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Immobilized metal ion affinity electrophoresis

A study with several model proteins containing histidine

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ABSTRACT

Immobilized metal ion affinity electrophoresis (IMA-Elec) is one among the many methods derived from the immobilized metal ion affinity chromatography. Two approaches for incorporating the metal ligand, were studied. One was in the form of insoluble particulate material based on Sepharose 6B and the other in the form of soluble polymer based on polyethylene glycol (PEG) 5000. Both the polymers coupled with iminodiacetate and metallized with copper or zinc were used as ligands, incorporated into soluble agarose as the electrophoretic gel. Several histidine-containing model proteins were studied with both the systems and their metal binding strengths were determined as the dissociation constants, K_d. The results clearly demonstrated that the mechanism of protein recognition by immobilized copper or zinc via the accessible histidyl residues was maintained in the IMA-Elec system. Proteins with increasing numbers of histidine residues showed increasing binding strength (lower K_d values). While this basic mechanism was conserved, the supporting polymers (Sepharose 6B and the PEG 5000) showed significant differences in the metal binding to the protein. The polysaccharide Sepharose 6B enhanced the binding strength compared with PEG 5000. The optimum electrophoretic parameters were determined to be current intensities up to 20 mA and pH *ca.* 7.0. At pH > 8.0, a significant decrease in the affinity was observed, this decrease being greater with PEG 5000 than Sepharose 6B as supporting material.

INTRODUCTION

Immobilized metal ion affinity electrophoresis (IMA-Elec) has recently been applied to study the interactions of proteins with immobilized copper ions in a quantitative manner [1]. This approach was derived both from the seminal concept of immobilized metal ion affinity chromatography (IMAC) [2] and from affinity electrophoresis using biospecific affinity ligands [3], lectins [4], triazine dyes [5], etc. This preliminary approach used the inclusion of insoluble Sepharose beads coupled with the metal chelate, which served as the affinity ligand in the agarose electrophoretic gel [1]. However, this approach has drawbacks such as limitations on the ligand concentration that could be used and the extra care needed to be taken to prepare highly homogeneous gel beds.

Nevertheless, this system made use of the same microenvironment for the immobilized copper ion as in the IMAC system, namely Sepharose 6B–IDA–Cu(II) (IDA = iminodiacetate). It has been estab-

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lished that, in protein recognition by immobilized transition metal ions via the accessible histidyl residues, both the microenvironment of the immobilized metal ion and that of the interacting histidyl residues determine the selectivity and the strength of interactions. If we are using IMA-Elec as an analytical tool for probing the histidyl residues of the proteins, these two factors should be taken into consideration. Hence we tried to study the different possibilities of using polymer-supported chelated metal ions on an electrophoretic gel. Our attempts to prepare metal-chelated soluble agar and chelated acrylamide and to use them as electrophoretic gels with integrated metal ligands were not very encouraging. On the other hand, it has been reported [6,7] that polyethylene glycol (PEG)-supported metal chelates used in immobilized metal ion affinity partition systems (IMAP) recognize proteins in the same manner as Sepharose 6B–IDA–Cu(II) used in IMAC.

In this paper we present an IMA-Elec system with agarose containing soluble PEG 5000–IDA–Cu(II) as the affinity ligand and compare this system with that reported previously [1].

EXPERIMENTAL

α-Chymotrypsinogen A (from bovine pancreas, type II; 6× crystallized), α-chymotrypsin (from bovine pancreas type II; 3× crystallized), α-chymotrypsin inactivated with diisopropyl phosphofluoridate (DIPF) (type VI; 3× crystallized), cytochrome *c* from *Candida krusei* (type VII) from horse heart (type VI) and from tuna heart (type XI), ribonucleases A (type II-a) and B (type XII-b) from bovine pancreas, α_2-macroglobulin and human serum-albumin (HSA), agarose (type VII), Coomassie Brilliant Blue G250 and methoxy-PEG 5000 were obtained from Sigma (St. Louis, MO, USA), α_2-glycoprotein and α_2-HS glycoprotein from Calbiochem (San Diego, CA, USA) and Sepharose 6B and gel-bond film from Pharmacia (Uppsala, Sweden).

