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# Synthetic metal-binding protein surface domains for metal ion-dependent interaction chromatography

# II. Immobilization of synthetic metal-binding peptides from metal ion transport proteins as model bioactive protein surface domains

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ABSTRACT

This preliminary investigation tests the premise that biologically relevant (1) peptide–metal ion interactions, and (2) metal ion-dependent macromolecular recognition events (*e.g.*, peptide–peptide interactions) may be modeled by biomimetic affinity chromatography. Divinylsulfone-activated agarose (6%) was used to immobilize three different synthetic peptides representing metal-binding protein surface domains from the human plasma metal transport protein histidine-rich glycoprotein (HRG). The synthetic peptides represented 1–3 multiple repeat units of the 5-residue sequence (Gly–His–His–Pro–His) found in the C-terminal of HRG. By frontal analyses, immobilized HRG peptides of the type $(GHHPH)_nG$, where $n$ = 1–3, were each found to have a similar binding capacity for both Cu(II) ions and Zn(II) ions (31–38 μmol/ml gel). The metal ion-dependent interaction of a variety of model peptides with each of the immobilized HRG peptide affinity columns demonstrated differences in selectivity despite the similar internal sequence homology and metal ion binding capacity. The immobilized 11-residue HRG peptide was loaded with Cu(II) ions and used to demonstrate selective adsorption and isolation of proteins from human plasma. These results suggest that immobilized metal-binding peptides selected from known solvent-exposed protein surface metal-binding domains may be useful model systems to evaluate the specificity of biologically relevant metal ion-dependent interaction and transfer events *in vitro*.

## INTRODUCTION

Despite remarkable increases in the number of solutions to various biopolymer structures, the surface chemistry of biospecific macromolecular interactions remains elusive in the majority of cases. Development of stationary phase surfaces with immobilized ligands of predetermined biochemical specificity and affinity for specific peptides and protein surface structures may provide useful *in vitro* models to investigate a variety of macromolecular recognition events.

Metal ion-dependent protein–protein and protein–DNA interactions occur frequently in nature and are now being recognized as events of major regulatory significance in biology (*e.g.*, refs. 1 and 2). The interactions of peptides and proteins with surface-immobilized transition metal ions *in vitro* may present an important opportunity to investigate and model the subtleties of metal ion-dependent macromolecular recognition motifs. Proteins with well-defined surface structures have been used

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to explore differences in the predicted and observed interactions of these proteins with immobilized transition metal ions under a variety of experimental conditions [3–6]. We propose that this approach may be especially informative if the model surface of stationary metal ions is comprised of synthetic peptides which represent naturally occurring metal-binding domains identified from the surface of known metal transport proteins.

The human plasma protein referred to as histidine-rich glycoprotein (HRG) is a 75 000-dalton Zn(II)- and Cu(II)-binding protein rich in His (11–13 mol%) and Pro (14–16 mol%) [7–13]. A 5-residue sequence (GHHPH) in the His-rich C-terminal region of HRG has been identified tentatively as the Zn(II)- and heme-binding motif in this protein [14–17]. This 5-residue primary sequence is found in tandem repeats of up to 25 residues in length. We have synthesized $(GHHPH)_nG$ for $n = 1$–3 to investigate the affinity of this protein surface metal-binding domain for free metal ions (see also refs. 18–21, added in proof) and to evaluate metal ion-dependent macromolecular recognition of this peptide.

The purpose of this communication is to discuss concepts, outline problems and present preliminary data which may serve as examples to increase the interest in using a biologically directed approach to the design and investigation of metal ion-dependent biomolecular interactions.

## EXPERIMENTAL

### *Synthesis of HRG metal-binding peptides (GHHPHG tandem repeats)*

The 6-residue HRG peptide $(GHHPH)_1G$ (1-mer), the 11-residue HRG peptide $(GHHPH)_2G$ (2-mer), and the 16-residue HRG peptide $(GHHPH)_3G$ (3-mer) were synthesized on an Applied Biosystems Model 430A automated peptide synthesizer using 9-fluorenylmethyloxylcarbonyl (Fmoc) chemistry. Peptide purity was verified by reversed-phase high-performance liquid chromatography. Peptide sequences were verified by sequential Edman degradation (see ref. 22) with an Applied Biosystems Model 473A automated peptide sequence analyzer. Determination of synthetic peptide mass was performed with a Vestec UV laser desorption time-of-flight mass spectrometer [18–21,23].

