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Chiral copper–chelate complexes alter selectivities in metal affinity protein partitioning

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ABSTRACT

Proteins can be distinguished by exploiting complementarity between a histidine's microenvironment and a metal–chelate ligand in metal-affinity separations. The partitioning behavior of three myoglobins was investigated in aqueous two-phase polyethylene glycol–dextran systems containing polyethylene glycol derivatized with Cu(II) complexes of the L- and D-isomers of methionine and aspartate. TSK chromatographic supports derivatized with the methionine complexes were used to study retention of these proteins in metal-affinity chromatography. In the partitioning studies, the amino acid metal chelates exhibit selectivities for the myoglobins that are different from that of Cu(II)-iminodiacetate. Significant differences in selectivity based on the chiral nature of the amino acid complexes were also observed. The chromatographic selectivities of the chelating ligands exhibit little variation, however, suggesting that interactions occurring in solution but not on a surface play an important role in protein binding to the Cu(II)-amino acid–PEG complexes. In solution, the Cu(II)-amino acid complexes are sensitive probes of the microenvironments of surface histidines. The choice of the metal chelate affinity ligand offers a powerful means by which the selectivity of metal-affinity separations can be altered.

INTRODUCTION

The development of cost-effective affinity separations will eventually require that expensive and labile biological affinity ligands be replaced by small chemicals capable of selective binding interactions. Metal-affinity separations, which use chelated metals to bind proteins and other biological materials, afford an excellent example of the advantages of a separation process based on small chemical affinity ligands. Metal chelate ligands are inexpensive and stable; they can be chemically modified and formulated into columns with extremely high loading capacities; they are easy to regenerate and recycle; and product elution is carried under conditions that do not normally harm sensitive biological products. As a result, the applications of metal-affinity separations are rapidly expanding. To be most effective, however, small chemical ligands must exhibit affinities and selectivities for the molecules of interest that are comparable to biological binding interactions. A major drawback of metal-affinity separations for large-scale purification is the limited selectivity that can be achieved with currently-used materials.

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In order to expand the potential applications of metal-affinity techniques in biological separations, we are investigating methods by which the selectivity for a particular protein can be modified and enhanced. Various means for altering selectivity, including engineering metal-binding residues into recombinant proteins, have recently been reviewed [1]. In this paper we demonstrate that significant changes in selectivity in metal-affinity partitioning can be effected through the choice of the metal chelate affinity ligand.

Metal-affinity chromatography with iminodiacetate-bound copper ion [Cu(II) IDA] distinguishes proteins primarily based on their surface histidine content. Proteins with several surface histidines are easily separated from those with no exposed histidines. However, to easily differentiate among proteins bearing similar numbers of exposed histidines, modifications which influence the strength of individual metal–histidine interactions are required. Since Cu(II)IDA is small, hydrophilic, achiral, and bears no net charge, its affinity for histidine is minimally influenced by the protein environment in the vicinity of the histidine. However, this environment is necessarily chiral and will have a particular character imparted to it by the nature of the surrounding amino acid residues. To investigate the feasibility of exploiting the chiral nature and specific properties of the microenvironments of surface histidine residues for protein recognition, we synthesized polyethylene glycol (PEG) derivatives of the L- and D-isomers of aspartic acid and methionine and metallated them with Cu(II) for use in metal-affinity aqueous two-phase partitioning [2]. The L- and D-isomers of methionine were also immobilized onto TSK supports and loaded with Cu(II) in order to determine their binding selectivities in metal-affinity chromatography.

