Primary Recovery of Biologically Active Compounds Using Macroporous Monolithic Hydrogels

Hanora, Amro

2005

Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1087 (2005) 38–44
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Screening of peptide affinity tags using immobilised metal affinity chromatography in 96-well plate format

Amro Hanora[a,d], Florent Bernaudat[b], Fatima M. Plieva[a,c], Maria B. Dainiak[a,c], Leif Bülow[b], Igor Yu. Galaev[a], Bo Mattiasson[a,*]

[a] *Department of Biotechnology, Centre for Chemistry and Chemical Engineering, Lund University, P.O. Box 124, SE-22100 Lund, Sweden*
[b] *Department of Pure and Applied Biochemistry, Centre for Chemistry and Chemical Engineering, Lund University, P.O. Box 124, SE-22100 Lund, Sweden*
[c] *Protista Biotechnology AB, P.O. Box 86, SE-26722 Lund, Sweden*
[d] *Department of Microbiology and Immunology, Faculty of Pharmacy, Suez Canal University, Ismailia, Egypt*

Available online 11 May 2005

Abstract

A method for high throughput screening of Green Fluorescent Proteins carrying metal binding tags in bacteria was developed. A random four amino acids tag-peptide library was successfully generated in *E. coli*. A 96-microtiter plate assembled with metal-iminodiacetic acid small cryogel columns was used for library screening. For the first time we were able to simultaneously screen a metal binding peptide tags library obtained from *E. coli* against different metal ions. From screening 25 different tags, three clones were able to bind to all metal ions studied (Ni^{2+}, Zn^{2+}, Co^{2+} and Cd^{2+}). It was clearly demonstrated that the new construct could facilitate the screening of large peptide libraries.

Keywords: Peptide library; *E. coli*; High throughput screening; Immobilised metal affinity chromatography; Cryogels; Green fluorescent protein

1. Introduction

The Green Fluorescent Protein (GFP) was first extracted from the jellyfish *Aequorea victoria* for the study of bioluminescence in photobiology [1,2]. Recently, GFP has been widely used as a reporter protein since it provides a fluorescent phenotype upon UV excitation [3,4]. GFP has also been used as fusion partner, together with different affinity tags at the N- or C-terminus (or both), providing a molecular recognition site for selective interaction with ligands immobilised onto a suitable matrix. Particularly, immobilised metal affinity chromatography (IMAC) makes use of matrix-bound metals, to purify biomaterials on the basis of their interaction with the immobilised metal ions [5–10]. Six histidine tags (His_6) have been frequently used to facilitate protein purification. However, various available literature reports, describe the purification of a target protein using metal binding tails other than His_6 [11–13]. Advances in peptide and protein library technology have made it possible to select new ligands with novel binding specificities and high endurance to chemical and physical conditions. Iminodiacetic acid (IDA) is by far the most widely used chelating ligand. Matrices with coupled IDA are commercially available from many producers, although in the past several years, other chelators have also been tried for immobilisation to chromatographic media. In general, tetradentate ligands, such as NTA, have higher affinities for metal ions than the tridentate chelator IDA, but they exhibit lower protein binding due to the loss of one coordination site for the metal ion [14]. When using IDA, the affinities of many retained proteins and their respective retention times are in the following order: $Cu^{2+}(II) > Ni^{2+}(II) > Zn^{2+}(II) \geq Co^{2+}(II)$ [10]. Several screening methods have been developed for selection of molecules with high affinity and specificity from different proteins, peptides or nucleic acids libraries [15,16].

Abbreviations: *E. coli*, *Escherichia coli*; IPTG, isopropyl-β-D-thiogalactopyranoside; LB, Luria–Bertani medium; IDA, iminodiacetic acid; NTA, nitrilotriacetic acid; CV, column volume; GFP, Green Fluorescent Protein; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; BSA, bovine serum albumin; SPR, Surface plasmon resonance

* Corresponding author. Tel.: +46 46 2228264; fax: +46 46 2224713.
E-mail address: bo.mattiasson@biotek.lu.se (B. Mattiasson).

0021-9673/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2005.04.029

In this paper, we constructed a random peptide library fused to GFPuv, an enhanced mutant of GFP, expressed in *E. coli* cells. Using IMAC in a novel miniature chromatographic mode, the fused peptides could be screened against different metals immobilised with IDA ligands. The chromatographic step was developed into a 96-well format allowing processing of samples without the need for artificial pressure drop as the matrix is supermacroporous [17]. The matrix used to pack the column presents also the advantage of being protected against running dry.

