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Recognition and separation of isoenzymes by metal chelates

Immobilized metal ion affinity partitioning of lactate dehydrogenase isoenzymes

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ABSTRACT

Poly(ethylene glycol) (PEG)-bound chelated metal ions partition preferentially into the top, PEG-rich, phase of a PEG–salt or PEG–dextran aqueous two-phase system. Extraction by this soluble affinity ligand of proteins is due to a selective interaction of the chelated metal ion with accessible histidine residues on the protein surface. Using Cu–iminodiacetate–PEG (Cu–IDA–PEG) the surface of lactate dehydrogenase (LDH) isoenzymes from different species was probed for the presence of metal chelate binding sites. It was demonstrated that the homotetramers (LDH-1)(H_4) from rabbit, bovine and pig displayed weak binding to chelated copper whereas the M_4-type isoenzymes (LDH-5) bound strongly to this ligand. The binding of the different heterotetramers increases as the number of M-type subunits increases. In contrast, the human isoenzymes are bound to chelated copper in a reversed sequence. The comparison of the affinity partitioning effect of Cu–IDA–PEG in PEG–salt and PEG–dextran systems revealed that the discriminatory effect of copper is promoted by high salt concentrations. Resolution of isoenzymes by multiple extraction using counter-current distribution provides valuable data on the partitioning of enzymes relative to that of the bulk proteins. The efficacy of metal chelate affinity partitioning for the purification of LDH from tissue samples by batchwise extraction was also demonstrated.

INTRODUCTION

Partitioning in aqueous two-phase systems is a well established method for the separation and fractionation of proteins, cells and cell particles [1,2]. The range of applicability of this method has been extended by utilizing the interaction of proteins with affinity ligands which were covalently attached to one of the phase-forming polymer, *e.g.*, poly(ethylene glycol) (PEG) [3–5].

Recently, chelated transition metal ions covalently linked to soluble or insoluble polymers have been used to increase the selectivity of separation in chromatography and phase partitioning [6–10]. This interaction is due to the coordination of immobilized metal ions with electron-rich ligands on the protein surface. It is now well established that accessible histidine residues localized in a favourable orientation on the protein surface serve as predominant metal binding sites [7,11,12]. Experiments with metal chelate-derivatized PEG in PEG–dextran and PEG–salt two-phase systems indicate that extraction of proteins by these ligands is histidine-mediated [8].

In this work, we used metal–IDA–PEG as

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probes for the recognition of surface differences in LDH isoenzymes from different sources and species. Substantial differences in the binding of isoenzymes to the chelated metal were found and can be exploited for isoenzyme separation by immobilized metal ion affinity partitioning. The difference in binding of isoenzymes to the affinity ligand is discussed on the basis of their primary structure with respect to the content and distribution of histidine residues.

EXPERIMENTAL

Materials

PEG 6000 and 1550 were obtained from Serva (Heidelberg, Germany), monomethoxy-PEG 5000 from Sigma (St. Louis, MO, USA) and Dextran T 70 from Pharmacia (Uppsala, Sweden). Lactate dehydrogenase (EC 1.1.1.27) isoenzymes from rabbit heart (LDH-1) and the human isoenzymes LDH-1, LDH-2, LDH-3 and LDH-5 were purchased from Sigma, LDH-3 from rabbit from Arzneittelwerk Dresden (Dresden, Germany) and LDH from pig muscle (LDH-5), pig heart (LDH-1), bovine heart (LDH-1) and rabbit muscle (LDH-5) from Boehringer (Mannheim, Germany).

Preparation of metal chelate–PEG

Iminodiacetate–PEG (IDA–PEG) was synthesized by the reaction of bromoacetic acid with aminomonomethoxy–PEG as described previously [10]. Charging of IDA–PEG with metal ions [*e.g.*, Cu(II), Zn(II) and Ni(II)] was performed in 50 m*M* sodium acetate buffer (pH 4.0). The product was extracted repeatedly with chloroform. The contents of Cu(II), Zn(II), and Ni(II) per mole of IDA–PEG were 0.83, 0.8 and 0.3 mol, respectively.