Preparation of electrophoretic agarose gels

Containing Sepharose 6B–IDA–M(II). The insoluble Sepharose 6B–IDA–M(II) beads (M = metal) were incorporated into the 1% (w/v) agarose solution in the corresponding buffer for the selected pH by adding various amounts of the suction-dried, metallized, chelated sepharose in the range 0.5–3.5 g of the suction dried gel representing metal [*e.g.*, Cu(II)] concentration in the range 3.0–20.0 m*M*. Then 3.5–10 ml (depending on the size of the electrophoresis plate) were poured and spread on a sheet of gel-bond film (8 × 4 cm or 12.3 × 5 cm) and used as described [1].

Containing PEG 5000–IDA–M(II). This was synthesized as described [7] using monomethoxy-PEG 5000 as the starting material and by two-step chemical reactions consisting of the preparation of amino-PEG 5000 and derivatization into iminodiacetate (IDA)-PEG 5000 using bromoacetic acid. Alternatively, direct derivatization of Cl-PEG 5000 into IDA-PEG 5000 was also used according to the method described by Wuenschell *et al.* [8]. Then the chelated PEG was metallized as described previously [7]. This soluble polymer containing the affinity ligand [IDA–M(II)] was incorporated into the 2% agarose gel melted at 70°C, in the concentration range 0–2.5% (w/v) of PEG 5000–IDA–M(II) representing metal [*e.g.*, Cu(II)] concentrations in the range 1.0–5.0 m*M*.

The remainder of the electrophoresis experiment was run in the conventional way using an SE 250 Mighty Small II vertical slab unit and PS 250/2.5 A power supply as the current source, both from Hoefer (San Francisco, CA, USA).

RESULTS

Study of the model proteins

Two groups of proteins were chosen, based on the data already available using IMAC or IMAP and the differences in their histidine contents: group I, cytochrome *c* from tuna heart, horse heart and *Candida krusei*; and group II, bovine ribonuclease A and B.

A double inverse plot of the differential migration distance ($d_0 - d$) as a function of ligand (L) concentration expressed as Cu(II) concentration in m*M* integrated in the form of PEG 5000–IDA–Cu(II) into the electrophoretic gel was plotted for each of these two groups of proteins (Fig. 1a and b).

The dissociation constants were calculated from these plots as described [1], based on Bøg-Hansen and Takeo [4].

As expected, cytochrome *c* from *Candida krusei* showed the maximum affinity for the immobilized