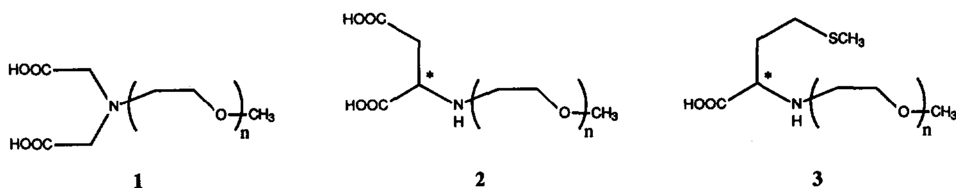
MATERIALS AND METHODS

MPEG-5000 [PEG monomethyl ether, relative molecular mass (M_r)5000] was purchased from Fluka, Dextran T-500 from Pharmacia, epichlorohydrin from Aldrich, and amino acids and proteins from Sigma. Materials were used as purchased, without further purification. TSK G6000PW (15–19 μm porous beads) was the kind gift of Dr. Y Kato, Tosoh. Microchemical analyses were performed at the Caltech Microanalytical Laboratory. Copper contents were determined spectrophotometrically by dissolving the samples in 0.10 *M* EDTA, pH 7, and comparing the absorbance at 800 nm with 0.10 *M* EDTA solutions of known Cu(II) concentration.

Synthesis of Cu(II)IDA–PEG

MPEG-5000 was chlorinated by the procedure of Buckmann *et al.* [3]. Cl-PEG (25.40 g, 5.1 mmols), anhydrous potassium carbonate (3.97 g, 28.7 mmols) and iminodiacetic acid (3.87 g, 29.1 mmols) were dissolved in 225 ml of distilled water and gently refluxed for 53 h. Anhydrous sodium sulfate (37.5 g) was dissolved in the warm reaction mixture, which was transferred to a separatory funnel to cool and separate into two phases. The lower phase, containing most of the inorganic salts and unreacted iminodiacetic acid, was discarded. The upper phase was diluted with an equal volume of distilled water and exhaustively dialyzed, first against dilute bicarbonate, then against distilled water, to remove the low-molecular-weight impurities. The dialyzed solution was lyophilized, giving the product as a white solid. Yield: 23.15 g (89%). IDA–PEG (**1**) and excess copper(II) sulfate pentahydrate were dissolved in

distilled water and exhaustively dialyzed. The dialyzed solution was lyophilized, giving the product as a light blue solid. Yield: 86%. Anal. ($C_{231}H_{459}NO_{117}Cu \cdot 3H_2O$) C, H, N, Cu.



Synthesis of Cu(II)Asp–PEG (L- and D-isomers)

Cl-PEG (5.04 g, 1.00 mmol), L-aspartic acid (0.82 g, 6.2 mmols), and anhydrous potassium carbonate (2.00 g, 14.5 mmols) were dissolved in 45 ml of distilled water and gently refluxed for 67 h. Anhydrous sodium sulfate (3.5 g) was dissolved in the warm reaction mixture, which was transferred to a separatory funnel to cool and separate into two phases. The two phases were treated as described above. Yield (L-isomer): 4.36 g (85%). The same procedure was performed using D-aspartic acid (0.79 g, 6.0 mmols). Yield (D-isomer): 4.38 g (85%). The specific rotation was determined for each product and found to be $-0.454°$ and $+0.453°$ for the L- and D-isomers, respectively (room temperature, 0.07 *M* phosphate, pH 7.0, using the sodium D line).

Asp-PEG (**2**) and excess copper(II) sulfate pentahydrate were dissolved in distilled water and exhaustively dialyzed. The dialyzed solution was lyophilized, giving the product as a light blue solid. Anal. L-isomer: ($C_{231}H_{459}NO_{117}Cu_{0.5} \cdot 2H_2O$) C, H, N, Cu. D-isomer: ($C_{231}H_{459}NO_{117}Cu_{0.5} \cdot 2H_2O$) C, H, N, Cu.