Partition experiments

Aqueous two-phase systems of PEG–dextran were prepared from stock solutions of dextran T 70 (20%, w/w), PEG 6000 (40%, w/w) and 0.1 *M* sodium-phosphate–1.5 *M* Na_2SO_4 (pH 7.0). Stock solutions used for PEG–salt systems were PEG 1550 (40%, w/w), PEG 6000 (40%, w/w), 20% (w/w) Na_2SO_4 and 0.1 *M* sodium phosphate buffer (pH 7.0). The solutions were weighed out in appropriate amounts to yield the required mass of the desired phase systems. PEG–dextran systems (total mass 2 g) used for partitioning had the following composition: 5% PEG 6000 (including metal–IDA–PEG), 7.5% dextran T 70, 0.15 *M* Na_2SO_4, 0.01 *M* sodium phosphate buffer (pH 7.0). PEG–salt systems of 2 g were composed of 10% PEG 1550, 2% PEG 6000 (including metal–IDA–PEG), 10% Na_2SO_4 and 0.01 *M* sodium phosphate buffer (pH 7.0). The phase systems were equilibrated at 22°C before adding the desired amounts of the enzymes. The systems were mixed by 20 inversions and phase separation was achieved by brief centrifugation (2 min at 2000 *g*). Aliquots were immediately withdrawn from the top and bottom phase and analysed for enzyme activity. The partitioning of the enzyme between the two phases is defined as K, expressing the ratio of the concentrations (activities) of the enzyme in the top and bottom phases. The change in the partition coefficient of the enzyme which is due to binding to metal chelate–PEG is expressed as $\Delta \log K$, given as $\Delta \log K = \log K_{aff} - \log K_0$, where K_{aff} and K_0 are the partition coefficients of the protein in the presence and the absence of the affinity ligand in the phase system, respectively, other conditions being identical.

Preparative extraction of LDH

Heart and skeletal muscle extracts from rabbit were prepared by homogenizing minced muscle in 50 m*M* sodium phosphate buffer (pH 7.0) using a Potter homogenizer. After centrifugation (30 min at 5000 *g*), the supernatant was used for partitioning experiments. The specific activity of LDH in heart and skeletal muscle extract was 1.9 and 16.3 U/mg protein, respectively.

The preparative two-step extraction was performed as follows. An 8-g phase system was prepared containing 10% PEG 1550, 2% Cu–IDA–PEG, 10% Na_2SO_4 and 2 ml of rabbit heart extract (27 U) or rabbit muscle extract (312 U). The phases having a volume ratio of 0.58 (top/bottom phase) were mixed at 22°C and separated by centrifugation (10 min at 4000 *g*). The enzymatic activity and the protein content were determined in the top phase (T1) and in the bottom phase (B1). For extraction of heart

LDH, the bottom phase (B1) was then mixed again with fresh top phase of a pre-equilibrated two-phase system of the same composition but without protein. The phases T2 and B2 thus obtained after centrifugation were subjected to analysis. With rabbit skeletal muscle LDH, T1 was re-extracted with fresh bottom phase to remove material with low partition coefficients. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to Laemmli [13]. The type of isoenzymes was determined by the ability of the enzyme to reduce 2-oxobutyrate [14].

Counter-current distribution

Repeated extraction of LDH was performed by counter-current distribution (CCD) using a standard apparatus with 60 cavities as described by Albertsson [1]. The chambers were loaded with 0.78 ml of the lower phase and 0.78 ml of the upper phase of a pre-equilibrated two-phase system composed of 10% PEG 1550, 10% Na_2SO_4, 2% PEG 6000 (or 2% Cu–IDA–PEG) and 0.01 *M* sodium phosphate buffer (pH 7.0). Sample systems (1.56 g) were prepared by including 200 μl of rabbit heart or muscle extract into the phase systems which were loaded into the chamber 0. Twenty-seven transfers were carried out at 22°C. The shaking time was 60 s and the settling time 6 min per transfer cycle. After the run 300 μl of water were added to each chamber, yielding a homogeneous phase. The fractions were analysed for enzyme activity and for protein using the method of Bradford [15].