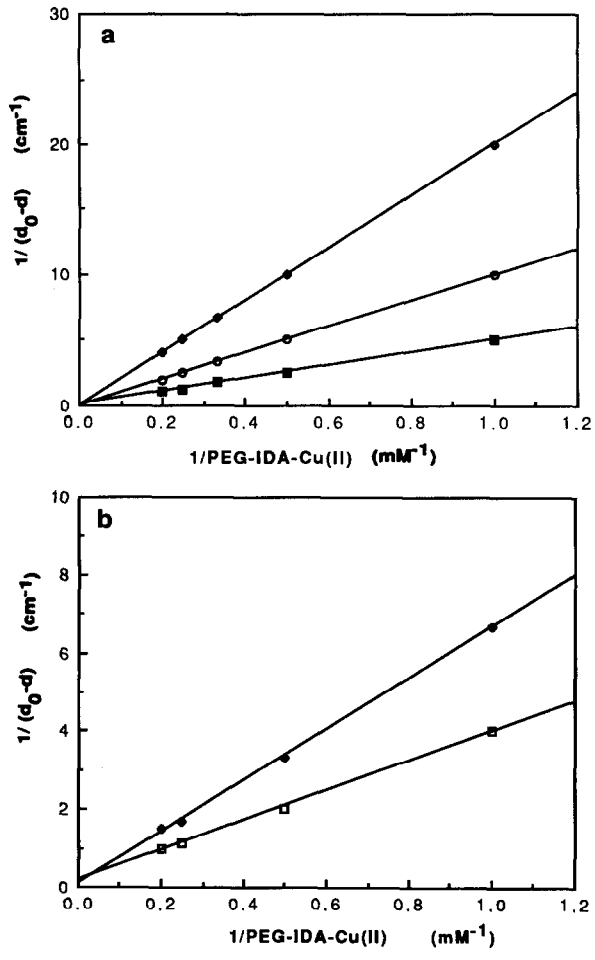

Fig. 1. Double inverse plot of differential migration ($d_0 - d$) *versus* integrated ligand (L) concentration expressed in m*M* copper incorporated in the form of PEG 5000–IDA–Cu(II). Electrophoretic buffer, 0.1 *M* Tris–acetate (pH 7.2); migration time, 7 h at 20 mA. (a) Cytochrome *c* from different sources: ◆ = Cytochrome *c* from tuna heart; ○ = cytochrome *c* from horse heart; ■ = cytochrome *c* from *Candida krusei*; (b) ribonuclease A and B: □ = ribonuclease A; ◆ = ribonuclease B.

copper as compared with cytochrome *c* from horse heart or from tuna heart. Similarly, bovine RNase A showed a higher affinity than bovine RNase B.

To a first approximation, proteins are retained on metal affinity columns (IMAC) or extracted into the liganded phase on metal affinity phase partition (IMAP) based on the number of accessible histidines [6]. It has been shown [6,9] that cytochrome *c* from tuna heart, horse heart and *Candida krusei* were bound to polymer-supported IDA–Cu(II) in the order of strength of binding tuna < horse < *Candida krusei*, the *Candida* having two histidyl residues at the surface, horse heart one and tuna heart no histidine. The data in Fig. 1a confirm the same order of strength of binding. This shows that the mechanism of protein binding to IDA–Cu(II) is almost the same in IMA-Elec as in other IMA systems such as IMAC and IMAP. However, in quantitative terms, the values calculated for the dissociation constants according to Bøg-Hansen–Takeo plots [4] (Table I) showed a significant decrease in the affinity. K_d values of 2.2–4.5 m*M* were reported [8] for the interaction of a protein with a single histidine (2.2 m*M* for horse heart cytochrome *c*), which is in agreement with the stability constants for complexes of IDA–Cu(II) and histidine derivatives in solution. However, the K_d values for cytochrome *c* on IMA-Elec are in the region of 63 m*M*, showing a decrease of more than one order of magnitude. It is known that the metal affinity ligand can sense the environment surrounding a histidine through hydrophobic and electrostatic interactions. Hence the driving force used in the metal affinity system would be expected to have an impact on the protein binding strength to the immobilized metal ligand. The use of an electrical field as the driving force and the absence of a high salt concentration, known to favour histidine-mediated binding in IMAC and IMAP, can at least partly account for this decrease in the binding strength. Nevertheless, we can conclude that the basic mechanism of histidine–mediated protein recognition by the IDA–M(II) (Cu and Zn) is conserved in IMA-Elec.

The difference in the affinity of RNase A and B to IDA–Cu(II) is very striking. It has been reported [10] that RNase A was slightly more retarded than RNase B on a Sepharose 6B–IDA–Cu(II) column. In fact, we found that when RNase A and B were run on a Sepharose 6B–IDA–Cu(II) column using a starting buffer at pH 5.5 and a protonation protocol for elution with a linear pH gradient from pH 5.5 to 4.0, RNase A was eluted precisely at pH 4.1 and RNase B at pH 4.5 (unpublished data). This clearly reflects the difference in the affinity due to differences in the microenvironment of the histidine. Both RNase A and B have four histidine residues, of which two are in the active site. However, RNase B is glycosylated at Asn 34 whereas RNase A is non-

TABLE I

INFLUENCE OF pH AND SUPPORTING POLYMERS ON THE AFFINITY OF MODEL PROTEINS TO IDA–Cu(II)

Dissociation constants in m*M*. ND = Not determined; BT = below threshold, *i.e.*, the value of $d_0 - d$ is near zero.