Synthesis of Cu(II)Met–PEG (L- and D-isomers)

Cl-PEG (5.46 g, 1.09 mmols), L-methionine (0.86 g, 5.6 mmols), and anhydrous potassium carbonate (0,84 g, 6.1 mmols) were dissolved in 40 ml of distilled water and gently refluxed for 96 h. Anhydrous sodium sulfate (7.0 g) was dissolved in the warm reaction mixture, which was transferred to a separatory funnel to cool and separate into two phases. The two phases were treated as described above. The dialyzed solution was lyophilized, giving the product as an off-white solid. Yield (L-isomer): 4.36 g (85%). The same procedure was performed using Cl-PEG (5.43 g, 1.08 mmols), D-methionine (0.83 g, 5.8 mmols), and anhydrous potassium carbonate (0.81 g, 5.8 mmols). Yield (D-isomer): 4.84 g (88%). The specific rotations was determined for each product and found to be $+0.315°$ and $-0.336°$ for the L- and D-isomers, respectively (room temperature, 0.07 *M* phosphate, pH 7.0, using the sodium D line).

Met–PEG (**3**) and excess copper(II) sulfate pentahydrate were dissolved in water and exhaustively dialyzed. The dialyzed solution was lyophilized, giving the product as a light blue solid. Anal. L-isomer: ($C_{232}H_{464}NO_{115}SCu_{0.5} \cdot 4H_2O$) C, H, N, Cu. D-isomer: ($C_{232}H_{464}NO_{115}SCu_{0.5} \cdot 4H_2O$) C, H, N, Cu.

Protein partitioning

Aqueous two-phase partitioning experiments were performed as described previously [4]. Two-phase systems were generated by combining appropriate amounts of

stock solutions: (1) 13.54% (w/w) dextran T-500 in distilled water, (2) 0.5 mg/ml protein in 0.046 *M* sodium phosphate, 0.45 *M* sodium chloride, pH 7.6 (3) 40% (w/w) MPEG-5000 in distilled water [used for the determination of the partition coefficient of the protein in the absence of the metal complex (K_0)], and (4) 40% (w/w) MPEG-5000 containing PEG-derivatized Cu(II) chelate to $5.6 \cdot 10^{-3}$% [used for the determination of the protein partition coefficient in the presence of the metal complex (K)]. Each phase system consisted of 1.30 g of the dextran solution (1), 2.00 g of buffered protein solution (2), and 0.70 g of the appropriate PEG solution, (3) or (4). This gave a final 4.00 g phase system composed of 4.4% (w/w) dextran, 1 mg total protein, 7% (w/w) total PEG, and $9.8 \cdot 10^{-4}$% ($1.6 \cdot 10^{-4}$ M) copper, buffered to pH 7.6. The total concentrations of Cu(II) were identical in all partitioning experiments.

The phase systems were gently mixed for 30 min, then centrifuged for 30 min to separate the phases. An aliquot of 200 μl of each phase was diluted to 3.00 ml with distilled water and their absorbances were read at the Soret maximum for the heme proteins (*ca.* 409 nm). The partition coefficient is the ratio of the absorbance derived from the upper phase to the absorbance derived from the lower phase.

Surface histidine contents of the myoglobins were determined from the X-ray crystal structure of sperm whale myoglobin and from the sequences of homologous proteins, as described previously [2]. A histidine was defined as exposed if it exhibited at least 1 Å^2 of surface accessible to a 3 Å radius probe.

Preparation of metal-affinity TSK derivatives

The TSK G6000PW beads were epoxidated using a method similar to that of Matsumoto *et al.* [5]. After extensive washing with distilled water, 9.3 g beads in 9 ml water were combined with 5 ml of 15 *M* NaOH and 6 ml epichlorohydrin, and the mixture was stirred for 2 h at room temperature. The activated beads were washed extensively with water and suction dried before coupling to the chelating agents. For coupling, 3 g of the dried epoxidated beads were added to 6 ml of L- of D-methionine (0.5 *M*, pH 10). An amount of 3.4 g beads were combined with 7 ml iminodiacetic acid (0.5 *M*, pH 11). The three reaction mixtures were shaken at 55°C overnight. (In a separate control experiment, L-methionine showed no tendency towards racemization under these conditions.) The derivatized beads were extensively washed with water.