Enzyme assay

The activity of the LDH isoenzymes was assayed at 25°C as described by Bergmeyer [16] using 0.6 m*M* pyruvate and 0.18 m*M* NADH as substrates in 50 m*M* sodium phosphate buffer (pH 7.5). One unit of the activity is defined as the amount of the enzyme catalysing the conversion of 1 μmol of substrate per minute at 25°C.

RESULTS

Fig. 1 illustrates the effect of immobilized copper (Cu–IDA–PEG) on the partitioning of LDH isoenzymes from rabbit in PEG–salt systems. In the absence of the metal ion the partition coefficient, *K*, does not vary greatly among the isoenzymes and was measured to be between 0.010 and 0.013. This was to be expected because in the presence of high salt concentrations the influence of charges on the partitioning of proteins is suppressed. Addition of Cu–IDA–PEG increases the partitioning of the isoenzymes into the top, PEG-rich, phase and dramatically changes the partitioning of LDH-5 (M_4-type). Owing to the binding to the affinity ligand, the *K* value of LDH-5 is changed 50 000-fold, from 0.01 to about 500, yielding $\Delta \log K = 4.6$ at the maximum ligand concentration. The isoenzyme LDH-3 (H_2M_2-type) displayed much weaker binding to the polymer-bound metal than the M_4-isoenzyme. The lowest binding was found for the homotetramer H_4. Despite the lack of LDH-4 and LDH-2 in our study, it could be observed that the binding of LDH isoenzymes to chelated copper increases with increasing number of the M-type subunit in the oligomer.

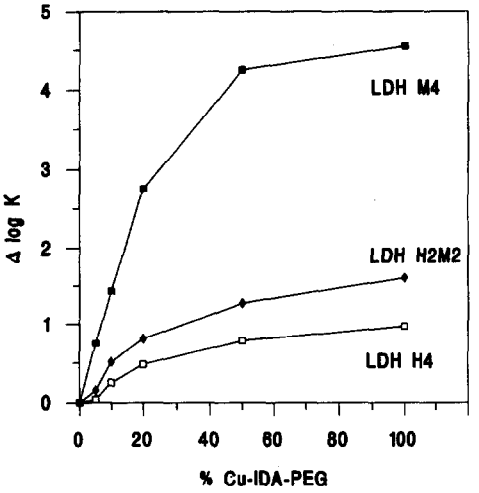

Fig. 1. Metal chelate affinity partitioning of LDH isoenzymes from rabbit. 5–10 units of each purified isoenzyme were partitioned in two-phase systems (2 g) composed of 10% PEG 1550, 10% Na_2SO_4, 2% PEG 6000 (or increasing substituting concentrations of Cu–IDA–PEG) and 0.01 *M* sodium phosphate buffer (pH 7.0). The concentration of Cu–IDA–PEG is expressed as a percentage of the total PEG 6000 that is replaced by Cu–IDA–PEG. □ = LDH-1 (H_4-type); ◆ = LDH-3 (H_2M_2-type); ■ = LDH-5 (M_4-type). Partitioning was performed at 22°C.

The partitioning of human LDH isoenzymes in

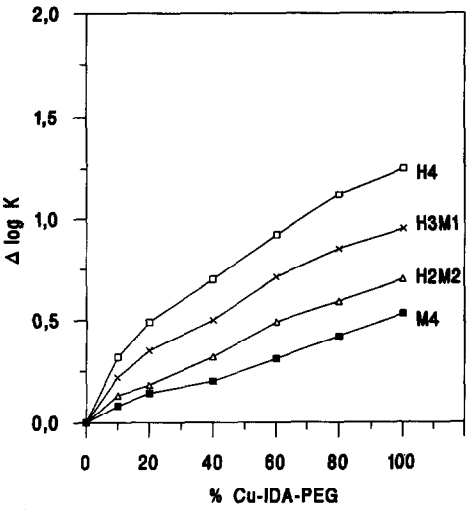

Fig. 2. Metal chelate affinity partitioning of human LDH isoenzymes. 2–6 units of each purified isoenzyme were partitioned in two-phase systems (2 g) composed of 10% PEG 1550, 10% Na_2SO_4, 2% PEG 6000 (or increasing substituting concentrations of Cu–IDA–PEG) and 0.01 *M* sodium phosphate buffer (pH 7.0). The concentration of Cu–IDA–PEG is expressed as a percentage of the total PEG 6000 that is replaced by Cu–IDA–PEG. □ = LDH-1 (H_4-type); × = LDH-2 (H_3M_1-type); △ = LDH-3 (H_2M_2-type); ■ = LDH-5 (M_4-type). Partitioning was performed at 22°C.

