Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters
ELSEVIER
Bioorganic & Medicinal Chemistry Letters 14 (2004) 1987–1990

Facile synthesis of metal-chelating peptides on chip for protein array

Ching-Wen Cheng,[a] Kuo-Chin Lin,[b] Fu-Ming Pan,[b] Supachok Sinchaikul,[b] Chi-Huey Wong,[b] Wei-Chih Su,[c] Ching-Hsiang Hsu[c,d] and Shui-Tein Chen[a,b,*]

[a]*ALPS Biotech Co. Ltd, Taipei, Taiwan*
[b]*Institute of Biological Chemistry and Genomics Research Center, Academia Sinica, Taipei, 11529, Taiwan*
[c]*Genmont Biotech Inc., Tainan, 741, Taiwan*
[d]*Allergy, Immunology and Rheumatology Department of the Pediatrics Division, China Medical University, Taichung, 404, Taiwan*

Received 18 November 2003; accepted 19 January 2004

Abstract—A unique peptide sequence of HGGHHG screening from a combinatorial synthetic peptide library showed a good chelating ability to bind a transition metal on a chip better than hexa-His peptide. It was directly conjugated with a His-Tagged proteins onto a chip in a mild aqueous solution and can be used this chip as a high throughput technique for protein array in order to detect and purify the His-Tagged proteins.

Along with the wide spread use of DNA array as a powerful tool for genome research, protein array has emerged as an exciting technology for the broad characterization of the activities and interactions of proteins.[1–12] Recombinant antibody and antibody fragment have also been used for array.[13,14] Unfortunately, there is no protein equivalent of PCR and proteins are not specifically hybridized to the complementary of amino acid sequences. The conjugation of proteins on a chip for array can be achieved by many methods such as ion exchange chip,[15] EDC coupling,[16] adsorption to borosilicate,[17] affinity capture reagents,[18] and streptavidin sensor surface self-assembled.[19,20] However, the protein loss of non-specific binding proteins on chips is the severe problem for reproducibility of chip. A new technology of protein immobilization on chips without biological activity loss is currently interesting for developing the protein array. Using peptide library and transitional metal-Tag to construct a peptide library or protein on chips based on immobilized metal-affinity chromatography (IMAC) provide a new throughput technique of protein array. The briefly mechanism of His-Tag based on IMAC is the selective interaction between a TentaGel resin as solid matrix immobilized with either metal ions (Cu^{2+}, Co^{2+}, Ni^{2+}, or Zn^{2+}) and a polyhistidine Tag (His-Tag) fused to either the N- or C-terminal of proteins. The metal is held by chelation with reactive groups covalently attached to a solid matrix and the proteins containing a polyhistidine Tag are selectively bound to the matrix while other cellular proteins are washed out.[21,22] In this research, we prepared a protein array on a commercial available chip following to six steps of procedure: (1) synthesize a combinatorial hexapeptide library on TentaGel resin; (2) screen the high affinity hexapeptide from the combinatorial hexapeptide library by using a FITC-labeled His-Tag; (3) determine the sequences of the high affinity hexapeptide; (4) test the binding affinity of the new peptide; (5) resynthesize the high affinity peptide on a chip; (6) test for protein array.

The combinatorial hexapeptide library was synthesized on a TentaGel NH_2 resin (Advanced ChemTech, Louisville, KY) using the one-bead one-sequence protocol.[23] The binding affinity of hexapeptides on resins was monitored by binding with a synthetic FITC-labeled His-Tag in a mild aqueous solution containing 50 mM NaH_2PO_4, 300 mM NaCl and 10 mM Ni^{2+}, pH 8.0 as shown in Figure 1.[24] The bright fluorescent resins were selected and the amino acid sequences were determined. Four hexapeptides (HGGHHG, HHGHHH,

* Corresponding author. Tel.: +886-2-27855696x7071; fax: +886-2-27883473; e-mail: bcchen@gate.sinica.edu.tw