TABLE I

IMMOBILIZED METAL-BINDING HRG PEPTIDES

Bound transition metal ions: Cu(II), Zn(II)

| |
|---|
| Immobilized GHHPHG |
| Immobilized GHHPHGHHPHG |
| Immobilized GHHPHGHHPHGHHPHG |

### *Immobilization of synthetic HRG peptides*

Sepharose 6B (Pharmacia) was activated with divinylsulfone (DVS) as described earlier by Porath and Axen [24]. Equivalent molar quantities of each of the HRG peptides were coupled (10 μmol/g gel) in 0.5 *M* sodium bicarbonate at pH 8–9 for 20 h at 23–25°C. Remaining vinyl groups were blocked by incubation overnight with 10% glycine in 0.5 *M* sodium carbonate at pH 9.4. Control preparations included DVS-activated agarose gel treated in an identical manner except for the addition of HRG peptide. Metal binding capacities were confirmed by frontal analyses with copper sulfate (0.8 m*M*) or zinc sulfate (1.0 m*M*) in 25 m*M* 4-(2-hydroxyethyl)-1-piperazinethanesulfonic acid (HEPES) buffer (pH 6.0–6.5). The void (dead) volumes were determined by pumping the same metal ion solutions into control columns of DVS-cross-linked agarose of the same dimension and bed volumes but prepared without immobilized peptides (*i.e.*, glycine inactivated).

### *Interaction of model peptides with immobilized HRG peptide–Zn(II) and Cu(II) ions*

Affinity columns (2.3 cm × 1.0 cm I.D.) of the three different immobilized HRG peptides were loaded with a 0.8 m*M* solution of Cu(II) ions or a 1.0 m*M* solution of Zn(II) ions in 25 m*M* HEPES buffer (pH 6.0–6.5). This is an important distinction from the metal ion loading procedures described previously for work with immobilized iminodiacetate (IDA) or tris(carboxymethyl)ethylenediamine (TED) metal chelate columns where metal ions were loaded in water [13,25–27]. Metal-free peptide samples were added (50–100 μl) to immobilized HRG peptide–Cu(II) or immobilized HRG peptide–Zn(II) equilibrated with 20 m*M* sodium phosphate buffer (pH 7.0) containing 0.5 *M* NaCl. Unbound peptides were removed by elution with column equilibration buffer. Bound peptides were eluted

with a pH gradient from pH 7.0 (20 m*M* sodium phosphate, 0.5 *M* NaCl) to pH 3.8 (50 m*M* sodium phosphate, 0.5 *M* NaCl) unless stated otherwise. The ionic strength of both the column equilibration buffer and the pH gradient elution buffers was elevated by inclusion of 0.5 *M* NaCl to eliminate nonspecific electrostatic interactions otherwise observed with immobilized metal ion affinity chromatography [25,26]. The absorbance at 220 nm and/or 280 nm and pH were monitored in each fraction (1 ml).

*Interaction of human plasma proteins with immobilized HRG peptide-Cu(II) ions*

An affinity column (2.3 × 1 cm) of the 11-residue HRG peptide (2-mer) was loaded with Cu(II) ions and equilibrated with 20 m*M* sodium phosphate buffer (pH 7.0) containing 0.5 *M* NaCl. An aliquot of human plasma (0.5 ml, dialyzed against 50 m*M* EDTA, then column equilibration buffer) was injected into the column and washed extensively (over 250 column volumes) until the absorbance at 280 nm had reached baseline. The bound proteins were then eluted with a pH gradient from pH 7 to 3.8 and finally with 50 m*M* EDTA in 20 m*M* sodium phosphate pH 7.0.

*Sodium dodecyl sulfate (SDS)–polyacrylamide gradient gel electrophoresis (PAGGE)*

Samples were heated with 2 to 5 volumes of solubilization buffer (2% SDS, 3% mercaptoethanol, 50 m*M* Tris–HCl, pH 6–8, and 10% glycerol) at 95°C for 2 min and then separated on a 8–12% polyacrylamide gradient gel according to Lacmmli [28]. Silver staining was according to Morrisey [29].