Metal-affinity chromatography

A 40 × 5 mm column was packed with a quantity of the derivatized beads to give approximately equal total copper loading of 4 μmol for each experiment. The columns were then subjected to the following protocol to determine copper loading and to regenerate the column between experiments (0.4 ml/min flow-rate): 0.1 *M* EDTA, pH 8.0: 10 min; 0.2 *M* $CuSO_4$: 10 min; distilled water: 2 min; 0.05 *M* sodium phosphate, 0.5 *M* NaCl, 0.02 *M* imidazole, pH 7.6: 10 min; 0.05 *M* sodium phosphate, 0.5 *M* NaCl, pH 7.6: 10 min.

The amount of copper loaded onto each column was determined by washing with 0.1 *M* EDTA, pH 7.0, and comparing the absorbance at 800 nm with standard copper solutions. The columns were loaded with approximately 100 μg ($5 \cdot 10^{-9}$ mol) protein, folowed by an imidazole gradient at a flow-rate of 0.2 ml/min. The experiments were repeated with samples of mixed proteins to ensure that the correct elution order was maintained.

RESULTS AND DISCUSSION

Protein recognition by copper chelates can be readily investigated through the measurement of partition coefficients in aqueous dextran–PEG two-phase systems containing metal chelate covalently bound to PEG. Histidines exposed on protein surfaces are available for interaction with chelated copper at neutral and alkaline pH. Since the side chains of other amino acids bind copper with much less affinity (see discussion below), metal-affinity partitioning with Cu^{2+} chelated by IDA at neutral pH reflects the protein's surface histidine content. The addition of Cu(II)IDA–PEG to a two-phase PEG–dextran of PEG–salt system increases the partitioning of proteins which contain surface histidines, and the increase in the logarithm of the partition coefficient in the presence of the the metal-affinity ligand, $\ln(K/K_0)$, is linearly proportional to the number of histidines exposed on the protein surface over a very wide range [2]. This behavior is expected when surface histidines bind equally and independently to the metal complex [4]. Similar behavior is observed in metal-affinity chromatography; proteins are eluted from Cu(II)IDA supports according to their surface histidine contents [6].

The partitioning of three myoglobins from horse, whale, and sheep were studied using copper complexes of IDA–PEG and chiral aspartate and methionine derivatives of PEG. The results are summarized in Table I. The contribution to partitioning that is due to interactions between the protein and the metal-affinity ligand is indicated by $\ln(K/K_0)$, the difference between the logarithms of the partition coefficient in the presence (K) and absence (K_0) of affinity ligand [4,7]. As observed in earlier studies, the metal-affinity partitioning of horse myoglobin to the PEG phase with Cu(II)IDA–PEG is less favorable [as measured by $\ln(K/K_0)$] than the partitioning of whale or sheep myoglobin.

The relative affinities of these proteins for the PEG-rich phase are dramatically

TABLE I

MYOGLOBIN PARTITION COEFFICIENTS IN PEG–DEXTRAN TWO-PHASE SYSTEMS CONTAINING PEG-DERIVATIZED Cu(II) CHELATES

K_0 = partition coefficient in the absence of PEG–copper chelate, K = partition coefficient in the presence of PEG–copper chelate. Each partition coefficient is an average of at least five measurements, with a standard deviation of approximately 3% of the mean. Mb = Myoglobin.