PEG–salt systems and the effect of Cu–IDA–PEG are demonstrated in Fig. 2. In the absence of the affinity ligand all isoenzymes partition preferentially into the bottom, salt-rich, phase with *K* values near 0.01. As can be seen, on average, the interaction of the human isoenzymes to immobilized copper is significantly lower compared with the rabbit species. Further, the sequence of extraction of the different isoenzymes is reversed, meaning that the homotetramer H_4 displayed stronger binding to copper than the homotetramer M_4. The heterotetrameric forms of LDH are extracted in the sequence of their increased number of H-type subunits in the molecule.

The species-dependent affinity partitioning of LDH isoenzymes is summarized in Table I. In addition to the effect of copper, the influence of zinc and nickel on isoenzyme partitioning has also been included. Typically, all isoenzymes bound most strongly to chelated copper. Note again the low affinity of bovine LDH-1 and pig LDH-1 and the high affinity of pig LDH-5 to this metal. In contrast, zinc had only marginal effects on the enzyme partitioning and could not dis-

TABLE I

EFFECT OF CHELATED METAL IONS ON PARTITIONING OF ISOENZYMES FROM DIFFERENT SPECIES

Between 2 and 10 units of LDH isoenzymes were partitioned in PEG–salt systems (2 g) composed of 10% PEG 1550, 2% PEG 6000 (or 2% metal–IDA–PEG), 10% Na_2SO_4 and 0.01 *M* sodium phosphate buffer (pH 7.0). Temperature, 22°C.

Species	Isoenzyme	Δ log *K*		
		Cu–IDA–PEG	Zn–IDA–PEG	Ni–IDA–PEG
Rabbit	H_4	0.9	0.2	0.5
	H_2M_2	1.6	0.2	0.8
	M_4	4.5	0.2	1.2
Bovine	H_4	0.5	0.3	0.3
Pig	H_4	0.4	0	0.2
	M_4	3.8	0.2	0.8
Human	H_4	1.3	0.3	0.6
	H_3M_1	1.1	0.4	0.6
	H_2M_2	0.9	0.2	0.8
	M_4	0.5	0.2	0.6

criminate between different isoenzymes. Interestingly, binding to Ni–IDA–PEG is less compared with the effect of copper, but some similarities in the sequence of extraction could be observed.

A comparison of affinity partitioning of isoenzymes in PEG–salt and PEG–dextran systems is shown in Fig. 3. As can be seen, the inverse sequence of extraction of rabbit and human isoenzymes in PEG–salt systems is apparent again. The very similar Δ log K values found for LDH-1 (rabbit) and LDH-1 (human) may argue for a similar number of metal chelate binding sites on the surface of these proteins. Partitioning in PEG–dextran systems at lower salt concentrations revealed that the affinity of the rabbit isoenzymes to copper is considerably reduced. Further, the discriminatory effect of copper for isoenzyme recognition is also decreased. For example, the differences in the Δ log K values of rabbit LDH-1 and LDH-5 amount to 1.85 in the PEG–dextran system, which is much less than the value of 3.6 obtained in the PEG–salt system. Cu–IDA–PEG was not capable of recognizing obvious differences in the protein surface of human isoenzymes in PEG–dextran systems. This means that high concentrations of sulphate promote the protein–metal chelate interaction and increase the discriminatory ability of copper in isoenzyme recognition.

Counter-current distribution of LDH isoenzymes

To increase the separatory effect of two-phase systems, multiple-step extractions were performed by CCD. The resolution of LDH from rabbit muscle and heart in the presence and absence of Cu–IDA–PEG is shown in Fig. 4. In the absence of the metal chelate the LDH activity was found at the far left site of the train with peak position in fractions 1 and 2. The bulk proteins partitioned also in favour of the salt-rich phase (Fig. 4A).