## RESULTS

Fig. 1 shows the frontal analyses of Cu(II) ion interaction with the immobilized 6-residue HRG peptide $(GHHPH)_1G$, the immobilized 11-residue HRG peptide $(GHHPH)_2G$ (2-mer), and the immobilized 16-residue HRG peptide $(GHHPH)_3G$ (3-mer) (Table I). Frontal analyses were performed on two different sets of each immobilized peptide column. Despite differences in the number of internally homologous repeat units, there were no significant differences in the total Cu(II) ion binding capacity (31 μmol/ml gel) among the three different immobilized HRG peptide affinity columns. Nonetheless, these same columns differed appreciably in their affinity for a given set of model peptide ligates.

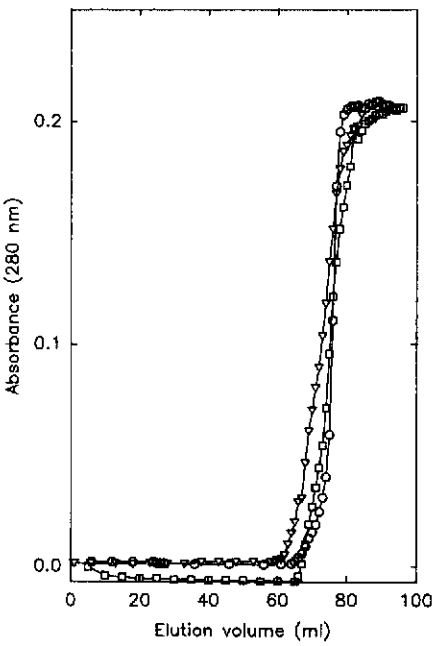


Fig. 1. Frontal analyses of Cu(II) ion interaction with the immobilized 6-residue HRG peptide $(GHHPH)_1G$ (1-mer; □), the immobilized 11-residue HRG peptide $(GHHPH)_2G$ (2-mer; ▽) and the immobilized 16-residue HRG peptide $(GHHPH)_3G$ (3-mer; ○). The columns (2.3 cm × 1.0 cm I.D.) were equilibrated in 25 m*M* HEPES buffer pH 6.0. A solution of $CuSO_4$ (800 μ*M*) in 25 m*M* HEPES (pH 6.0) was pumped continuously into the columns. Copper elution was monitored by absorbance at 280 nm.

Fig. 2 illustrates the Cu(II) ion-dependent interaction of angiotensin I with separate columns of immobilized 6-residue HRG peptide GHHPHG (1-mer), the immobilized 11-residue HRG peptide $(GHHPH)_2G$, and the immobilized 16-residue HRG peptide $(GHHPH)_3G$. In no case was any portion of the applied angiotensin I ever observed to elute unbound in the column flow-through fractions (*i.e.*, equilibration buffer). When elution of bound angiotensin I from each of the three different immobilized HRG peptide–Cu(II) columns was initiated with an identical gradient of descending pH (from pH 7 to pH 4), significant differences in angiotensin I elution were apparent (Fig. 2); differences in angiotensin I elution from these columns were also observed by stepwise pH elution (data not

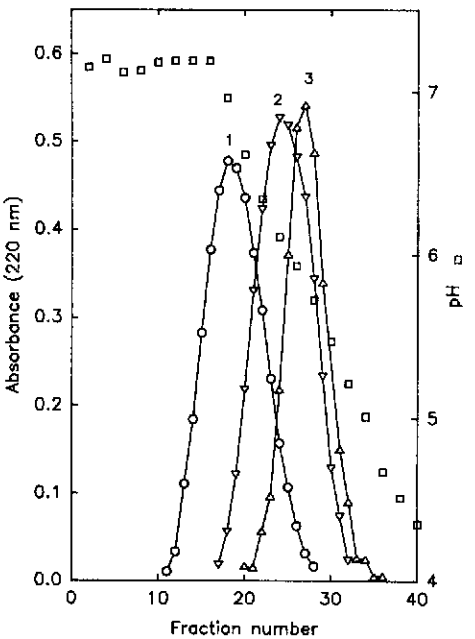


Fig. 2. Cu(II) ion-dependent interaction of angiotensin I with immobilized 1-mer (peak 1; ○), 2-mer (peak 2; ▽) and 3-mer (peak 3, △). After loading with Cu(II) ions as described in Fig. 1, the columns (2.3 cm × 1.0 cm I.D.) were equilibrated with 20 m*M* sodium phosphate (pH 7.0) containing 0.5 *M* NaCl. Elution of bound peptide was initiated with a descending pH gradient from pH 7 to pH 4 (□). Fractions of 1.0 ml each were collected. Peptide elution was monitored by absorbance at 220 nm.