	Derivative	Horse Mb	Whale Mb	Sheep Mb
K_0		0.39	0.47	0.38
K	IDA	0.75	0.97	1.10
	L-Asp	0.53	0.60	0.52
	D-Asp	0.54	0.53	0.50
	L-Met	0.73	0.55	0.73
	D-Met	0.49	0.53	0.49
$\ln(K/K_0)$	IDA	0.66	0.73	1.07
	L-Asp	0.32	0.25	0.32
	D-Asp	0.33	0.13	0.28
	L-Met	0.64	0.17	0.66
	D-Met	0.24	0.12	0.27

altered when the chiral metal-chelating polymers derived from aspartic acid and methionine are used. $\mathrm{Ln}(K/K_0)$ for whale myoglobin is the least favorable of the three proteins in all four two-phase systems containing the amino acid-copper–PEG complexes. This is particularly striking for partitioning with the methionine-derivatized PEG; the apparent affinity of both optical isomers of Cu(II)Met–PEG towards whale myoglobin is approximately equivalent to (Cu(II)IDA–PEG binding to a protein possessing only one exposed histidine as opposed to five. In contrast, the apparent affinity of the Cu(II)-L-Met–PEG complex towards the myoglobins from horse and sheep in only slightly smaller than that of Cu(II)IDA–PEG. Furthermore, there is a distinct chiral effect in the protein partitioning; partitioning with complexes derived from L-isomers of the amino acids results in $\ln(K/K_0)$ values significantly different from those obtained using the D-isomers. For the methionine-based complexes, this chiral effect is most significant for the myoglobins from horse and sheep.

To determined whether the altered selectivities observed for metal-affinity partitioning also occur in metal-affinity chromatography, a TSK chromatographic support was derivatized with Cu(II)IDA, Cu(II)-L-Met, and Cu(II)-D-Met. The concentrations of imidazole required to elute the three myoglobins from these supports are reported in Table II. For these experiments, the column lengths were adjusted to maintain similar copper loadings. On all three Cu(II)-chelating supports, the whale protein requires a greater concentration of imidazole for elution than do the horse and sheep proteins. Since the elution order remains the same for the myoglobins on all three supports, there are no significant differences in binding selectivity for the copper chelates in the solid phase. If anything, the selectivity of Cu(II)-methionine is reduced with respect to Cu(II)IDA; the horse and sheep myoglobins are indistinguishable on both methionine-based supports. The fact that the myoglobins require smaller concentrations of imidazole for elution from the L- and D-methionine supports as compared to Cu(II)IDA probably reflects the lower Cu(II)-binding affinity of methionine. Cu(II) is more easily removed from these columns than from the IDA-derivatized material.

Imidazole competes with binding sites on the protein for coordination to the bound copper ion. Elution with a gradient of imidazole at pH 7.6 allows us to identify the relative binding affinities based on interactions between the copper chelates and

TABLE II

COLUMN CHARACTERISTICS AND CONCENTRATIONS OF IMIDAZOLE REQUIRED TO ELUTE MYOGLOBINS FROM TSK METAL-AFFINITY COLUMNS

Column diameter is 0.5 cm, flow-rate = 0.2 ml/min.

Chelating ligand	Column volume (ml)	Copper loading (μmol)	Concentration of imidazole at elution (mM)		
			Horse	Sheep	Whale
IDA[a]	0.63	4.2	7.3	8.5	9.3
L-Met[b]	0.51	3.6	4.9	4.9	6.4
D-Met[b]	0.78	3.7	5.3	5.3	6.1

[a] Gradient: 0–10 mM imidazole in 150 min, pH 7.6.
[b] Gradient: 2–8 mM imidazole in 100 min, pH 7.6.

surface histidine residues. Direct binding by chelated Cu(II) to other functional groups such as the N-terminus or lysine residues are small because these groups are still largely protonated at this pH. Copper coordination to carboxylic acid moieties is weak relative to histidine [8]. There is no evidence that copper directly coordinates aromatic side chains; the apparent influence of aromatic residues in metal-affinity chromatography is due to indirect effects on solvation and metal electronegativity [1,9]. Thus it is unlikely that direct binding interactions with residues other than histidine are contributing to the observed altered selectivity order in metal-affinity partitioning. While Cu(II)IDA appears to bind all accessible histidines with more-or-less equal affinity [4], the altered selectivities of Cu(II)-L-Met and Cu(II)-D-Met most likely reflect the effects of neighboring amino acid residues on Cu(II)-histidine binding. However, these interactions manifest themselves only in solution, not upon interaction with the surface-immobilized chelates.