Addition of Cu–IDA–PEG causes extraction of muscle LDH into the top phase occupying the far right site of the train (Fig. 4B). The peak of activity preferentially contained LDH-5 and LDH-4, which is due to their high affinity to the ligand. Even the bulk proteins displayed binding to the ligand and were co-extracted with the enzyme. CCD of the heart extract revealed that Cu–IDA–PEG pulls out the bulk proteins into the top phase whilst having little effect on partitioning of the enzyme (Fig. 4C and D). The splitting of the activity peak is attributed to the partial separation of LDH-1 and LDH-2. The results demonstrate that separation of isoenzymes by CCD is possible.

Extractive purification of LDH

The results of the CCD experiments gave valuable information for carrying out batchwise

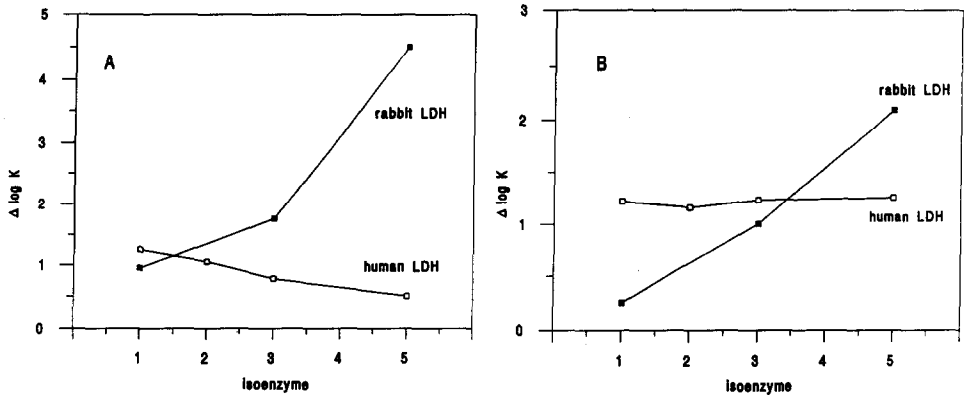

Fig. 3. Effect of Cu–IDA–PEG on partitioning of LDH isoenzymes in PEG–salt and PEG–dextran systems. Between 2 and 10 units of each isoenzyme were partitioned in two-phase systems (2 g) composed of (A) 10% PEG 1550, 10% Na_2SO_4, 2% PEG 6000 (or 2% Cu–IDA–PEG) and 0.01 M sodium phosphate buffer (pH 7.0) and (B) 5% PEG 6000 (or substituted totally by Cu–IDA–PEG for human LDH, or substituted by 10% Cu–IDA–PEG for rabbit LDH), 7.5% dextran T 70, 0.15 M Na_2SO_4 and 0.01 M sodium phosphate buffer (pH 7.0).