Protein	IMA-Elec with Sepharose 6B–IDA–Cu(II)			IMA-Elec with PEG 5000–IDA–Cu(II)		
	pH 8.2	pH 7.2	pH 5.5	pH 8.2	pH 7.2	pH 5.5
RNase A	6.25	4.07	ND	69.00	22.00	BT
RNase B	11.10	4.74	ND	BT	67.00	BT
Cyt. *c* from *Candida krusei*	8.20	3.72	45.00	BT	63.00	BT
Cyt. *c* from horse heart	Abnormal	10.96	66.70	BT	160.00	BT
Cyt. *c* from tuna heart	20.70	148.00	250.00	BT	400.00	BT

glycosylated. This glycosylation can influence at least through long range forces and/by steric hindrance the microenvironment of histidines 119 and 48. The K_d values calculated from Fig. 1b for RNase A and B are 22 and 67 m*M*, respectively. These values are close to that obtained for cytochrome *c* from *Candida krusei*, having two histidines on the surface.

Influence of the nature of IDA–Cu(II) supporting polymers

Two types of supporting polymers were studied. The insoluble particulate polymer Sepharose 6B and the soluble polymer PEG 5000 were both derivatized with IDA and metallized. IMA-Elec was run with gels incorporated with Sepharose 6B–IDA–Cu(II) (in the concentration range 0–20%, w/v) and with PEG 5000–IDA–Cu(II) (in the concentration 0–2.5%, w/v), using two sets of model proteins, namely cytochrome *c* from *Candida krusei*, horse heart and tuna heart as one set and bovine RNase A and B as a second set.

Figs. 1a and 2a show the double inverse plots according to Bøg-Hansen and Takeo [4] for the first set of three related proteins the cytochrome *c* from the above-mentioned three sources, using soluble (Fig. 1a) and insoluble (Fig. 2a) polymer-supported IDA–Cu(II) at pH 7.2. The K_d values calculated do show differences (Table I). However, the basic mechanism of recognition of protein based on the surface histidine residues is conserved in both instances, as the cytochrome *c* from *Candida krusei* was the strongest binding in both instances.

Sepharose 6B–IDA–Cu(II) showed an increased

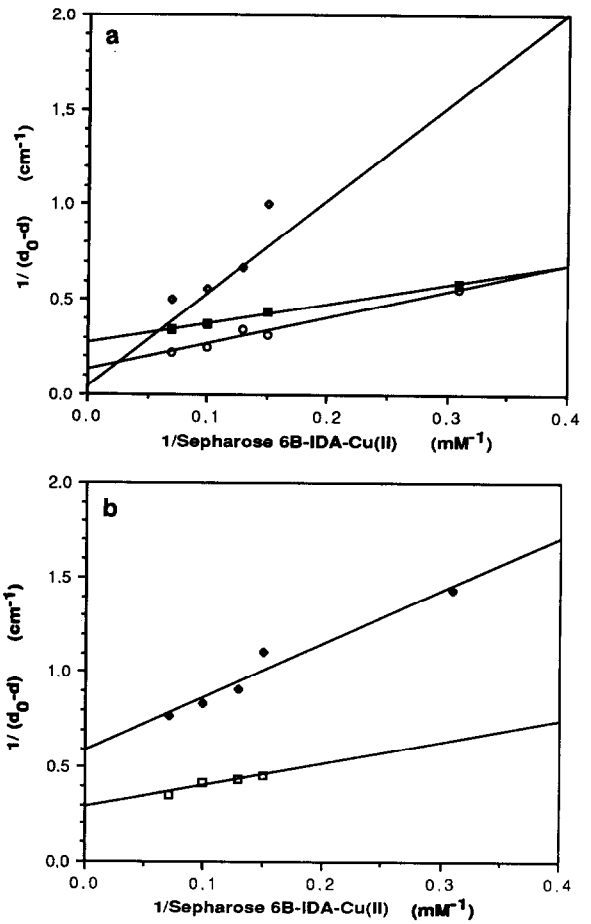

Fig. 2. Double inverse plot of differential migration ($d_0 - d$) *versus* integrated ligand (L) concentration expressed in m*M* copper incorporated in the form of Sepharose 6B–IDA–Cu(II). Other details as in Fig. 1.

affinity and the K_d values for the two histidine-containing *Candida krusei* cytochrome *c* was 3.7 m*M* compared with 63 m*M* for the same protein when PEG 5000–IDA–Cu(II) was used as the ligand in this electrophoretic system. Similar differences are observed for the other cytochrome *c* (horse heart with one histidine and tuna heart with no histidine) containing fewer histidine residues.

