and RNA contaminants

Table 1
Qualitative summary of adsorption results

DNA type	NaCl conc. (m*M*)	Compaction agent (2.5 m*M*)	Effect
Salmon sperm DNA	600	Spermidine	No effect
	600	Spermine	Enhanced binding
	600	$Co(NH_3)_6$	Enhanced binding
	300	Spermidine	No effect
	300	Spermine	No effect
	300	$Co(NH_3)_6$	No effect
	100	Spermidine	No effect
	100	Spermine	Precipitation
	100	$Co(NH_3)_6$	Precipitation
pBGS19luxwt	600	Spermidine	Enhanced binding
	600	Spermine	Enhanced binding
	600	$Co(NH_3)_6$	No effect
	300	Spermidine	Enhanced binding
	300	Spermine	Enhanced binding
	300	$Co(NH_3)_6$	No effect
	100	Spermidine	Enhanced binding
	100	Spermine	Precipitation
	100	$Co(NH_3)_6$	Precipitation

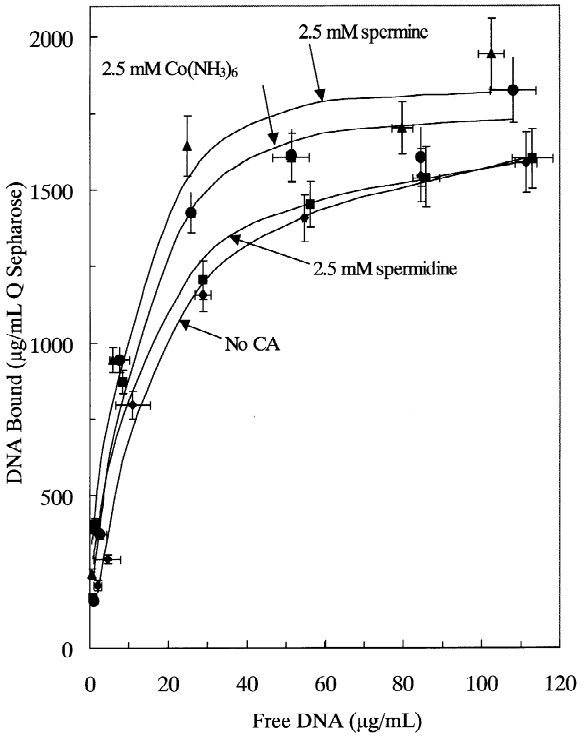

Fig. 2. Salmon sperm DNA equilibrium adsorption isotherm on Q Sepharose in the presence of various compaction agents at a 2.5 m*M* concentration in 10 m*M* Tris–HCl, pH 8.0 with 600 m*M* NaCl.

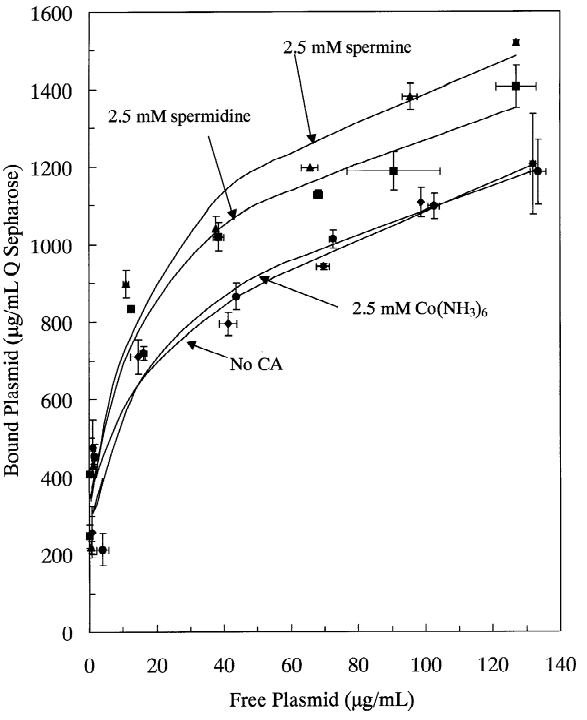

Fig. 3. pBGS19luxwt adsorption on Q Sepharose in the presence 2.5 m*M* of various compaction agents concentration in 10 m*M* Tris–HCl, pH 8.0 with 600 m*M* NaCl.

as the basis of an efficient compaction–precipitation protocol for plasmid isolation [21,22].