Possible origins of the differences in binding selectivity observed for the various Cu(II) chelates in metal-affinity partitioning must be discussed in light of the structures of the protein-chelate complexes. Cu(II) and IDA form a 1:1 (metal–ligand) complex, whereas most amino acids form a 1:2 (bis-glycinate-like) complex in solution [10,11] (Fig. 1). When the derivatized PEGs are metallated with excess Cu(II), followed by exhaustive dialysis to remove unbound metal, the free, dialyzable Cu(II) becomes negligible as the Cu(II)–(IDA–PEG) ratio approaches 1:1. In contrast, significant quantities of free copper are present until the Cu(II)–(Asp–PEG) and Cu(II)–(Met–PEG) ratios approach 1:2. This indicates that the stability constants [13] and structures of the soluble PEG-derivatized chelates are similar to those of their underivatized counterparts. 1:2 Cu(II)–amino acid complexes cannot form on the TSK surface because all the amino acids are covalently attached, and the distance between ligands (>40 Å) is much greater, on average, than the length of epoxide spacer arm and Cu(II)Met ligand (7.5 Å).

We expect a surface histidine to coordinate Cu(II)IDA equatorially in a 1:1 complex, similar to the coordination seen in the crystal structure of Cu(II)IDA–bipyridine [14]. Although two equatorial sites are available for histidine coordination,

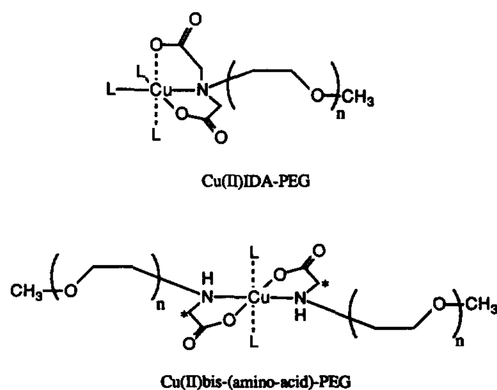


Fig. 1. Expected structures of Cu(II)IDA–PEG and Cu(II)bis(amino acid–PEG) complexes. Structures are assumed to be analogous to those of Cu(II)iminodiacetate dihydrate [10] and bis(L-methionato)Cu(II) [11,12]. "L" indicates sites available for ligand binding (protein or solvent). * = Stereogenic center.

protein complexation with Cu(II)IDA–PEG is sterically limited to single histidine residues, unless multiple histidines are arranged according to precise geometric requirements, such as the His-X_3-His arrangement in an α-helix [15,16]. Thus, in solution, the protein can associate with as many Cu(II)IDA–PEG molecules as there are accessible histidines. This simple binding behavior leads to the linear dependence of $\ln(K/K_0)$ on surface histidine content.

The nature of complex formation between a histidine residue and a Cu(II)bisglycinate-like amino acid complex is less clear. A histidine could coordinate to the weak axial site of the bis-complex. Such binding would be weaker than to a Cu(II) IDA complex, a result of both the weaker nature of the axial bond and the greater steric hindrance from the bisamino acid complex, which depends on the histidine microenvironment. However, this weaker binding may not necessarily be apparent in the partitioning, since two PEG molecules associated at a single histidyl site could offset the negative effects of the reduced affinity on partitioning. Alternatively, the histidine could displace one amino acid to take up a more strongly-bound equatorial position, yielding a 1:1 Cu(II)chelate–histidine complex similar to that expected for Cu(II)IDA. Cu(II) complexes of amino acids (1:1 mixed) are known: the crystal structure of Cu(II)aspartic acid–phenanthroline is similar to that of the Cu(II)IDA–bipyridine complex cited above [17]. However, a complex of this type, which can form on the solid support as well as in solution, cannot explain the difference between partitioning and chromatography.