In the case of RNase A and B, similar increase in the affinity (lower K_d values) were observed when the insoluble polymer was used for supporting the IDA–Cu(II) compared with the values obtained with PEG 5000–IDA–Cu(II) (Figs. 1b and 2b). Moreover, the difference between the K_d values of RNase A and B was more significant with the PEG 5000–IDA–Cu(II) system rather than in the Sepharose 6B–IDA–Cu(II) system (Table I). However, one has to take into consideration the fact that the maximum metal concentration that could be incorporated using PEG 5000–IDA–Cu(II) was only 5 m*M* compared with 20.0 m*M* in the case of Sepharose 6B–IDA–Cu(II).

The data suggest that while the basic mechanism of IDA–Cu(II) recognizing the protein via the accessible histidyl residues is conserved, the more hydrophilic Sepharose (polysaccharide) polymer increases the strength of the interactions, as compared with the relatively hydrophobic PEG as the supporting polymer.

In fact, when proteins such as lipase having a moderate number of histidine residues were purified by IMAC and IMAP, the IMAC experiment gave a better resolution than the IMAP using IDA–Cu(II) as the metal ligand [11].

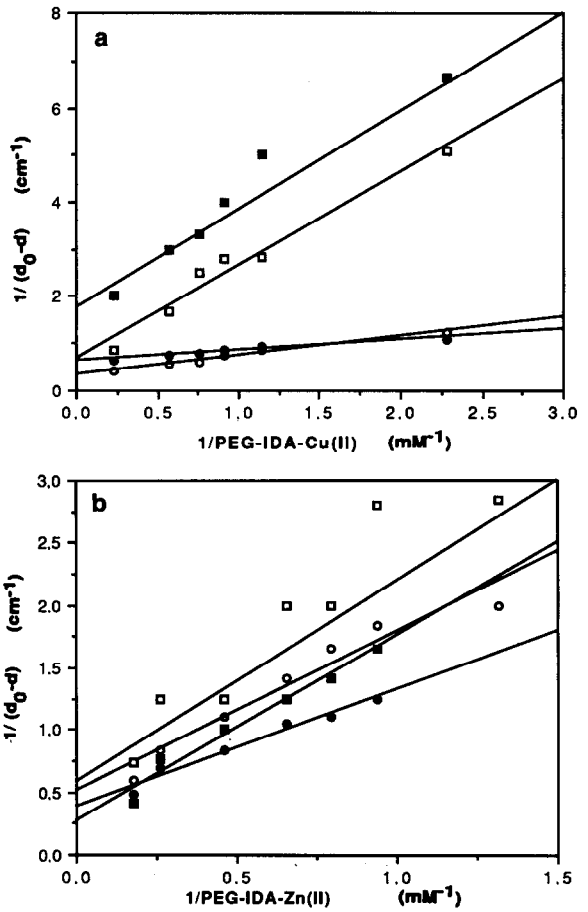

Fig. 3. Differential electrophoretic migration of four histidine-rich model proteins as a function of ligand concentration incorporated (a) as PEG 5000–IDA–Cu(II) and (b) as PEG 5000–IDA–Zn(II). ● = α_2-Macroglobulin; ○ = α_2-glycoprotein; □ = α_2-HS-glycoprotein; ■ = human serum albumin.

Influence on the nature of the metal ion

This study is limited to PEG 5000-supported IDA–M(II). A group of proteins rich in histidine were chosen as models to investigate their relative affinities to IDA–Cu(II) and IDA–Zn(II) supported by PEG 5000 and integrated into the electrophoretic agarose gel. These proteins were run at different integrated ligand concentrations and the double inverse plots according to ref. 4 are shown in Fig. 3a and b. The K_d values calculated from these plots are presented in Table II.