0960-894X/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2004.01.084

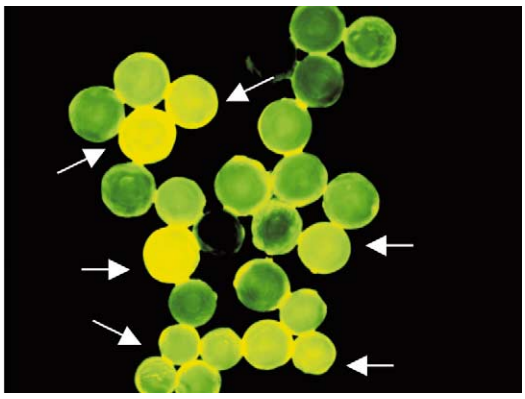

Figure 1. Fluorescent binding test of hexapaptide library on TentaGel resins with FITC-labeled hexa-His. Colors: yellow, high affinity binding of hexapaptide library and FITC-labeled hexa-His; green, low affinity binding; transparent green, non-binding. Arrows show the selected resins for amino acid sequencing of hexapeptide.

HGHHGH, HGHHGG) showing high affinity with FITC-labeled hexa-His were selected and resynthesized on TentaGel resins to further confirm their binding to the FITC-labeled hexa-His. Finally, the HGGHHG hexapeptide was found to be the one with the highest affinity, which showed the most brighten fluorescence on the screening resin, and thus used for comparison with His-Tag and subsequent study of protein array.

For the competitive binding test of HGGHHG on hexa-His chip, hexa-His (HHHHHH) as competitive control was synthesized on a commercial amine chip (SuperAmine, TeleChem International Inc.) using a homemade reactor on PS3 Synthesizer (Rainin). Accompany with an amine chip, a small amount of TentaGel resin (5 mg, 0.1 mmole) was added in the reactor. Simultaneously, a few synthesized resins were used to test the completeness of peptide coupling by ninhydrine.[25] For the study of competitive binding, the synthesized FITC-HGGHHG and hexa-His (10^{-5} mmol) were orderly added onto a hexa-His chip in a mild aqueous solution containing 50 mM NaH_2PO_4, 300 mM NaCl and 10 mM Ni^{2+}, pH 8.0 and incubated at room temperature for 30 min. Interestingly, the HGGHHG showed the high competitive binding on hexa-His chip rather than hexa-His with a higher fluorescent intensity (Fig. 2). Hexa-His showed the competitive binding on hexa-His chip less than HGGHHG and the mixture of HGGHHG and hexa-His with 19.9% and 3.4% less intensities, respectively. As the competitive binding, the mixture of HGGHHG and hexa-His showed the intensity less than HGGHHG followed with hexa-His about 16.6%. In addition, we found that some factors such as sequence similarity and binding sequence order were affected to the competitive binding.

In the same manner, hexa-His and HGGHHG were synthesized on a commercial SuperAmine chip and also compared the affinity binding by binding with a recombinant DP5P proteins, which are the allergic proteins and expressed in *E. coli* system containing a His-Tag at C-terminus,[26] in a mild aqueous solution containing 50 mM NaH_2PO_4, 300 mM NaCl and 10 mM Ni^{2+}, pH 8.0. Using the first antibody against the DP5P proteins (1 mg/mL) and its secondary antibody labeled with FITC (1/50 dilution), there were clearly monitored the fluorescent spots on chips as shown in Figure 3.[27] In addition, the affinity binding of bound DP5P proteins on both chips was examined by elution with EDTA as chelating reagent. The recombinant DP5P proteins on HGGHHG and hexa-His chips were washed with different concentrations of EDTA for competitive binding on chip and removing the DP5P proteins. The FITC labeled-second antibody was against with DP5P protein on both chips and monitored by using a fluorescence scanner (Typhoon 9200 image scanner, Amersham Pharmacia Biotech). The DP5P proteins bound on HGGHHG chip was found to have higher affinity and stability than on hexa-His chip after elution with 10×elution buffer containing 0.5 M NaH_2PO_4, 3 M NaCl and EDTA, pH 8 for 20 min. Proteins bound on the HGGHHG chip were stable in a concentration range of 0.1 M–1 M EDTA with only 1% loss. In contrast, the DP5P protein on hexa-His chip was lost by 9, 12 and 15% after elution with EDTA at 0.1, 0.5 and 1 M, respectively.