3.2. Hill analysis for salmon sperm DNA and pBGS19luxwt isotherms

The Hill plots [23] for adsorption of salmon sperm DNA on Q Sepharose at 600 m*M* NaCl were linear (Fig. 4). These Hill plots were fit using the method described by Jones [24] to extract well-defined values of n_H and K_D (Table 2). When the same Hill analysis was applied to the plasmid isotherms, however, the curves had a very distinct curvature suggestive of considerable complexity (Fig. 5 and Table 3).

Data analysis was done using Hill fits to the isotherm data. From this n_H (the Hill cooperativity parameter) and K_D (dissociation constant) were found and adsorption differences were determined from differences in the logarithms of the dissociation constants. The ultimate DNA binding capacity (*m*) was taken as 2200 μg/ml of matrix for salmon sperm DNA and 1500 μg/ml for plasmid DNA (fit parameters were found to be relatively insensitive to the capacity value chosen).

The percent binding increases (log K_D decreases) summarized in Tables 2 and 3 illustrate the effects of compaction agent addition. For plasmid in 600 m*M* NaCl, the addition of both the minor groove-binders spermidine and spermine enhanced binding by about 36%, while the major groove-binding hexamine cobalt did not enhance binding. On the other hand, with salmon sperm DNA at 600 m*M* NaCl spermine enhanced binding 42%, hexamine cobalt enhanced binding 21%, and spermidine enhanced binding 15%.

Curvature of the plasmid Hill plots may arise from heterogeneity of binding sites, lateral adsorbate interactions, or the existence of multiple adsorbate

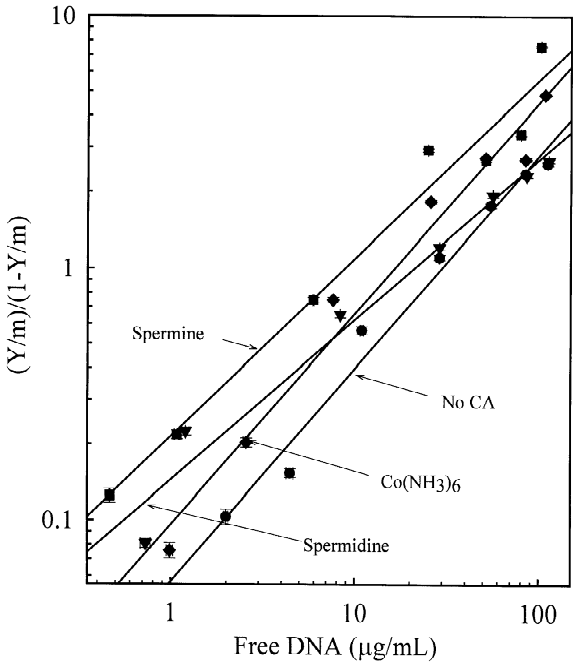

Fig. 4. Hill plot for adsorption of salmon sperm DNA on Q Sepharose in 600 m*M* NaCl (Fig. 2). Y stands for μg of DNA bound per ml of Q Sepharose, and *m* is the ultimate DNA binding capacity of the matrix (μg/ml).

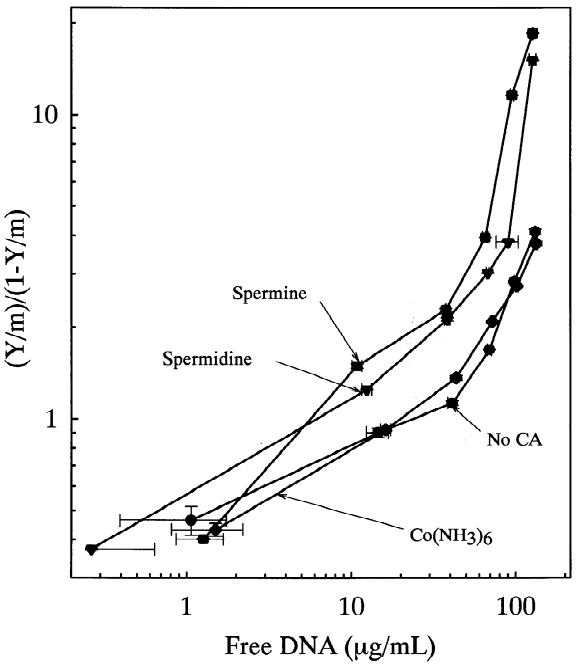

Fig. 5. Hill plot for adsorption of plasmid pBGS19luxwt on Q Sepharose in 600 m*M* NaCl (Fig. 3). Y stands for μg of DNA bound per ml of Q Sepharose, and *m* is the ultimate DNA binding capacity of the matrix (μg/ml).