It is obvious that α_2-macroglobulin, which has 156 histidine residues per mole and at least 60 of which are accessible, gave the highest affinity (K_d = 0.34 m*M*). This is in accordance with the high extracting power (1000-fold) for this protein in the IMAP system using IDA–Cu(II) as the ligand [7]. The most interesting feature was that only α_2-HS glycoprotein had similar K_d values with both IDA–Cu(II) and IDA–Zn(II) as the ligand. According to Sulkowski [10], copper can recognize even a single histidine residue or dispersed histidine residues on the protein surface, whereas zinc needs histidine clusters with His-$(x)_{2,3}$-His or histidines brought closer by an α-helix. Hence we can predict that α_2-HS-glycoprotein has the histidine clusters necessary for zinc recognition whereas the other proteins,

TABLE II

INFLUENCE OF THE NATURE OF THE METAL ION ON THE DISSOCIATION CONSTANTS OF HISTIDINE RICH PROTEINS

The metal ions were incorporated into the electrophoretic gel in the form of PEG 5000–IDA–Cu(II) or as PEG 5000–IDA–Zn(II).

Protein	K_d (mM)	
	PEG–IDA–Cu(II)	PEG–IDA–Zn(II)
α_2-Macroglobulin	0.34	2.42
α_2-Glycoprotein	1.21	2.55
α_2-HS glycoprotein	2.85	3.31
Human serum albumin	1.28	5.45

including the high histidine-containing α_2-macroglobulin, may not have this structural element.

Influence of the electrophoretic conditions

Selection of the optimum electrophoretic conditions (current intensity, pH) and the relative migration of the free protein and the protein–ligand complex are essential for the reliable determination of the affinity strength and for the eventual usefulness of this system for the quantitative determination of a protein showing metal affinity.

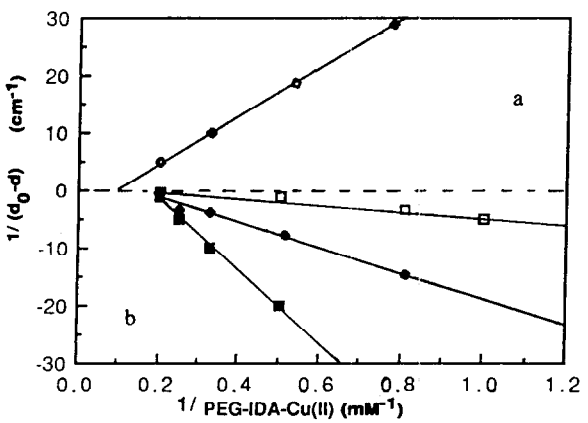

Fig. 4. Influence of the applied current intensity on the differential migration ($d_0 - d$) of α-chymotrypsinogen A, α-chymotrypsin and DIFP-inactivated α-chymotrypsin as a function of ligand [PEG 5000–IDA–Cu(II)] concentration. (a) 20 mA; (b) 50 mA. The values of $d_0 - d$ for α-chymotrypsinogen and α-chymotrypsin at 20 mA were almost zero and only DIFP-inactivated α-chymotrypsin is shown at a 20-mA current intensity. □ = Chymotrypsinogen, 50 mA; ■ = chymotrypsin, 50 mA; ◆ = Chymotrypsin + DFP, 50 mA; ◇ = chymotrypsin + DFP, 20 mA.