Furthermore, there has been found that the transitional metal ions have the different affinity binding and specificity to the chelating ligands and His-Tagged proteins.[21,22] Thus, the affinity binding and specificity of metal ions were examined by changing the chelating metal ions such as Cu^{2+}, Co^{2+}, Ni^{2+} or Zn^{2+} (10

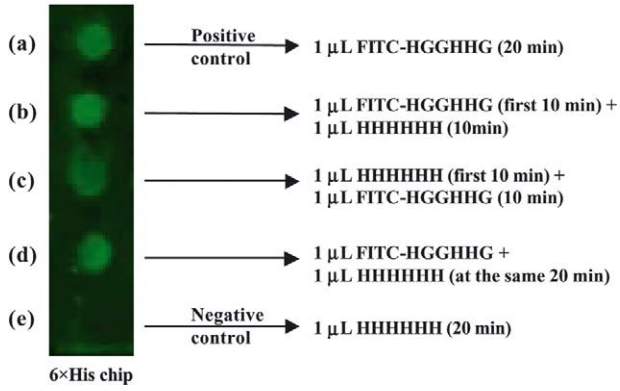

Figure 2. Competitive binding test of HGGHHG and HHHHHH hexapeptides on hexa-His (6×His) chip by fluorescent detection.

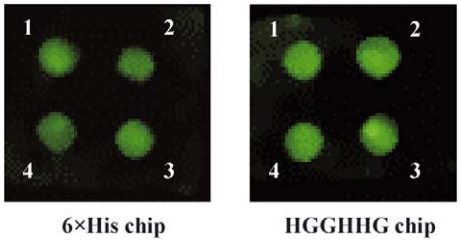

Figure 3. Immunofluorescent binding test of recombinant DP5P protein containing His-Tag at C-terminus bound on hexa-His and HGGHHG immobilized chips, which were against with FITC-labeled DP5P antibody. The chips were washed with different concentrations of EDTA for 30 min (1, 0.1 M EDTA; 2, 0.5 M EDTA; 3, 1 M EDTA; 4, no EDTA).

mM), and by eluting with imidazole (1.5 M) instead of EDTA in different times of 30, 60, 90 and 120 min as shown a model in Figure 4. We found that all transitional metal ions had the different coordination ability in binding with DP5P proteins on chips. The distinctly difference of metal ions showed at 60 min elution time (Fig. 5). Cu^{2+} as a chelating metal ion formed the stable complex with proteins on both chips better than other metal ions along 120 min elution time, in which the observed metal-binding orders on hexa-His and HGGHHG chips were $Zn^{2+} \geq Cu^{2+} > Ni^{2+} \geq Co^{2+}$ and $Cu^{2+} > Zn^{2+} > Co^{2+} \geq Ni^{2+}$, respectively. It indicated that the different metal-binding order on both chips varied to specificity on different sequence order and affinity binding with proteins after elution. This observed metal-binding order is the useful information, which can be used to be the suitable condition for protein conjugation. Although the HGGHHG showed the specificity and affinity metal binding with His-Tagged DP5P proteins than hexa-His after elution with imidazole, the affinity binding of protein in the presence of Cu^{2+} was found to have higher affinity and stability on both chips along 120 min elution than other metal ions, in which the HGGHHG chip showed the highest affinity with the His-Tagged proteins. In contrast to the other metal ions such as Co^{2+}, Ni^{2+}, or Zn^{2+}, HGGHHG was found to have lower affinity with His-Tagged proteins than hexa-His. This evidence showed that some factors such as ordered sequence, chelating reagents such as EDTA and imidazole, and washing and elution times were affected to the affinity binding of protein on chips.[21,22] However, this technique can be used for protein array and will be further optimized to get a higher affinity and stability in different conditions.