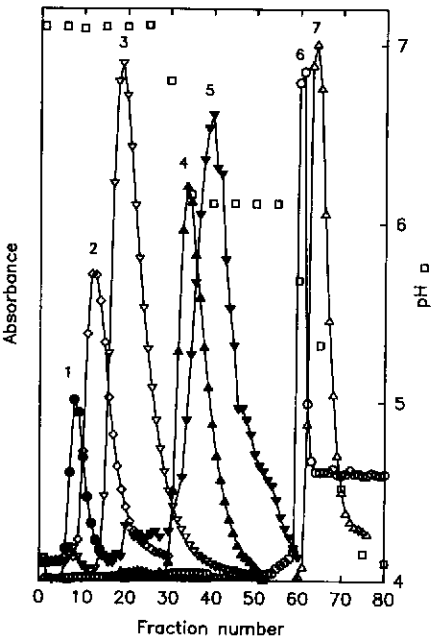


Fig. 3. Cu(II) ion-dependent interaction of several different metal-binding peptides with the immobilized 11-residue HRG peptide $(GHHPH)_2G$ (2-mer). A 1.8-ml column (2.3 cm × 1 cm I.D.) of the immobilized $(GHHPH)_2G$ was loaded with Cu(II) ions and equilibrated with 20 m*M* sodium phosphate buffer (pH 7.0) containing 0.5 *M* NaCl. The various peptides to be analyzed were loaded onto the immobilized $(GHHPH)_2G$–Cu(II) ion column separately (50–100 μl). After removing unbound and loosely retained peptides by washing with 22 fractions (1 ml each) of column equilibration buffer, bound peptides were eluted with a discontinuous pH gradient (□) from pH 7.0 to pH 5.8 (20 m*M* sodium phosphate, 0.5 *M* NaCl) and from pH 5.8 to pH 3.8 (50 m*M* sodium phosphate, 0.5 *M* NaCl). Absorbance at 220 nm or 280 nm in each fraction was monitored as a function of measured elution pH. The numbered peaks indicate the elution positions of (1) bovine gastric inhibitory peptide, (2) angiotensin II, (3) the HRG peptide $(GHHPH)_1G$, (4) angiotensin I, (5) parathyroid hormone 1–34, (6) human gastric inhibitory peptide and (7) the HRG peptide $(GHHPH)_2G$.

shown). Therefore, a larger series of different model peptides were evaluated for indications of selectivity for immobilized HRG peptide–Cu(II).

Results shown in Fig. 3 illustrate further the Cu(II) ion-dependent interaction of several different peptides with only the immobilized 11-residue HRG peptide $(GHHPH)_2G$. The column of immobilized HRG metal-binding peptide $(GHHPH)_2G$ was loaded with Cu(II) ions and equilibrated with 20 m*M* sodium phosphate buffer (pH 7.0) containing 0.5 *M* NaCl. The separate numbered elution peaks shown in Fig. 3 reflect the ability of the immobilized $(GHHPH)_2G$, only in the presence of bound Cu(II) ions, to resolve bovine gastric inhibitory peptide, angiotensin II, the 6-residue HRG peptide $(GHHPH)_1G$, angiotensin I, parathyroid hormone 1–34 and human gastric inhibitory peptide. When the 11-residue HRG peptide $(GHHPH)_2G$ was added as free peptide to the column of immobilized $(GHHPH)_2G$ it was tightly bound and, upon elution at low pH, was only barely distinguished from human gastric inhibitory peptide. None of the peptides were retained on the column of immobilized $(GHHPH)_2G$ in the absence of metal ions (data not shown). We next investigated the affinity of each HRG peptide affinity column to bind Zn(II) ions.

Frontal analyses of Zn(II) ion interaction with the immobilized 6-residue HRG peptide $(GHHPH)_1G$, the immobilized 11-residue HRG peptide $(GHHPH)_2G$, and the immobilized 16-res-