Applied current intensity. Fig. 4a and b show the relative migration of the three closely related proteins α-chymotrypsinogen, α-chymotrypsin and DIPF-denatured α-chymotrypsin at (a) 20 mA and (b) 50 mA current intensities. Owing to very low affinity of α-chymotrypsinogen and chymotrypsin, we could not obtain significant $d_0 - d$ values for these two proteins even at 20 mA current intensity. However, if we compare the case of DIFP-inactivated α-chymotrypsin at 20 and 50 mA, it is very striking that high current intensities (50 mA) (b) make the protein move faster even in the presence of the affinity ligand, giving negative affinity values. In fact, it has been shown in the case of enzyme-substrate affinity electrophoresis [12] that at high applied currents the enzymes move too fast so that their mobility becomes the limiting factor for the enzyme–substrate complex to be formed. Moreover, we have shown previously [1] that an increasing current intensity even as low as 30 mA resulted in a deviation from linearity of the plot of $1/(d_0 - d)$ *vs.* 1/[L].

Moreover, one has to take into consideration the kinetics of metal coordination with IDA and the kinetics of IDA–M(II) binding to protein. Even if the latter is rapid, at higher current intensities metal coordination to IDA may be the limiting factor. In fact, at times we observed a faint blue band of copper ion moving on the surface of the electrophoretic gel when >50 mA current was applied.

pH. The immobilized metal ion–protein interactions are favoured by deprotonation of the protein, hence higher efficiencies of binding or extraction in IMAC or IMAP systems, respectively, have been reported [9,13] at higher pH (7.0 or 8.0). Although we observed an increase in the binding strength with increasing pH values of 5.5, 6.2 (data not shown) and 7.2, IMA-Elec showed a decrease in the binding strength at pH 8.2 compared with pH 7.2. This decrease was even more striking when PEG 5000–IDA–Cu(II) was used as the ligand (Table I). This pH effect will depend on the relative cathodic or anodic migration of the protein and that of the protein–ligand complex at the given pH [12]. The cytochrome *c* samples from different sources each have a pI value of 10.6. Hence an electrophoretic pH

increase from 7.2 to 8.2 can result in modification of the migration velocity of the protein and hence decrease in the binding strength. It is noteworthy that proteins with acidic p*I* values gave positive values for the dissociation constants (Table II) at pH 8.2 when anodic migration was used. However, a significant decrease in the affinity was observed at pH 5.5. It is difficult to explain this only on the basis of protonation of the proteins. Although the present set of data indicate an optimum pH of 7.2, further investigations with pH values ranging from 5.0 to 8.0 are needed.

CONCLUSION

In electroimmunoassay and other affinity electrophoresis systems, using lectins or enzyme substrates, the affinity ligand is usually directly incorporated into the agarose gel either as a freely moving ligand or as an immobilized ligand [3,4]. The affinity ligand in the immobilized metal ion affinity systems is in fact IDA–M(II) and not the free metal. Moreover, Porath [14] has shown that IDA–M(II) interactions with proteins are water-mediated. The metal affinity systems systems such as IMAC and IMAP use IDA–M(II) coupled to polyhydroxylic polymers such as Sepharose and PEG.

These two polymers were used in this study to constitute the ligand support in the IMA-Elec applications. Both systems ensured effective immobilization of the interacting ligand, namely IDA–Cu(II) or IDA–Zn(II), as shown by the absence of any metal leaching or the heterogeneous distribution of the metal under optimized electrophoretic conditions. This is also borne out by the fact that only the plot of $1/(d_0 - d)$ *vs.* 1/[L] corresponding to the immobilized ligand gave a linear relation as compared with that of $1/d$ *vs.* 1/[L] applicable to the free-moving ligand in the system, as discussed by others [15].

It is now clearly established that the metal–protein recognition in IMAC and IMAP is via the accessible histidine residues on the surface of the protein [9,13]. More recent work using 2D NMR to study the IDA–Cu(II) interaction with proteins in solution has emphasized this fact [16]. Our data here with the different groups of model proteins studied demonstrate that the IDA–M(II) recognition of the protein is via the accessible histidine residues of the protein. Hence it is clear that the basic mechanism of protein recognition is conserved even in the electrical field.