2. Material and methods

2.1. Bacterial strains and plasmids

E. coli strain TGI (supE thi-1 Δ(lac-proAB) Δ(mcrB-hsdSM)5 (r_K^- m_K^-) [F′ *traD36 proAB lacI*^{q}*ZΔM15*]) was used as a host in all experiments and plasmid pTrc99a (GE Healthcare, Uppsala, Sweden) was used as expression vector. The construction of plasmids pTGFP and pTH6GFP, expressing GFPuv and a hexa-histidine tagged GFPuv, respectively, were described previously [18,19].

2.2. Enzymes, chemicals and reagents

Restriction endonucleases, T4 DNA ligase, DNA molecular weight marker III, "Expand High Fidelity PCR System" and *Taq* DNA polymerase were obtained from Roche (Mannheim, Germany). The Qiaprep, spin miniprep kit and the QIAquick, Gel Extraction Kit was obtained from Qiagen (Basel, Switzerland). PopCulture reagent from Novagen (Madison, WI, USA). Chelating Sepharose Fast Flow was from Amersham Biosciences (Uppsala, Sweden). Ni^{2+}-NTA Sepharose was obtained from IBA (Göttingen, Germany). *N*,*N*-Dimethylacrylamide (DMAA, 99%), ammonium persulfate (APS) and allyl glycidyl ether (AGE, 99%) were bought from Aldrich (Steinheim, Germany). *N*,*N*′-Methylenebis(acrylamide) (MBAAm) was from Acros (Geel, Belgium); iminodiacetic acid (IDA) was from Fluka (Buchs, Switzerland). *N*,*N*,*N*′,*N*′-Tetramethylethylenediamine (TEMED) and imidazole were purchased from Sigma (Stockholm, Sweden). All other chemicals were of analytical grade and commercially available

2.3. Library construction

Amplification of the *gfpuv* gene was performed using two primers, obtained from MWG Biotech AG (Ebersberg, Germany) and plasmid pTGFP as a template. The sense primer is identical to the coding strand of *gfpuv* gene but includes a 5′ overhang containing the random sequence, sense primer: 5′-ATTAGAATTCCAC**NNSNNSNNSNNS**ACCATGAGTAA-AGGA GAAGAACTTTTCACTGG-3′, where N stands for (G, C, A, T) and S for (G, C), antisense primer: 5′-TCTGCGTTCTGATTTAATCTGTATCAGGC-3′. The PCR reaction was performed using the "Expand High Fidelity PCR System".

Table 1
Colony-PCR programme

Cycle	Temperature (°C)	Time	Number of cycles
1	94	10 min	1
2	94	30 s	25
	50	30 s	
	72	1 min	
3	72	10 min	1

The amplified PCR product was isolated after electrophoresis on 1% agarose gel. Bands corresponding to the amplified PCR product were extracted using QIAquick, Gel Extraction Kit from Qiagen, (Basel, Switzerland). Plasmid DNA pTrc99a and DNA originating from the amplification step were restricted using the enzymes *Eco*RI and *Pst*I. The restricted DNAs were then isolated as described above and finally fused overnight at 4 °C. The new constructed plasmids were used to transform competent *E. coli* cells and spread over LB agar plates containing 100 μg/ml ampicillin and 1 mM IPTG. All cloning procedures were performed as described by Maniatis et al. [20]. The fluorescent clones were checked for correct insertion by colony PCR. Colonies were directly picked from agar plates and mixed in PCR tubes with primer pTrc (5′-CATCCGGCTCGTATAATGTGTGGAATT-3′), which anneals upstream to the random insert, the antisense primer described previously and *Taq* polymerase. The mixture was subjected to the following PCR programme (Table 1) and PCR products were isolated by 1% agarose gel electrophoresis to check for amplification. Clones having a correct insert were streaked on LB agar plates for further investigation.

2.4. Cell culture and protein purification

Cells were cultivated in 100 ml flasks containing 20 ml of modified LB broth (10 g/L peptone, 10 g/L NaCl, and 5 g/L yeast extract) supplemented with 100 μg/ml ampicillin and 1 mM IPTG. Flasks were incubated overnight (12–18 h) at 37 °C in a shaking incubator (170 rpm). Cells were harvested by centrifugation at 3000 × *g* for 5 min. The cell pellets were washed and reconstituted in 5 ml of 20 mM Tris–HCl, 0.5 M NaCl, pH 8.0 (buffer A). Cells were then sonicated using a W380 Heat System Ultrasonic, output 5 (1 s pulses with 2 s intervals) for 15 min. The cell homogenate was divided into two parts, one part was left without further treatment as sonicated lysate. In the other part, the cell debris were removed by centrifugation at 20,000 × *g* for 15 min. The crude homogenate obtained was heated at 70 °C for 15 min and centrifuged as above to remove denatured proteins, giving a clarified extract. The supernatant was stored at 4 °C until further used.