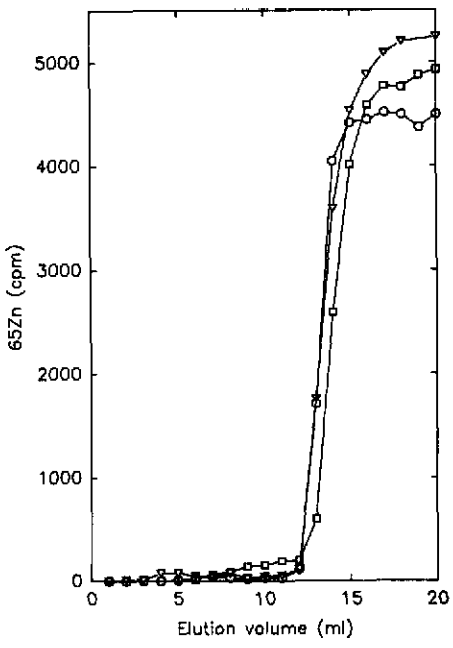


Fig. 4. Frontal analyses of Zn(II) ion interaction with the immobilized 6-residue HRG peptide $(GHHPH)_1G$ (1-mer; ○), the immobilized 11-residue HRG peptide $(GHHPH)_2G$ (2-mer; ▽) and the immobilized 16-residue HRG peptide $(GHHPH)_3G$ (3-mer; □). The columns (2.5 cm × 1.0 cm I.D.) were equilibrated in 25 m*M* HEPES buffer pH 6.5. A solution of $ZnSO_4$ (1 m*M*; labeled with $^{65}ZnSO_4$) in 25 *M* HEPES (pH 6.5) was pumped continuously into the column. Fractions of eluate (1.0 ml each) were monitored for radioactivity to quantitate eluted Zn(II).

idue HRG peptide $(GHHPH)_3G$ is shown in Fig. 4. As in the case of Cu(II) ions, despite a three-fold difference in the number of homologous repeat units, there were no significant difference in the Zn (II)ion binding capacity (38 μmol/ml gel) among the three different immobilized HRG peptides. The immobilized HRG peptide–Zn(II) columns did, however, demonstrate a systematic variation in their affinity for several different model peptides.

Fig. 5 reveals variations in the Zn(II) ion-dependent interaction of three different peptides with columns of the immobilized $(GHHPH)_1G$ (Fig. 5A), the immobilized $(GHHPH)_2G$ (Fig. 5B), and the immobilized $(GHHPH)_3G$ (Fig. 5C). The 16-residue HRG peptide $(GHHPH)_3G$ was bound most tightly to the immobilized 16-residue HRG peptide $(GHHPH)_3G$ and was clearly resolved from angiotensin I and the 11-residue HRG peptide $(GHHPH)_2G$. This resolution was not observed, or was less well defined, in the case of chromatography on the immobilized 6-residue peptide $(GHHPH)_1G$, or the immobilized 11-residue peptide $(GHHPH)_2G$.

Finally, the immobilized 11-residue HRG peptide–Cu(II) column was investigated for its ability to interact selectivity with proteins present in unfractionated human plasma. Fresh human plasma was dialyzed in column equilibration buffer and loaded onto a column of HRG peptide–Cu(II) affinity column. An estimated 85–90% (based on absorbance at 280 nm) of the applied plasma proteins did not bind to the column and were eluted directly in the flow-through fractions. The column was then washed overnight (approx. 250 column volumes) with equilibration buffer to remove all low-affinity proteins. Introduction of a descending pH gradient resulted in the elution of several plasma proteins (Fig. 6). Subsequent introduction of 50 m*M* EDTA eluted another set of bound proteins. The composition of the proteins in each of these pools was investigated by SDS–polyacrylamide gradient gel electrophoresis (Fig. 7). The immobilized HRG peptide–Cu(II) column was able to selectivity absorb specific plasma proteins. These proteins are different from some of the plasma proteins adsorbed to other types of immobilized metal ion affinity columns [25,26]. The identity of the adsorbed proteins awaits amino acid sequence and/or immunoblot analyses. The plasma protein adsorption selectivity was metal ion-dependent; more than 98% of the plasma proteins were recovered in the flow-through fractions when applied to an identical column of immobilized peptide in the absence of bound Cu(II) ions (not shown). A detailed comparison of differences in plasma protein adsorption selectivity by the six different types of immobilized HRG peptide–metal ion columns (*i.e.*, HRG 1-mer, 2-mer, 3-mer with bound Cu or Zn) presented here is in progress.