Although the recent reports show that both Sepharose- and PEG-supported IDA–M(II) can be used for protein purification and protein studies, no data are available in terms of the quantitative differences in the binding strength of the proteins to these metallized polymers, which can be due to the synergistic or antagonistic effects of these OH-containing polymers on the protein binding to IDA–M(II). The binding strengths (K_d) have been extensively reported for PEG–IDA–M(II) in the IMAP system, but not in a comparative manner with reference to the corresponding IMAC using Sepharose 6B–IDA–M(II). Our data here show that while the basic mechanism of protein recognition via the histidyl residues is conserved, Sepharose 6B as the supporting matrix enhances the binding strength for a given protein (Table I). All electrophoretic conditions (pH, temperature, current intensity) being similar, the K_d values are almost one order of magnitude lower with Sepharose 6B–IDA–Cu(II) than PEG 5000–IDA–Cu(II). This is also supported by the fact that lipase from *Chromobacterium viscosum* could be successfully retained and eluted with 5.0–10.0 m*M* imidazole from a Sepharose 6B–IDA–Cu(II) column, whereas the same affinity extraction of using PEG 5000–IDA–Cu(II) was not very efficient.

Moreover, the K_d values calculated by the IMA-Elec method are in general higher than those calculated by IMAP. This decrease in the binding strength could be attributed to the effect of the electrical field applied. However, the maximum ligand [IDA–Cu(II)] concentration that could be incorporated as PEG 5000–IDA–M(II) in IMA-Elec is limited. Nevertheless, a K_d value of 10.6 m*M* was obtained with IMA-Elec using Sepharose 6B–IDA–Cu(II) for α-chymotrypsin [1], whereas the K_d value calculated by a partition experiment (IMAP) using PEG–IDA–Cu(II) for the same enzyme was 20 m*M* [17]. This clearly demonstrates that the metal–protein binding strength is enhanced by Sepharose compared with PEG as the supporting polymer. This synergistic effect is in fact conserved even in the electrical field.

Limitations and perspectives of the method

The range of pH values at which the system can be run will depend on the relative migration of the protein and the protein–ligand complex at the given pH. As is shown in Table I, pH 7.2 seems to be the optimum for the system.

This sets up a serious limitation on the possibilities of studying pH influences on the protein binding to IDA–M(II). Moreover, in both IMAC and IMAP, an increase in pH increased the binding strength between IDA–M(II) and the protein. However, in IMA-Elec with the present set of data, only pH 7.2 seems to be optimum, as considerable decreases in affinities were observed both at pH 8.2 and at pH 5.5 (Table I).

However, IMA-Elec run under the optimum conditions can be a very useful technique for determining the relative binding strength of unknown proteins based on their surface accessible histidine residues. Moreover, as shown with RNase, A and B, the subtle differences in the microenvironment of the histidine residues induced by the glycosylation of the proteins can be probed by this technique. Further, as demonstrated by others [18], using IMAP one can follow the protein folding and unfolding with the help of IMA-Elec at microgram–nanogram protein concentrations.

As the basic mechanism of protein recognition by IDA–M(II) is conserved, IMA-Elec is a very useful analytical tool for protein surface histidine residues and for the conception of preparative IMAC or IMAP purification methods for proteins.

It has been shown that eukaryotic cells can be recognized by polymer-supported IDA–M(II), based on the differences in their surface protein structures in both IMAC and IMAP [19,20]. On the other hand, affinophoresis of red blood cells using soluble polyionic polymers coupled with affinity ligands was reported by Shimura *et al.* [21]. Hence, soluble polymer-supported IDA–M(II) [*e.g.*, PEG–IDA–M(II)] can be used as an affinophore for whole cell studies.

Another logical evolution of this IMA-Elec method would be its use in capillary electrophoresis systems either with polymer-coated capillary tubes constituting the affinity system or with the soluble polymer-supported IDA–M(II) used in the free zone.

This study was limited to IDA as the chelating ligand and Cu(II) and Zn(II) as the metals. A vast variety of metals and chelators specific to other systems such as phosphorylation [22] and chiral recognition [23] can be used in IMA-Elec as in IMAC or in IMAP.

IMA-Elec is one among the many branches evolved from the original concept of IDA–M(II) protein recognition proposed by Porath *et al.* [2]. This constitutes a very valuable analytical tool both for protein studies and for designing preparative protein and whole cell purification methods.

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