In conclusion, we introduced a new technique of facile synthesis of metal-chelating hexapeptide on chip that is very useful to prepare a specific metal binding peptide on chips and can highly bind the His-Tagged DP5P proteins as model on chips as protein array. In addition, we successfully investigated a good HGGHHG hexapeptide, which was found to have higher affinity and specificity with His-Tagged protein than hexa-His in the presence of Cu^{2+}. Therefore, this technique is very

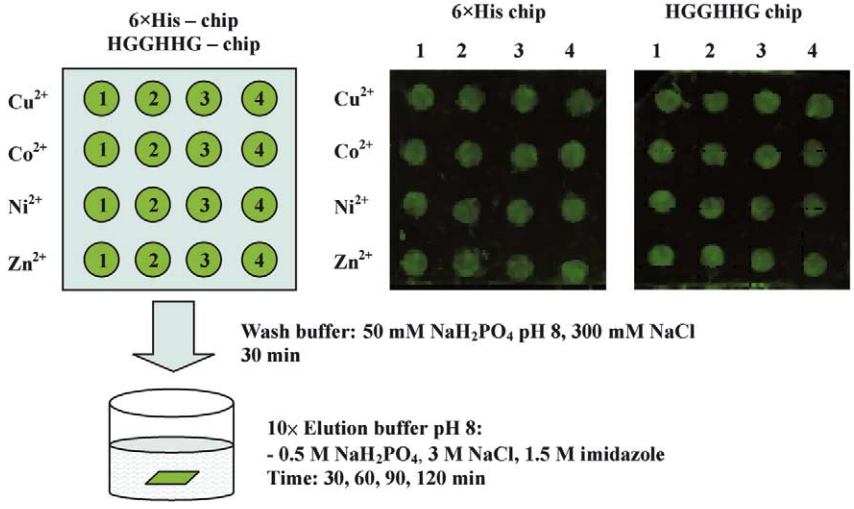

Figure 4. Immunofluorescent binding test of His-Tagged DP5P protein bound on hexa-His and HGGHHG chips after chelating with different metal ions (10 mM) and eluting by imidazole for 120 min. Each chelating metal ions were repeated in four spots (no 1–4).

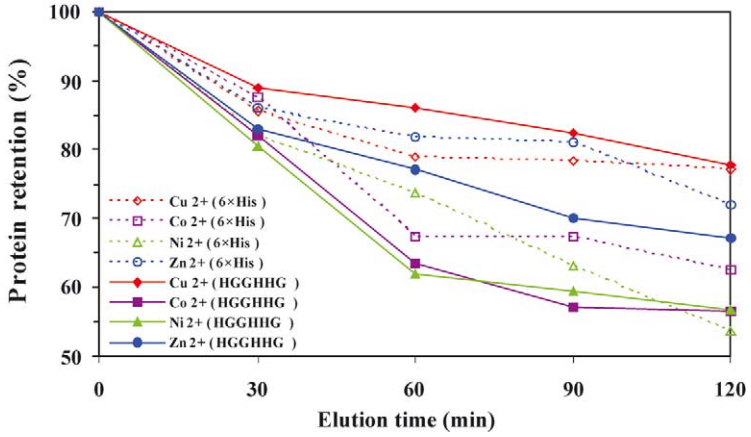

Figure 5. Protein retention of His-Tagged DP5P protein bound on hexa-His and HGGHHG chips after chelating with different metal ions and eluting with imidazole in different elution times. The protein intensities in four experimental spots are averaged and the initial protein intensity at 0 min is defined as 100% protein retention.

useful and can be used for rapid detection and purification of other His-Tagged proteins or any targets containing His-Tag.

Acknowledgements

We gratefully acknowledge the Main Subject Programs, Academia Sinica, Taiwan and the National Research Program for Genomic Medicine, National Science Council, Taiwan, (NSC 91-3112-13-001-002) for supporting this research.