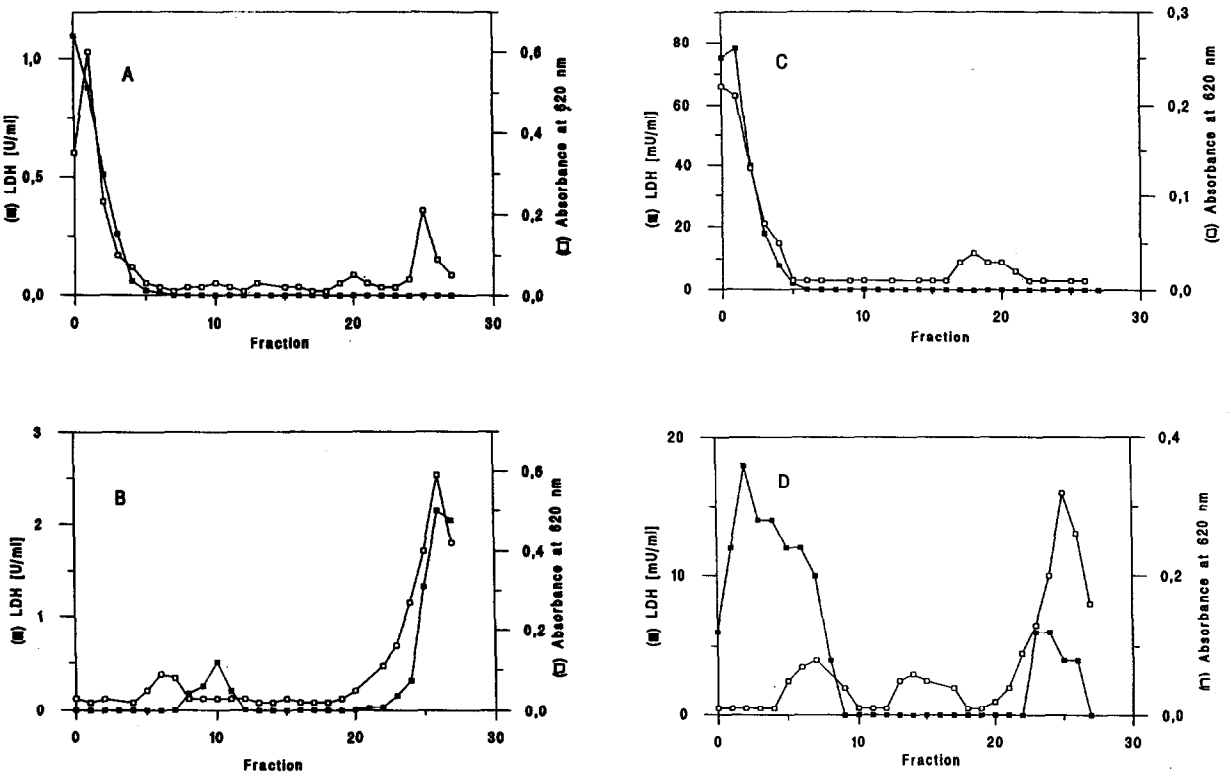

Fig. 4. Counter-current distribution of rabbit LDH from heart and skeletal muscle extract. Distribution pattern of (A and B) muscle and (C and D) heart extract, (A and C) in the absence and (B and D) in the presence of Cu–IDA–PEG. The phase systems were composed of 10% PEG 1550, 10% Na_2SO_4, 2% PEG 6000 (or 2% Cu–IDA–PEG) and 0.01 *M* sodium phosphate buffer (pH 7.0). After 27 transfers at 22°C the fractions were analysed for (■) enzyme activity and (□) protein content.

extraction by showing how bulk proteins partition relative to the target enzyme.

Table II illustrates the efficacy of purification of LDH from heart extract by batchwise two-step affinity extraction in PEG–salt systems. As can be seen, Cu–IDA–PEG, in the first extrac-

TABLE II

EXTRACTIVE PURIFICATION OF RABBIT LDH BY TWO-STEP AFFINITY PARTITIONING

Heart extract was partitioned at 22°C in a PEG–salt system composed of 10% PEG 1550, 2% Cu–IDA–PEG, 10% Na_2SO_4 and 0.01 *M* sodium phosphate buffer (pH 7.0). The bottom phase obtained after the first partitioning step was re-extracted with fresh ligand-containing top phase in the second partitioning step as described under Experimental. The specific activity of LDH in the starting sample was 1.9 U/mg.

Extraction step	Phase volume (ml)	Activity (U/ml)	Total activity (U)	Protein (mg/ml)	Specific activity (U/mg)
T1	2.8	0.8	2.2	3.6	0.2
B1	4.8	3.8	18.3	0.4	9.5
T2	2.8	0.88	2.4	0.1	8.8
B2	4.8	3.0	14.4	0.12	25.0

tion step, pulls out the bulk proteins into the top phase. This results in a fivefold increase in the specific activity of LDH in the bottom phase. Re-extraction of material from the bottom phase by mixing with fresh ligand-containing top phase increases the purity of LDH further to about thirteenfold. The recovery of enzyme activity after the second extraction step was about 62%. The major loss of activity was found in the first extraction due to partial coprecipitation of LDH with bulk proteins at the interphase.