2.5. Protein expression in 96-well plates

LB medium (0.1 ml supplemented with 100 μg/ml ampicillin and 1 mM IPTG) was inoculated with individual clones in 96 deep-well microtiter plate with 2 ml cavities and incubated overnight in a shaking incubator at 37 °C at 200 rpm. 0.9 ml of LB (100 μg/ml ampicillin, 1 mM IPTG) was added to each well and the plate was incubated for an additional 3 h.

The production of tagged GFP was confirmed by monitoring the fluorescence under UV. The cells were harvested and the pellets were reconstituted in 250 μl of buffer A. The cells were then lysed either by sonication or addition of 40 μl of PopCulture and let to incubate at room temperature for 15 min.The samples were stored at 4 °C until further used.

2.6. Preparation of 96-well IMAC plate

Holes of 0.3 cm diameter were drilled in 96-well plates (Deep Well Plate, Masterblock, round bottom, polypropylene obtained from Greiner Bio-One, Germany) and the wells were packed with poly(dimethylacrylamide)-based supermacroporous monolithic columns containing IDA ligands. The preparation of supermacroporous gels is described in details in [17,21]. Briefly, DMAA (2.1 ml) and MBAAm (0.75 g) were dissolved in 40 ml of deionised water. Then 0.275 ml AGE was added to the mixture under continuous stirring and the volume was adjusted to 50 ml with deionised water. The mixture was degassed under vacuum for 10 min. Free radical polymerisation was initiated by adding TEMED (35 μl) and APS (30 mg) and the reaction mixture was stirred gently for 30 s. A half milliliter of the reaction mixture was then added very quickly into each well of a 96-well cold mould. The solution in the mould was frozen at −10 °C within 30 min in a cooling chamber, maintained at that temperature overnight and thawed at room temperature. The supermacroporous cryogel columns were washed by passing 10 ml of deionised water through each well. The monoliths were transferred from the mould to a 96-well plate. Iminodiacetic acid was coupled to cryogel columns as described in [17]. Briefly, 4 ml of 0.5 M Na_2CO_3 solution was applied through each well filled with cryogel column, equilibrated with 0.5 M IDA in 1.0 M Na_2CO_3, pH 10.0, then the 96-well plate was placed into a vessel containing 400 ml of the same solution and incubated overnight at room temperature with gentle shaking. Finally, the modified cryogels were washed with water until pH became neutral.

2.7. Screening for peptide ligand from the library

Five column volumes (CV = 0.5 ml), of 100 mM metal chloride (Cu^{2+}, Ni^{2+}, Zn^{2+}, Co^{2+}, Cd^{2+}) solution in water were applied to the columns. The columns were subsequently washed with 6 CV water and 6 CV of 200 mM imidazole to remove the excess of unbound and loosely bound metal ions. Columns were washed with 5 CV water and equilibrated with 6 CV of buffer A. Cell homogenates obtained by either PopCulture reagent or sonication (250 μl) were applied and the columns were washed with 6 CV of buffer A. Elution was carried out with a stepwise gradient of 10, 20, 30, 40, and 200 mM imidazole. Columns were then regenerated by applying 5 CV of 50 mM EDTA and followed by washing with 6 CV of water. The presence of GFP protein in flow-through, washing or elution was detected by monitoring fluorescence under UV irradiation.

2.8. DNA sequencing

pTrc primer was used as template for DNA sequencing using BigDye-terminators version 3.0 from Applied Biosystems (Warrington, UK), according to the supplier's instructions. The results were analysed on an ABI 3100 DNA sequencer by the BM unit at Lund University.

2.9. Comparison of the binding strength of selected tag-GFPs

The chromatographic experiments were performed using a Tricorn column (0.5 cm × 10 cm) coupled to a Biologic DuoFlow Chromatographic System (Bio-Rad, Hercules, CA, USA). The column was filled with either 1.2 ml IDA or NTA Sepharose gel loaded with Ni^{2+}-ions and equilibrated with running buffer A at a flow rate of 1 ml/min. One millilitre of heat-treated samples was applied to the columns. Washing was performed with running buffer A, elution was carried out using a continuous imidazole gradient (0–100 mM) and fractions of 1 ml were collected. The presence of GFP was monitored in a fluorescence system from Photon Technology International (West Sussex, UK), using a photomultiplier detection system model 814. The fluorescence intensity was measured at 508 nm (excitation at 400 nm) and expressed in arbitrary units. For each clone, the three most fluorescent fractions collected from the IDA columns, were combined and dialysed overnight in water using a 3.5 kDa cut-off Spectra/Por dialysis membrane from Spectrum Laboratories (Rancho Dominguez, CA, USA). Proteins were then concentrated by lyophilisation, re-suspended in 200 μl deionised water and analysed by SDS-PAGE using a 15% slab gel [20].