species in solution. When a Hill plot is curved, an overall slope and intercept cannot be calculated. The parameters that are calculated are the K_{50} and the n_{50} where K_{50} is the dissociation constant and n_{50} is the Hill cooperativity parameter defined at 50% loading. The plasmid Hill plots were used to calculate the relative amounts of binding affinity increase from the percentage K_{50} decrease. A sensitivity analysis done

Table 2
Hill constants and percent binding increase with various compaction agents for salmon sperm DNA

	NaCl (m*M*)	Compaction agent	Log K_D (μg/ml)±SD	% Log K_D decrease±SD	n_H±SD
Salmon sperm DNA	600	None	1.36±0.02	–	0.92±0.01
	600	Spermidine	1.16±0.02	15±3	0.69±0.01
	600	Spermine	0.78±0.07	42±7	0.83±0.07
	600	$Co(NH_3)_6$	1.07±0.02	21±3	0.95±0.02
	300	None	2.00±0.09	–	0.90±0.04
	300	Spermidine	2.04±0.03	−2±9	0.73±0.01
	300	Spermine	2.02±0.01	−1±9	0.78±0.00
	300	$Co(NH_3)_6$	2.10±0.05	−5±10	0.55±0.01
	100	None	2.70±0.15	–	0.47±0.03
	100	Spermidine	2.52±1.26	7±127	0.44±0.22
	100	Spermine		Precipitated	
	100	$Co(NH_3)_6$		Precipitated	

Table 3
Hill constants and percent binding increase with various compaction agents for plasmid DNA (pBGS19luxwt)

	NaCl (m*M*)	Compaction agent	Log K_{50} (μg/ml)±SD	% Log K_{50} decrease±SD	n_{50}±SD
pBGS19luxwt Plasmid DNA	600	None	1.3±0.1	–	1.1±0.1
	600	Spermidine	0.8±0.1	36±5	1.1±0.1
	600	Spermine	0.8±0.2	36±9	1.3±0.1
	600	$Co(NH_3)_6$	1.3±0.1	0±8	1.1±0.1
	300	None	1.8±0.1	–	1.0±0.1
	300	Spermidine	1.7±0.1	3±5	1.0±0.1
	300	Spermine	1.4±0.2	20±9	1.0±0.1
	300	$Co(NH_3)_6$	1.6±0.1	9±5	1.0±0.1
	100	None	2.1±0.1	–	1.0±0.1
	100	Spermidine	1.8±0.1	15±5	1.0±0.1
	100	Spermine		Precipitated	
	100	$Co(NH_3)_6$		Precipitated	

on the fitted lines showed that small deviations in the fitted curves did not radically affect the calculated differences in loading capacity. These K_{50}, n_{50} and the percent binding increases for pBGS19luxwt plasmid DNA are shown in Table 3.

4. Discussion

Several factors affect the DNA/Q Sepharose system in different ways than previously explored protein anion-exchange systems. First, the apparent Hill binding affinity of DNA for Q Sepharose increases with increasing NaCl concentration, possibly due to reduced intermolecular repulsion between DNA molecules, allowing DNA to pack more closely on the surface of the Q Sepharose. Reduced kinetic trapping near the particles' surfaces may also play a role.

An unanticipated observation was the selectivity of some compaction agent–DNA interactions. These point to possible enhancements in column separations as an isotherm is a one-stage batch separation, while a column could amplify selectivity effects through multiple stages of separation.

Possible sources of Hill plot curvature for the plasmid include intrinsic heterogeneity of binding sites on the surface of the Q Sepharose, lateral interactions of plasmid molecules, and heterogeneity of the plasmid molecules. All of the Hill cooperativity parameters for the plasmid isotherms are approximately unity. Our previous work on anion-exchange adsorption of cytochrome b_5 [18], showed $n_h<1$ which was ascribed to adsorbent site heterogeneity, but Hill plots of these isotherms were linear.