The performance of extraction is shown by SDS-PAGE (Fig. 5). Most of the bulk proteins of the crude material (lane 2) were extracted into the Cu–IDA–PEG phase in the first extraction step, while the enzyme activity remained substantially enriched in the salt-rich bottom phase (lane 4). Isoenzyme determination in the bottom phase was performed by measuring the ability to reduce 2-oxobutyrate. The rate of conversion of 2-oxobutyrate was 92% compared with pyruvate. This indicates the predominance of LDH-1 in the salt-rich bottom phase.

On the other hand, attempts to purify LDH from muscle by two-phase partitioning failed because of the similar binding properties of this enzyme and the bulk proteins to chelated copper, as already demonstrated by CCD.

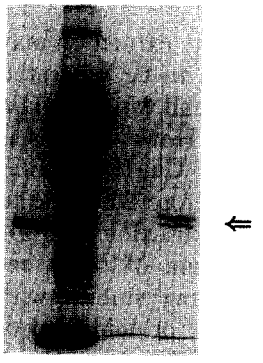

Fig. 5. SDS-PAGE of extracted LDH. LDH from heart extract was purified by two-step partitioning. SDS-PAGE was carried out in 8% polyacrylamide gels at 40 mA and 220 V for 2.5 h at room temperature. Lanes: 1 = 10 μg of commercial LDH-1; 2 = 300 μg of protein of heart extract; 3 = 28 μg of protein of the top phase (T2); 4 = 25 μg of protein of the bottom phase (B2). The arrow indicates the position of LDH.

DISCUSSION

Affinity partitioning in aqueous two-phase systems has been widely used for the study and separation of proteins [2,4,5] and cells [2,17]. Different polymer-bound ligands, *e.g.*, dyes [4,5], palmitate [3] and others [2], were applied to recognize binding sites on the protein surface. Recently, immobilized metal ions have been introduced as affinity ligands in the two-phase separation technique for the extraction of proteins [8–10] and cells [18,19]. As corroborated by a number of experimental data, the binding of chelated metal ions to proteins occurs via electron-donating side-chains of amino acids. Accessible histidine residues on the protein surface have been reported to serve as predominant metal-binding sites.

As shown in this study, PEG-bound metal ions were capable of recognizing and differentiating isoenzymes of LDH. The different partitioning behaviour of the isoenzymes studied can be explained by assuming that the binding ($\Delta \log K$) is proportionally related to the number of histidine residues. At present, owing to the lack of a crystal structure analysis of the isoenzymes used, we are unable to define the histidine residues that are exposed to the solvent and thereby accessible for metal–IDA–PEG interaction. Nevertheless, a correlation between the $\Delta \log K$ of various proteins and their total number of histidines has been demonstrated [20]. This assumption can be approximated because histidines have been shown to be distributed more or less evenly between the interior and the exterior of proteins [21].

The H-type subunit of rabbit LDH contains six and the M-type subunit contains eleven histidines [22]. The latter apparently binds much more strongly to chelated copper than the H-type subunit. Although demonstrated for only three isoenzymes, it can be deduced that the increase in the partitioning ($\Delta \log K$) is proportional to the number of M-type subunits in the different isoenzymes (Fig. 3). A similar tendency became apparent for LDH from pig.

In contrast, for human LDH a reversed binding of the H- and M-types to immobilized copper was found. The increments in affinity of these

isoenzymes to copper were found to be proportional to the number of H-subunits in the tetrameric molecule. This indicates that the relative contribution of each H-subunit for the binding is equal. The overall lower binding of human LDH to copper compared with the rabbit enzymes implies a reduced number of metal chelate binding sites. The human H- and M-types possess only seven histidines [23,24]. Hence a Δ log K that is near to the value for rabbit LDH-1 was to be expected. However, despite having a similar histidine content, fine differences in the metal chelate binding properties of the human isoenzymes were confirmed. The amino acid sequences of the H- and M-types show 74.3% identities. Out of seven histidines, six His residues (His_{67}, His_{181}, His_{186}, His_{193}, His_{231} and His_{271}) are in identical positions. The position of one histidine is different: the H-type contains His_{156} and the M-type His_{215}. One is tempted to assume that the different affinity of the human isoenzymes could arise from the different availabilities of these His residues for Cu–IDA–PEG. Nevertheless, these results show that Cu–IDA–PEG is a very sensitive probe to detect fine differences in surface properties as shown in the case of closely related isoenzymes.