2.10. Protein expression level

Cell lysate from selected clones were obtained as previously described for flask cultures, but in this case the cells were re-suspended to the same density after harvesting. The fluorescence intensities of the supernatant were determined both before and after heat-treatment. The different samples were analysed by SDS-PAGE by determining the GFP content in the bands found at 30 kDa on the gel using Alpha Innotech Corporation gel documentation system equipped with the AlphaImager 2200 software (San Leandro, CA, USA).

2.11. Surface plasmon resonance measurements

All surface plasmon resonance (SPR) measurements were performed in a BIAcore 3000 system (Uppsala, Sweden) using a NTA sensor chip immobilised with nickel ions. Proteins extracts purified on a Ni^{2+}-NTA Sepharose column and predialysed in running buffer (0.01 M HEPES, 0.15 M NaCl, pH 7.4). In the case of native GFP, the protein was purified by extraction with organic solvent as described in [22]. The proteins were injected onto the chip at a concentration 200 nM for 6 min at a flow rate of 30 μl/min and then allowed to dissociate. Between each run, the chip was regenerated and re-immobilised with nickel ions, according to the manufacturer's instructions.

3. Results and discussion

The peptide library was generated by the addition of the amino acid sequence MEFHXXXXH (where X represents any amino acid) at the N-terminal of the GFP. Twenty-three clones were selected for further studies. To screen the expressed proteins, a recently developed chromatographic format comprising of 96 monolithic supermacroporous IMAC columns has been used. Poly(dimethylacrylamide)-based supermacroporous monolithic columns were produced by polymerisation of the monomers in a moderately frozen system. When freezing the initial solution, the solutes are concentrated in a small non-frozen part of the sample despite that the whole sample looks as a solid block. The polymerisation proceeds in these non-frozen zones, resulting in the formation of polymer network around ice crystals, which act as templates. When the ice is melted, a system of large (1–100 μm in size) interconnected pores is left in place of the ice crystals. The entire polymer in the system is expelled in a relatively small volume of the pore walls. Hence the polymer concentration in the walls is fairly high, above 20% (w/v) as the initial total concentration of monomers used was about 5% (w/v) [23]. A high polymer content in the pore walls makes them strong and endorses the whole monolith with elasticity. Thus, it was possible to compress monoliths (diameter of 0.71 cm) slightly and insert in the wells (diameter of 0.69 cm) of the plastic 96-well plate. The degree of compression (i.e. the diameter of the well in the plastic plate) was optimised so that the compressed monolith is kept tightly in the well with no leakage occurring in between the monolith and the walls. Moreover, the monoliths in the wells retain the liquid due to the capillary forces, no drainage of the liquid occurred. The application of 0.5 ml liquid into the well packed with monolithic column resulted in displacing of 0.5 ml liquid from the bottom of the monolith. Thus the monolithic columns have running-dry protected properties. Cell debris can pass freely through the monolith columns, which allows library screening without the need to clarify the cell extracts (Fig. 1). Both native-GFP and His_6-GFP could be bound to Cu^{2+}-IDA-monolith and eluted almost at