## DISCUSSION

Each of the immobilized HRG peptides evaluated in this investigation has a metal binding capacity comparable to the commercially available immobilized chelating groups (IDA). The Pharmacia chelating Sepharose Fast Flow material consists of immobilized IDA groups and has a capacity for Cu(II)

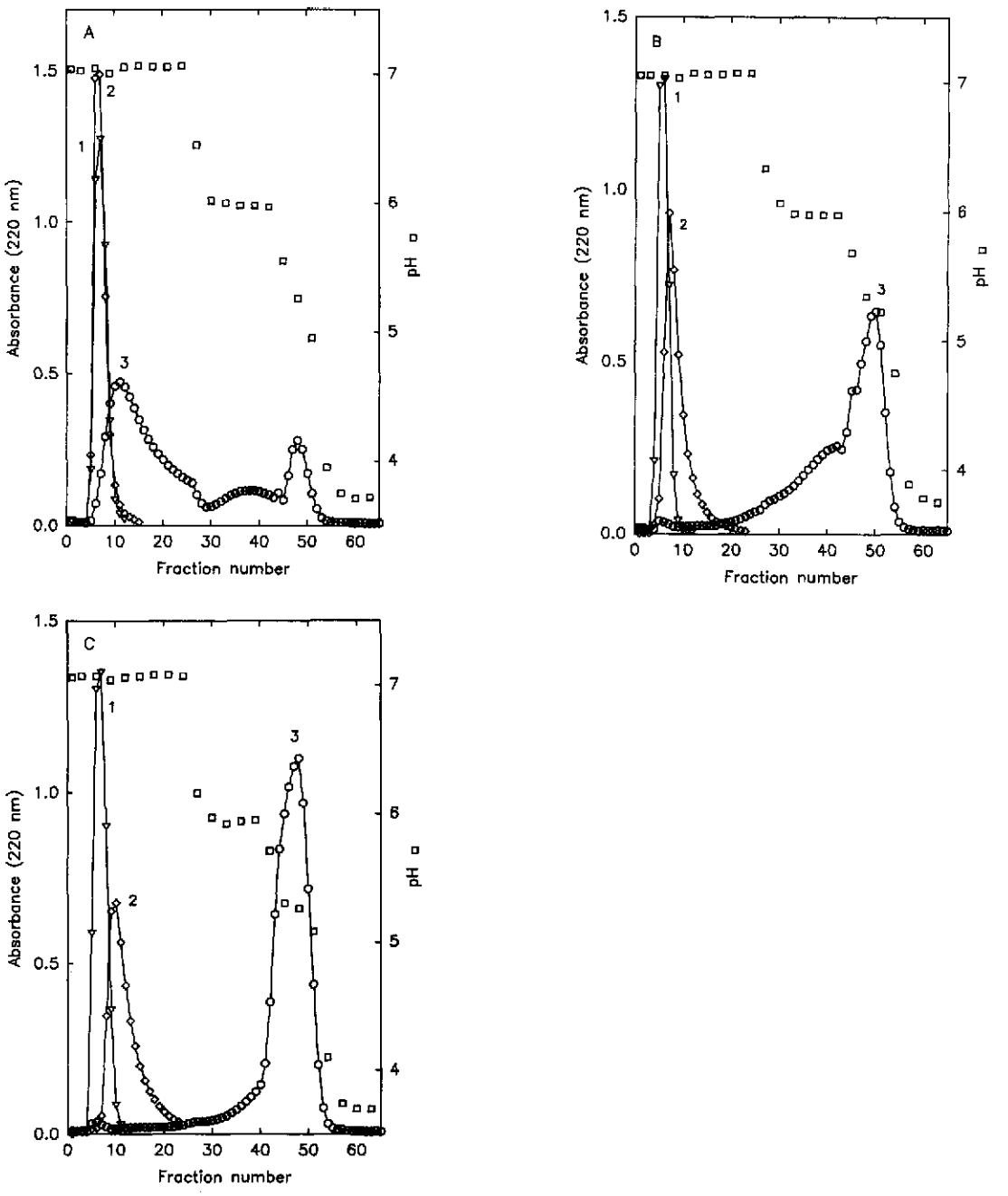


Fig. 5. Zn(II) ion-dependent interaction of angiotensin I (peak 1; ▽), the 11-residue HRG peptide $(GHHPH)_2G$ (peak 2; ◇) and the 16-residue HRG peptide $(GHHPH)_3G$ (peak 3; ○) on columns of the immobilized 6-residue HRG peptide $(GHHPH)_1G$ (A), the immobilized 11-residue HRG peptide $(GHHPH)_2G$ (B) and the immobilized 16-residue HRG peptide $(GHHPH)_3G$ (C). Separate 2.0-ml columns (2.5 cm × 1 cm I.D.) of the immobilized HRG peptide $(GHHPH)_nG$ were loaded with Zn(II) ions and equilibrated with 20 m*M* sodium phosphate buffer (pH 7.0) containing 0.5 *M* NaCl. The peptides were loaded onto the immobilized $(GHHPH)_nG$–Zn(II) ion columns separately (50–100 μl). Unbound and loosely retained peptides were removed by washing with 22 fractions (1 ml each) of column equilibration buffer. Bound peptides were eluted with a discontinuous pH gradient (squares) from pH 7.0 to pH 5.8) (20 m*M* sodium phosphate, 0.5 *M* NaCl) and from pH 5.8 to pH 3.8 (50 m*M* sodium phosphate, 0.5 *M* NaCl). Absorbance at 220 nm in each fraction was monitored as a function of measured elution pH.

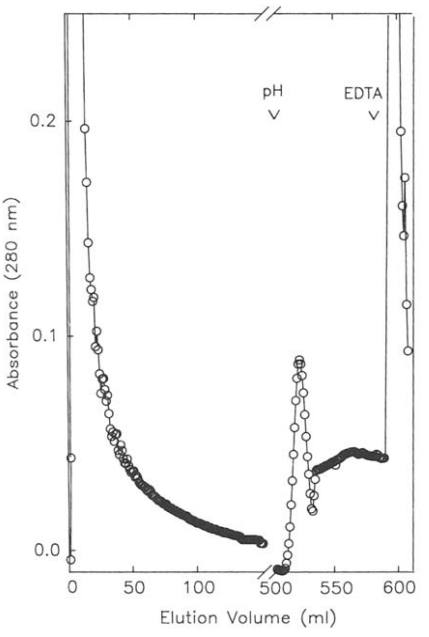


Fig. 6. Cu(II)-dependent interaction of human plasma proteins with the immobilized 11-residue HRG peptide $(GHHPH)_2G$ (2-mer). A 1.8-ml column (2.3 cm × 1 cm I.D.) of the immobilized HRG metal-binding peptide $(GHHPH)_2G$ was loaded with Cu(II) ions and equilibrated with 20 m*M* sodium phosphate buffer (pH 7.0) containing 0.5 *M* NaCl. Human plasma was dialyzed against 