The complexes derived from the L- and D-isomers of aspartic acid exhibit similar affinities for the different myoglobins in partitioning, although the affinity for whale myoglobin is somewhat reduced. Aspartic acid is isomeric with IDA and, if a 1:1 Cu(II)aspartate–histidine complex is formed, it would be expected to have properties very similar to those of the IDA complex and should exhibit little tendency to be influenced by the protein environment in the vicinity of the histidine. The properties of the methionine-derived complexes should be quite different from those derived from aspartic acid. These complexes bear a greater positive charge than do their aspartate-derived counterparts, and the methyl thioether side chain confers both steric bulk and a greater hydrophobic character to the complex. Both forms of the Cu(II) Met–PEG have low apparent affinity for whale myoglobin in metal-affinity partitioning.

Although the structures of myoglobins from horse, whale, and sheep are highly conserved [18], they differ slightly in the content and placement of surface histidine residues. Horse, whale and sheep myoglobin share five histidines that are accessible to solvent (amino acid positions 36, 48, 81, 113 and 116). The whale and sheep proteins each have one additional surface histidine. Histidines 113 and 116 in all three myoglobins are separated by a very short distance, and it appears that only one Cu(II)IDA–PEG can bind at this position [2]. As discussed previously [16], there is no evidence that the His 113–His 116 arrangement forms a special high-affinity binding site for Cu(II). Myoglobin partitioning with Cu(II)IDA is fully explained by binding interactions with non-interacting histidines [4].

Using protein sequence data for horse, sheep and whale myoglobins [7], and crystallographic data for sperm whale myoglobin [19,20], the surface environments within 6 Å of the exposed histidines were compared. The amino acid residues within this radius are 100% conserved for three of the exposed histidines (His 36, 48, and 81)

in horse and sheep myoglobin. In contrast, the environment is 100% conserved for only one of the surface histidine residues in whale myoglobin (His 81). In two of four of the surface histidine environments which differ from those of the sheep and horse proteins (His 36 and 48), the whale myoglobin either has an additional positive charge in the vicinity (a lysine residue), or lysine has been substituted by a larger, positively-charged arginine. The effect of these substitutions may be to move a positive charge closer to, or add an additional charge in the vicinity of, the potential histidine binding sites. The reduction in affinity for whale myoglobin is much more pronounced for the more positively charged Cu(II)methionine complexes than for the Cu(II) aspartate ones. The increase in positive charge near the histidine residues in the whale myoglobin could effectively reduce the number of histidines available for complexation to Cu(II)Met–PEG.

Cu(II)-L-Met–PEG has a higher apparent affinity for the horse and sheep myoglobins than does the complex derived from D-methionine in the partitioning experiments. The protein environment surrounding a histidine is chiral, and it is certainly feasible that these surroundings can differentiate between copper chelate complexes that differ in their chirality. [The effective size of the chelate that is available for interactions with the protein surface and, therefore, the distinction between the D- and L-forms are enhanced if the protein is binding a 1:2 copper–(amino acid–PEG) complex.] However, at this point we are unable to identify specific structural origins for the differences in the abilities of the complexes derived from the D- and L-amino acids to bind to exposed histidines. Further work will be necessary to explain the observed chiral selectivity.

We have shown that selectivities in protein binding by copper chelate complexes can differ significantly from solution to solid phase. A possible explanation for this behavior is that, in solution, the amino acid chelates form larger 1:2 copper–chelate complexes which are capable of discriminating among histidines on the surface of the myoglobins. Cu(II)Met–PEG and Cu(II)Asp–PEG complexes are sensitive probes of the microenvironments of surface histidines in metal-affinity partitioning.

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