References and notes

1. Cahill, D. J.; Nordhoff, E. *Adv. Biochem. Eng. Biotechnol.* **2003**, *83*, 177.
2. Jain, K. K. *Pharmacogenomics* **2003**, *4*, 383.
3. Liotta, L. A.; Espina, V.; Mehta, A. I.; Calvert, V.; Rosenblatt, K.; Geho, D.; Munson, P. J.; Young, L.; Wulfkuhle, J.; Petricoin, E. F. *Cancer Cell.* **2003**, *3*, 317.
4. Lee, Y. S.; Mrksich, M. *Trends Biotechnol.* **2002**, *20*, S14.
5. Wilson, D. S.; Nock, S. *Angew. Chem., Int. Ed. Engl.* **2003**, *42*, 494.
6. Cutler, P. *Proteomics* **2003**, *3*, 3.
7. Zhu, H.; Snyder, M. *Curr. Opin. Chem. Biol.* **2003**, *7*, 55.
8. Lal, S. P.; Christopherson, R. I.; dos Remedios, C. G. *Drug Discov. Today* **2002**, *7*, S143.
9. Ha, K. S. *Exp. Mol. Med.* **2001**, *33*, 127.
10. Angenendt, P.; Glokler, J.; Murphy, D.; Lehrach, H.; Cahill, D. J. *Anal. Biochem.* **2002**, *309*, 253.
11. Ng, J. H.; Ilag, L. L. *J. Cell. Mol. Med.* **2002**, *6*, 329.
12. Kusnezow, W.; Hoheisel, J. D. *Biotechniques* **2002**, *33*, S14.
13. Steinhauer, C.; Wingren, C.; Hager, A. C.; Borrebaeck, C. A. *Biotechniques* **2002**, *33*, 38.
14. Kingsmore, S. F.; Patel, D. D. *Curr. Opin. Biotechnol.* **2003**, *14*, 74.
15. Delehanty, J. B.; Ligler, F. S. *Biotechniques* **2003**, *34*, 380.
16. Elia, G.; Silacci, M.; Scheurer, S.; Scheuermann, J.; Neri, D. *Trends Biotechnol.* **2002**, *20*, S19.
17. Pavlickova, P.; Knappik, A.; Kambhampati, D.; Ortigao, F.; Hug, H. *Biotechniques* **2003**, *34*, 124.
18. Peluso, P.; Wilson, D. S.; Do, D.; Tran, H.; Venkatasubbaiah, M.; Quincy, D.; Heidecker, B.; Poindexter, K.; Tolani, N.; Phelan, M.; Witte, K.; Jung, L. S.; Wagner, P.; Nock, S. *Anal. Biochem.* **2003**, *312*, 113.
19. Wingren, C.; Ingvarsson, J.; Lindstedt, M.; Borrebaeck, C. A. *Nat. Biotechnol.* **2003**, *21*, 223.
20. Hallborn, J.; Carlsson, R. *Biotechniques* **2002**, 30.
21. Chaga, G. *J. Biochem. Biophys. Methods* **2001**, *49*, 313.
22. Gaberc-Porekar, V.; Menart, V. *J. Biochem. Biophys. Methods* **2001**, *49*, 335.
23. Rapp, W. E. In *Combinatorial Chemistry, Chapt. 4*; Wilson, S. R., Czarnik, A. W., Eds.; John Wiley & Sons: New York, 1997; pp 65–93.
24. Hochuli, E.; Dobeli, H.; Schacher, A. *J. Chromatogr.* **1987**, *411*, 177.
25. Kaiser, E.; Colescott, R. L.; Bossinger, C. D.; Cook, P. I. *Analyt. Biochem.* **1970**, *34*, 595.
26. Fall, B. I.; Eberlein-Konig, B.; Behrendt, H.; Niessner, R.; Ring, J.; Weller, M. G. *Anal. Chem.* **2003**, *75*, 556.
27. Sreekumar, A.; Chinnaiyan, A. M. *Biotechniques* **2002**, *33*, S46.