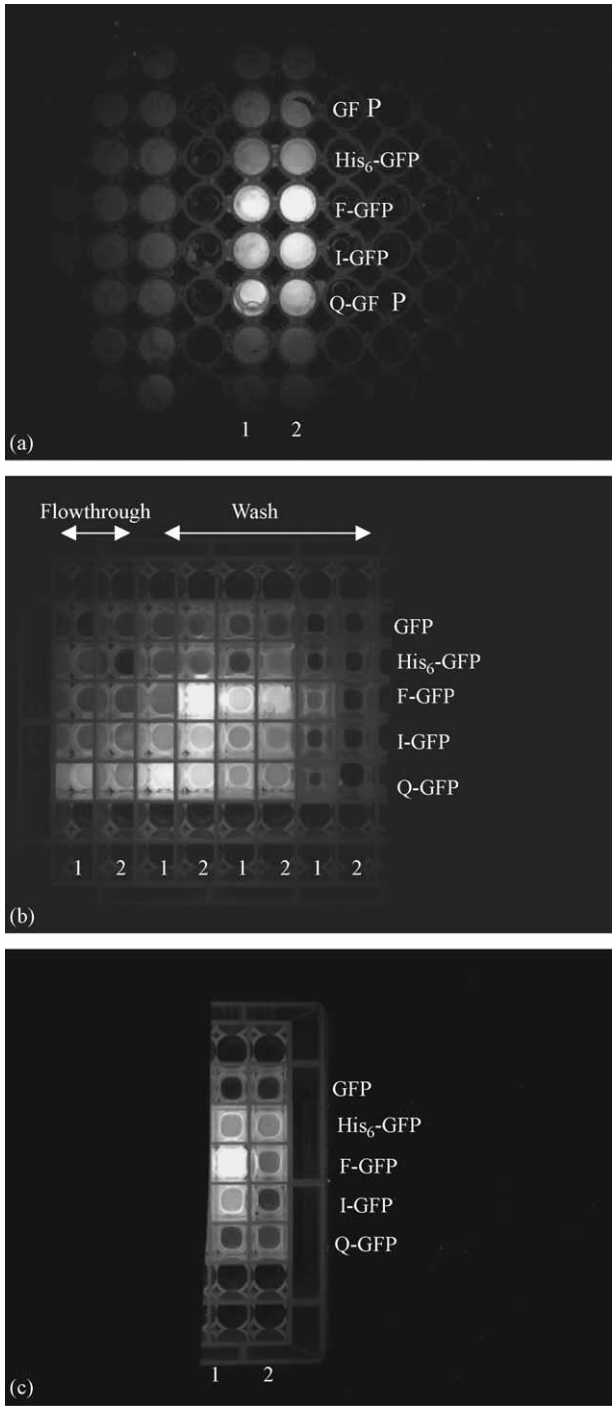

Fig. 1. (a) Photo of a 96-microtiter plate (Deep Well Plate, polypropylene, round bottom, with 3 mm holes drilled at the bottom) packed with supermacroporous poly(dimethylacrylamide)-based monoliths immobilised with nickel ions, after application of the protein extracts. Columns 1 and 2 represent respectively, the cells treated by PopCulture and the sonicated lysate. (b) Photo of a 96-microtiter plate containing the flow-through and wash fractions from the columns in picture a. (c) Photo of a 96-microtiter plate containing the eluted fractions from the columns in picture (a).

Table 2
Binding of proteins expressed by different clones to monolithic supermacroporous columns loaded with different metal ions (Ni^{2+}, Zn^{2+}, Co^{2+}, Cd^{2+})

Name	Tag sequence	Ni^{2+}-IDA	Zn^{2+}-IDA	Co^{2+}-IDA	Cd^{2+}-IDA
Native-GFP	MEFELGT				
His_6-GFP	MGHHHHHHGT	+	+	+	+
A-GFP	MEFH**VRLK**H	+			
B-GFP	MEFH**VCMH**H	+			
C-GFP	MEFH**QETE**H	+			
D-GFP	MEFH**PKLE**H	+			
E-GFP	MEFH**NWMD**H	+			
F-GFP	MEFH**FKS**H	+	+	+	+
G-GFP	MEFH**NAIL**H	+			
H-GFP	MEFH**NRSR**H	+			
I-GFP	MEFH**ANHM**H	+	+	+	+
J-GFP	MEFH**WRSR**H	+			
K-GFP	MEFH**NGSE**H	+			
L-GFP	MEFH**TRSG**H	+			
M-GFP	MEFH**NWMD**H	+			
N-GFP	MEFH**EIDV**H	+			
O-GFP	MEFH**WRAR**H	+			
P-GFP	MEFH**WGYL**H	+			
Q-GFP	MEFH**TSML**H	+	+	+	
R-GFP	MEFH**SRLS**H	+			
S-GFP	MEFH**QKVL**H	+			
T-GFP	MEFH**ALRG**H	+			
U-GFP	MEFH**FQFD**H	+			
V-GFP	MEFH**RSLA**H	+			
W-GFP	MEFH**VWMR**H				