The heterogeneity of the plasmid isotherms is believed to arise from the presence of multiple plasmid species in solution. There was some nicked and linear plasmid (ca. 5%), but the main difference was different degrees of supercoiling. The writhing number, which corresponds to the number of twists in a supercoiled plasmid (with open-circle DNA having a writhing number equal to 0), varies from about 0 to −10 in typical plasmid preparations.

In contrast to the pBGS19luxwt isotherms, the salmon sperm DNA did show evidence of negative cooperativity with $n_H<1$, and the amount of negative cooperativity decreasing with increasing salt concentration. We believe that the negative cooperativity effects for the linear salmon sperm DNA arise from surface heterogeneity, and from the extended linear DNA occupying a large area at low salt concentrations. At higher ionic strength, intermolecular repulsion is reduced, and the DNA can pack more closely on the surface.

Roush et al. [18] saw similar reductions in heterogeneity at higher salt concentrations. These were interpreted as evidence of either reduced lateral interaction, or selection of a reduced, more-uniform set of adsorbent sites as ''acceptable'' at higher salt

concentrations. The latter explanation was favored, as apparent negative cooperativity was seen even at very low adsorbent loadings.

5. Conclusions

The use of compaction agents to enhance binding capacities of DNA on anion exchangers was demonstrated. A maximum binding enhancement of 40% in equilibrium adsorption experiments was found. Compaction agents also have varying selectivities for different nucleic acids. This selective enhancement of adsorption could be an asset in the separation of linear and plasmid DNA. The use of conformational control to enhance adsorption may also be applicable to separations of other large biopolymers.

Acknowledgements

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References

[1] S. Gurunathan, D.M. Klinman, R.A. Seder, Annu. Rev. Immunol. 18 (2000) 927.
[2] D. Ferber, Science 294 (2002) 1638.
[3] A.D. Ellington, Curr. Biol. 4 (1994) 427.
[4] A. Pease, D. Solas, E. Sullivan, M. Cronin, C. Holmes, S. Fodor, Proc. Natl. Acad. Sci. USA 91 (1994) 5022.
[5] I. Drevin, L. Larsson, B.L. Johansson, J. Chromatogr. 477 (1989) 337.
[6] P.G. Arscott, A.Z. Li, V.A. Bloomfield, Biopolymers 30 (1990) 619.
[7] R. Hasan, M.K. Alam, R. Ali, FEBS Lett. 368 (1995) 27.
[8] J.S. Kieft, I. Tinoco, Structure 5 (1997) 713.
[9] J. Bednar, P. Furrer, A. Stasiak, J. Dubochet, E.H. Egelman, A.D. Bates, J. Mol. Biol. 235 (1994) 825.
[10] R. Wilson, V.A. Bloomfield, Biochemistry 18 (1979) 2192.
[11] D. Bancroft, L.D. Willams, A. Rich, M. Egli, Biochemistry 33 (1994) 1073.
[12] K. Maillard, Ph.D. Dissertation, University of Houston, Houston, TX, 1996.
[13] Manual of Dehydrated Culture Media and Reagents for Microbiological and Clinical Procedures, Difco Laboratories, 1953.
[14] J. Sambrook, E.F. Fritsch, T. Maniatis, Molecular Cloning. A Laboratory Manual, 3rd ed., Cold Spring Harbor Laboratory Press, New York, 1989, p. 2100.
[15] N. Horn, M. Marquet, J. Meek, G. Budahazi, US Patent, 5 576 196 (1996).
[16] I. Drevin, L. Larsson, B.L. Johansson, J. Chromatogr. 477 (1989) 337.
[17] D.S. Gill, D.J. Roush, R.C. Willson, J. Colloid Interface Sci. 167 (1994) 1.
[18] D.J. Roush, D.S. Gill, R.C. Willson, J. Chromatogr. A 653 (1993) 207.
[19] N. Singh, R.C. Willson, J. Chromatogr. A 840 (1999) 205.
[20] B.C. Hoopes, W.R. McClure, Nucleic Acids Res. 9 (1981) 5493.
[21] J.C. Murphy, J.A. Wibbenmeyer, G.E. Fox, R.C. Willson, Nat. Biotechnol. 17 (1999) 822.
[22] J.C. Murphy, G.E. Fox, R.C. Willson, Anal. Biochem. 295 (2001) 143.
[23] A.V. Hill, J. Physiol. 40 (1910) iv–vii.
[24] M.N. Jones, Biochemical Thermodynamics, Elsevier, New York, 1979, p. 403.