Suh *et al.* [9] have recently demonstrated that high stability constants for Cu–IDA–PEG–protein complexes are found when specific His–X_3–His configurations are available in α-helical regions of the proteins. There are no indications of such specific sites in the LDH isoenzymes studied. Thus, the strong binding of, *e.g.*, rabbit LDH-5 to Cu–IDA–PEG may be the result of metal binding to obviously a number of but single histidine side-chain residues. Special attention was paid to the difference in the partitioning behaviour of LDH isoenzymes in PEG–salt and PEG–dextran systems. The experiments clearly indicate that the partitioning in PEG–salt increases the discriminatory capability of chelated copper for isoenzyme recognition. Thus, sulphate ions seem to promote complex stability. Interestingly, phosphate ions when used to prepare PEG–salt systems were found to lack this property (unpublished results). Generally, water-stabilizing ions seem to increase the interaction of proteins and Cu–IDA–PEG [25]. Owing to its antichaotropic and water-stabilizing character, sulphate ions reduce the ability of water to coordinate to the metal and to compete with the histidines for binding to the metal ion. Therefore, sulphate ions may increase the complex stability by changing the structure of the solvent and by reducing its ability to coordinate itself. Similar effects to those found for sulphate have been reported for NaCl when used in high concentrations in metal chelate affinity chromatography [25].

The fact that high salt concentrations confer high stability of coordination to metals offers an excellent possibility to carry out preparative affinity extraction in inexpensive PEG–salt systems. We have tested the ability to separate isoenzymes that differ in their binding to copper by multi-stage and two-stage extraction. Counter-current distribution was demonstrated to be a very efficient method for the affinity extraction of isoenzymes. Valuable data on the partition coefficients of the target enzyme relative to the bulk proteins can be obtained. Hence it could be demonstrated that extractive purification of LDH-5 failed despite its strong binding to Cu–IDA–PEG because of the co-extraction of most of the bulk proteins. On the other hand, the thirteenfold enrichment of LDH-1 by two-stage affinity extraction provides an example of the efficacy of this step.

Purification by negative affinity extraction may be useful if bulk proteins have to be separated from proteins with low affinity and which remain in the salt-rich bottom phase. However, this is limited by the capacity of the Cu–IDA–PEG-containing top phase. It is therefore advisable to use such a fast and efficient extraction step for refined purification or separation of these enzymes. Nevertheless, partitioning studies with other proteins revealed that the protein load of the top phase containing the chelated metal ion is high, ranging between 10 and 20 mg/ml [20]. Increasing the top phase volume and/or increasing the Cu–IDA–PEG content would lead to a further increase in the extraction capacity.

For the separation of isoenzymes of LDH, electrophoretic methods, ion-exchange chromatography and affinity chromatography have been used, expoiting differences in charges or affinity

to certain immobilized ligands [26,27]. Recently, Kirchberger *et al.* [28] were able to separate LDH-1 and LDH-5 from rabbit by partitioning owing to their different affinities to PEG-bound dyes. Disadvantageously, this method cannot be performed in PEG–salt systems because of the breakdown of dye–protein complexes at high salt concentrations. Further, Cu–IDA–PEG was found to possess a much higher discriminating ability among the isoenzymes than the dyes.

CONCLUSIONS

Metal chelate affinity partitioning in aqueous two-phase systems was found to be a very attractive method for the separation of isoenzymes of LDH. Cu–IDA–PEG has been demonstrated to be a sensitive probe for the recognition of distinct surface differences of closely related proteins. From analytical studies suitably done by CCD, data can be obtained to create affinity phase systems designed for the preparative extraction of proteins on the basis of their different contents and distributions of histidine residues. It can be expected that Cu–IDA–PEG may also serve as an affinity ligand for the recognition of multiple forms of other enzymes and proteins.

ACKNOWLEDGEMENT

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