the same imidazole concentrations (20–30 mM imidazole), therefore Cu^{2+}–IDA cannot differentiate between, the native and His_6-tagged GFP, and was not used for screening. On the other hand, apart from copper, all other metal-IDA could differentiate between different tagged-GFPs. The screening results are presented in Table 2. Out of the 23 tagged-GFPs bound to Ni^{2+}-IDA, seven GFP variants were eluted at 20 mM imidazole, while the rest were eluted already at 10 mM imidazole. Three GFP variants (F, I and Q) could bind to Zn^{2+} and Co^{2+}-IDA columns, but only two of them, F-GFP and I-GFP, could even bind to Cd^{2+}-IDA columns. The tag expressed by F-GFP had only three amino acids in the random sequence instead of 4, resulting in a HXXXH sequence, which according to Fujii et al. [24], may form an α-helix which is properly oriented to bind to metals. Surprisingly, the protein expressed by B-GFP bound only to Ni^{2+}-IDA, even though it had a HXXXH sequence, as well as three histidine residues like the tag expressed by I-GFP. In the case of I-GFP, two of the histidines may have the same orientation as they are separated by only one amino acid and therefore be available for binding with metal ions. The respective chromatographic profile for the three proteins expressed by I-GFP, Q-GFP and F-GFP, revealed differences in their binding strength. The proteins were eluted from Ni^{2+}-IDA in the following order Q-GFP, F-GFP and I-GFP in sharp peaks, but at a lower imidazole concentration than His_6-GFP (Fig. 2). His_6-GFP binds strongly to Ni^{2+}-IDA and it is therefore difficult to elute it as a sharp peak, using this imidazole gradient. Native-GFP did not bind to the Ni^{2+}-IDA column and came out in the flow-through fraction. Only His_6-GFP and I-GFP could bind to Ni^{2+}-NTA in column format, which is due to the difference in selectivity between IDA and NTA. Ni^{2+}-NTA is more selective than Ni^{2+}-IDA, because in Ni^{2+}-NTA the nickel ion has less co-ordination sites available for the proteins to bind [14]. The SDS-PAGE, of the eluted fractions from Ni^{2+}-IDA, reveals a significant increase in tag-GFP purification, even though impurities are still present (Fig. 3). The tags have great influence on the expression levels of the proteins (Fig. 4) but no or little effect on the protein stability, as the fluorescence intensity remains high after heat treatment (Fig. 5). Furthermore, the comparison of the amounts of expressed proteins determined by SDS-PAGE analysis, with the fluorescence intensities of the same samples (Table 3), tends to prove that the tags do not influence the activity of GFP.

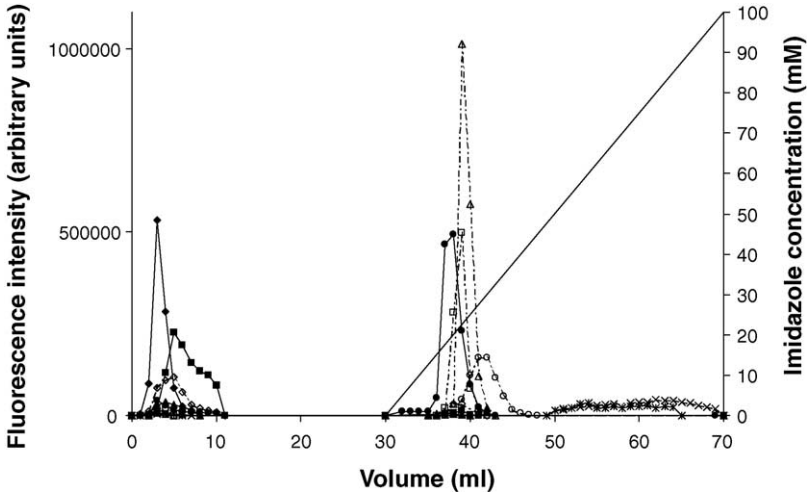

Fig. 2. Comparison of native-GFP (rhombs), His_6-GFP (cross), F-GFP (triangle), I-GFP (circle) and Q-GFP (square) on Ni-IDA Sepharose (dotted line and open symbols) and Ni-NTA Sepharose (straight-line and black symbols) gel columns. Elution was carried out by a continuous imidazole gradient ranging from 0 to 100 mM (straight line). One millilitre of heat-treated extracts was applied onto 1.2 ml of gel at a flow rate 1 ml/min.

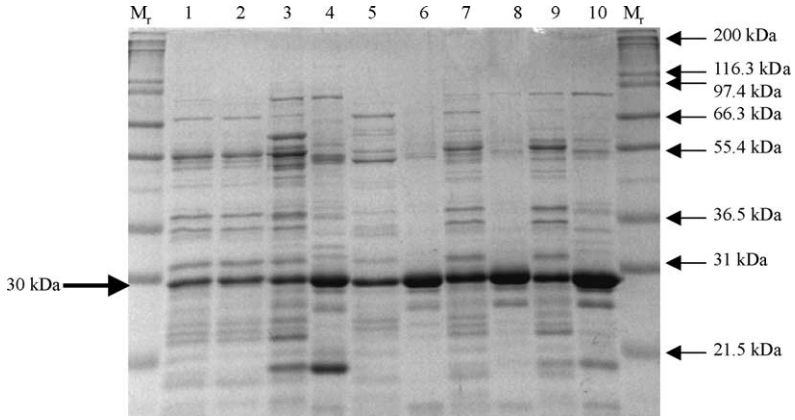

Fig. 3. SDS-PAGE of tagged GFPs before and after Ni^{2+}-IDA column purification. Lanes 1, 3, 5, 7 and 9 contain the applied samples of native-GFP, His_6-GFP, F-GFP, I-GFP and Q-GFP, respectively. Lane 2 the flow-through fraction of native-GFP. Lanes 4, 6, 8 and 10 contain the concentrated purified fractions for His_6-GFP, F-GFP, I-GFP and Q-GFP, respectively. (M_r) protein molecular mass markers.