50 m*M* EDTA, 20 m*M* sodium phosphate (pH 7) and then dialyzed against with 20 m*M* sodium phosphate, 0.5 *M* NaCl (pH 7). The dialyzed sample (0.5 ml) was applied and the column was washed extensively (overnight) with column equilibration buffer (250 column volumes) until a stable baseline was reached. Bound proteins were eluted with a continuous pH gradient from pH 7–4. Finally, the column was washed with 50 m*M* EDTA, 20 m*M* sodium phosphate (pH 7.0). Fractions of 1 ml each were collected for measurement of absorbance (280 nm) and pH.

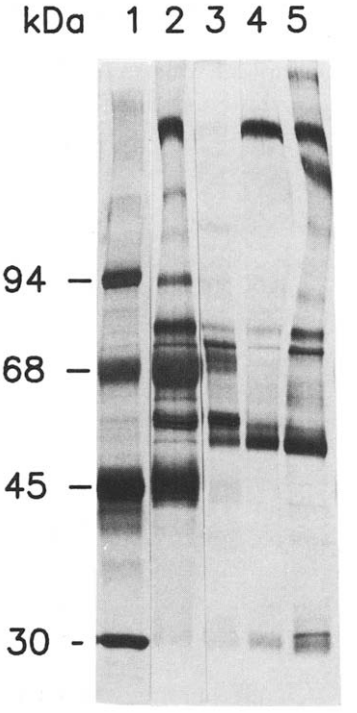


Fig. 7. SDS–polyacrylamide gel electrophoresis of eluted plasma proteins from the immobilized HRG peptide–Cu(II) affinity column. Peak fractions (5–50 μl) were boiled in SDS solubilization buffer with reducing agent, separated on an 8–12% acrylamide gradient SDS gel, and silver stained. Lanes: 1 = molecular mass markers (kDa = kilodalton); 2 = unretained (flow-through) fraction of plasma protein; 3 = trailing end of unretained peak; 4 = pH gradient eluted peak; 5 = EDTA peak.

and Zn(II) ions which is approximately 30 μmol/ml of gel. The Pharmacia chelating Superose FPLC matrix (IDA) has a metal binding capacity which is reportedly between 20 and 30 μmol metal ion/ml gel. The TSK chelate-5PW high-performance metal chelate (IDA) column (ToyoSoda, Japan) has a Cu(II) binding capacity of 23 μmol/ml of gel. Other types of immobilized chelating groups described in the literature bind transition metal ions with a capacity near that described here for the immobilized HRG peptides. For example, the TED–agarose column described by Porath and Olin [26] was reported to have a metal binding capacity of 47 μmol/ml of gel. The TED–agarose metal affinity gels synthesized in our laboratory have a somewhat higher capacity, approximately 50 to 65 μmol/ml of gel.