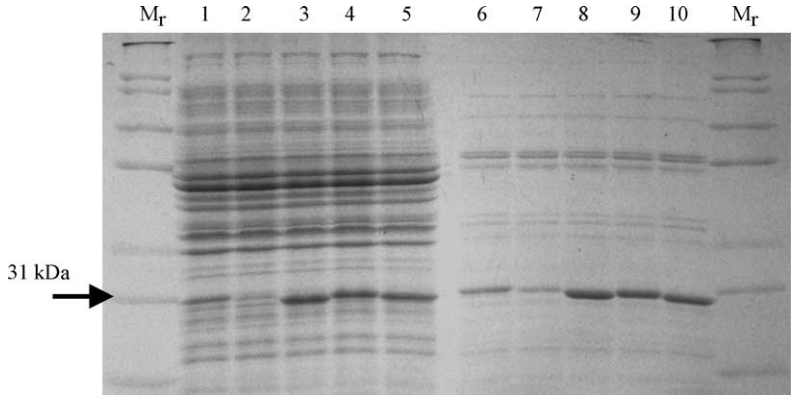

Fig. 4. SDS-PAGE of clarified and non-clarified protein samples. Lanes 1–5, non-clarified samples of native-GFP, His_6-GFP, F-GFP, I-GFP and Q-GFP, respectively. Lanes 6–10 clarified samples of native-GFP, His_6-GFP, F-GFP, I-GFP and Q-GFP, respectively. (M_r) protein molecular mass markers.

His_6-GFP and I-GFP, which both bind stronger to the Ni^{2+}-NTA Sepharose, were also tested for their binding to a Ni^{2+}-NTA bearing chips by SPR. As expected, His_6-GFP gave a much higher response as observed on the sensogram (Fig. 6). But the very weak binding showed by I-GFP, could be explained by a different chemistry of NTA ligand coupling on NTA Sepharose column and NTA-chip, as well as the high dynamic flow used in SPR instruments resulting in the different access of the I-GFP to NTA-ligand [25].

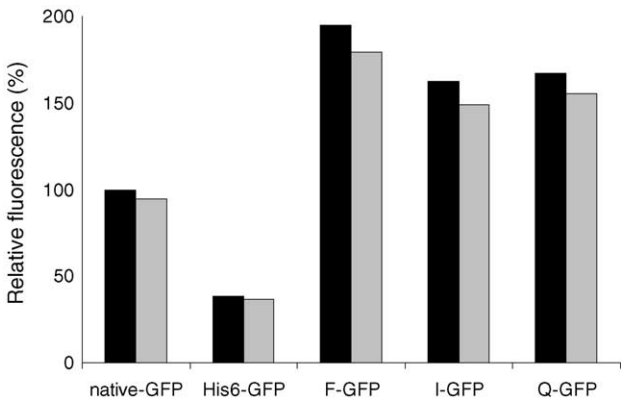

Fig. 5. Comparison of the fluorescence intensity of the different clones before (black) and after (grey) heat treatment. All values are normalised against the fluorescence intensity of non heat-treated native-GFP.

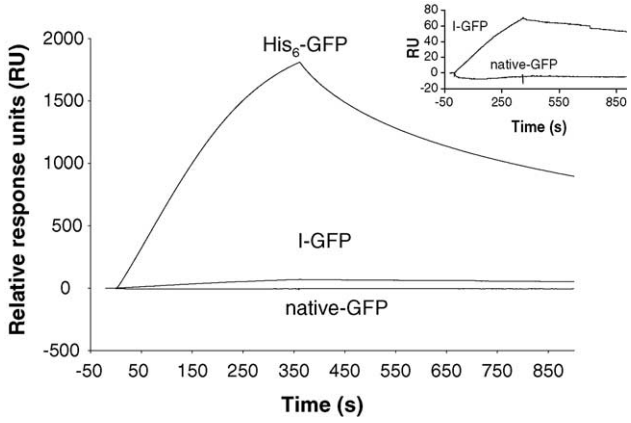

Fig. 6. SPR binding affinity comparison of native-GFP, His_6-GFP and I-GFP to a Ni^{2+}-NTA sensor chip. The insert shows the enlarged sensograms for native-GFP and I-GFP.

Table 3
Comparison of the relative GFP content in non heat-treated fractions determined by SDS-PAGE with the relative fluorescence intensity measured in the same fractions

Construct name	GFP relative content	Fluorescence relative intensity
Native-GFP	100	100
His_6-GFP	36	38
F-GFP	198	195
I-GFP	172	162
Q-GFP	176	167

All values are normalised against native-GFP.

4. Conclusion

The conventional procedures to screen a bacterial peptide library involve individual picking, cultivation and expression of selected clones. The developed 96-well chromatographic format based on supermacroporous monolithic columns allows simple parallel chromatographic processing of multiple samples. As the monolithic columns are drainage-protected, the system does not require any additional equipment to create excessive pressure or vacuum (to ensure the flow-through of the columns) or valves to prevent drainage of the columns [17]. The performance of the system has been demonstrated for the screening of a combinatorial library for metal binding peptide tags co-expressed with GFP, but should be applicable also for other systems.

Acknowledgement

This work was supported by the Swedish Centre for Bioseparation.

Contributions. Amro Hanora and Florent Bernaudat have made an equal contribution concerning the experimental works and analysis of the data.

References

[1] O. Shimomura, F.H. Johnson, Y. Saiga, J. Cell Comp. Physiol. 59 (1962) 223.
[2] D.C. Prasher, V.K. Eckenrode, W.W. Ward, F.G. Prendergast, M.J. Cormier, Gene 111 (1992) 229.
[3] R.Y. Tsien, Anal. Rev. Biochem. 67 (1998) 509.
[4] T. Misteli, D.L. Spector, Nat. Biotechnol. 15 (1997) 961.
[5] J. Porath, B. Olin, Biochemistry 22 (1983) 1621.
[6] E. Sulkowski, Bioessays 10 (1989) 170.
[7] T.T. Yip, T.W. Hutchens, Methods Mol. Biol. 59 (1996) 197.
[8] V. Gaberc-Porekar, V. Menart, J. Biochem. Biophys. Methods 49 (2001) 335.
[9] J. Porath, J. Carlsson, I. Olsson, G. Belfrage, Nature 258 (1975) 598.
[10] E.K.M. Ueda, P.W. Gout, L. Morganti, J. Chromatogr. A 988 (2003) 1.
[11] M.C. Smith, T.C. Furman, T.D. Ingolia, C. Pidgeon, J. Biol. Chem. 263 (1988) 7211.
[12] R.R. Beitle, M.M. Ataai, Biotechnol. Prog. 9 (1993) 64.
[13] C. Ljungquist, A. Breitholtz, H. Brink-Nilsson, T. Moks, M. Uhlen, B. Nilsson, Eur. J. Biochem. 186 (1989) 563.
[14] S.A. Lopatin, V.P. Varlamov, Appl. Biochem. Microbiol. 31 (1995) 221.
[15] D.B. Kaufman, M.E. Hentsch, G.A. Baumbach, J.A. Buettner, C.A. Dadd, P.Y. Huang, D.J. Hammond, R.G. Carbonell, Biotechnol. Bioeng. 77 (2002) 278.
[16] N.E. Labrou, J. Chromatogr. B 790 (2003) 67.
[17] I.Yu. Galaev, M.B. Dainiak, F.M. Plieva, R. Hatti-Kaul, B. Mattiasson, J. Chromatogr. A 1065 (2005) 169.
[18] S. Fexby, G. Hambraeus, F. Tjerneld, L. Bülow, Biotechnol. Prog. 20 (2004) 793.
[19] F. Bernaudat, Metal binding tags-characterisation, use in bioseparation and application of green fluorescent proteins, Ph.D. Thesis, Lund Institute of Technology, 2005.
[20] T. Maniatis, E.F. Fritsch, J. Sambrook, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1987.
[21] P. Arvidsson, F. Plieva, V. Lozinsky, I.Yu. Galaev, B. Mattiasson, J. Chromatogr. A 986 (2003) 169.
[22] A.V. Yakhnin, L.M. Vinokurov, A.K. Surin, Y.B. Alakhov, Protein Exp. Purif. 14 (1998) 382.
[23] F. Plieva, I. Savina, S. Deraz, I.Yu. Galaev, B. Mattiasson, J. Chromatogr. B 807 (2004) 129.
[24] I. Fujii, Y. Takaoka, K. Suzuki, T. Tanaka, Tetrahedron Lett. 42 (2001) 3323.
[25] D.J. O'Shannessy, K.C. O'Donnell, J. Martin, M. Brigham-Burke, Anal. Biochem. 229 (1995) 119.