Despite the difference in the number of histidyl residues in the three different immobilized HRG peptides (1–3 internally homologous repeat units), they were nearly indistinguishable in their capacity to bind a given metal ion. Further, the immobilized HRG 1-mer, 2-mer, and 3-mer columns were equivalent in their binding capacity for Cu(II) ions (Fig. 1) and Zn(II) ions (Fig. 4). Although the binding capacity and sequence homology of the three different immobilized HRG peptides were similar, the immobilized peptides displayed differences in both selectivity and affinity for other peptides. Further, the immobilized HRG peptide–Cu(II) ions were found to be a "stronger" interacting affinity ligand

than the equivalent Zn(II) adduct. For example, angiotensin I was retained on each of the HRG peptide–Cu(II) columns but not on the immobilized HRG peptide–Zn(II) columns. This result is consistent with the behavior of peptides and proteins on other immobilized metal ion affinity columns (*e.g.*, IDA and TED).

The selective interaction of model peptides with immobilized HRG peptide–Cu(II) affinity columns was similar to that observed for immobilized iminodiacetate–Cu(II) ions. For example, the elution order (1) bovine gastric inhibitory peptide (1 His/42 residues), (2) angiotensin II (1 His/8 residues), (3) HRG 1-mer (3 His/6 residues), (4) angiotensin I (2 His/10 residues), (5) parathyroid hormone 1–34 (3 His/34 residues), (6) human gastric inhibitory peptide (2 His/42 residues) and (7) HRG 2-mer (6 His/11 residues) is generally similar to that observed for these peptides from immobilized IDA–Cu(II) ions, except for the stronger retention of bovine gastric inhibitory peptide by immobilized IDA–Cu(II) ions [30].

It should be noted that the immobilized HRG peptide columns loaded with Cu(II) were stable to elution with up to 100 m*M* imidazole. The affinity elution of peptides and proteins from these columns with imidazole is under further investigation. However, even 100 m*M* imidazole could not elute the tightly bound plasma protein from the immobilized 2-mer–Cu(II) column (data not shown).

The immobilized HRG peptide affinity for Zn(II) ions was significantly less than that of Cu(II) ions. However, the $^{65}$Zn(II) ions were not transferred from the immobilized HRG peptide to any of the model peptides that were added to the column and eluted in the flow-through fraction. $^{65}$Zn(II) ions were, however, released when the column pH levels were reduced below pH 6.

The selectivity of the immobilized HRG peptide–Cu(II) ion affinity column for proteins in a complex biological fluid such as plasma was quite different from that observed for the immobilized synthetic organic ligand–metal ion affinity column [*e.g.*, TED–Zn(II)] (unpublished data) and demonstrates the need to investigate further the biologically relevant presentation of metal ions to protein surfaces. The use of immobilized biopolymers, especially peptides derived from known metal transport proteins, to investigate biospecific metal ion transfer events is under further investigation. Preliminary data (unpublished) suggest that alternative chemical methods of peptide immobilization will permit a detailed evaluation of metal ion transfer, one important aspect of understanding metal ion bioavailability. With the discovery of HRG in human colostrum and milk [31] and bovine milk (unpublished), the physiological significance of metal ion transfer from proteins like HRG is evident.

We now have the possibility of designing and building artificial biomimetic surfaces with biologically active protein surface domain structures. We have demonstrated here that the acitivity of these surface immobilized peptides is effectively modulated by bound metal ions. We have now demonstrated several other models of metal ion-dependent protein dimerization (*e.g.*, estrogen receptor subunit protein recognition of the immobilized 53-residue helical dimerization domain) and metal ion-dependent protein recognition of nucleic acids (*e.g.*, estrogen response element interaction with the immobilized 71-residue DNA-binding domain) (submitted).

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