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CELL SURFACE INTERACTIONS WITH METAL CHELATES

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SUMMARY

We have explored immobilized metal ion affinity adsorption as a means of discrimination between cells and to assess partially the types of interaction that might contribute to the adsorption of cells on the such adsorbents Erythrocytes from different sources were adsorbed on immobilized iminodiacetic acid charged with Cu^{2+}, Ni^{2+} or Zn^{2+} The affinity of the human erythrocytes for the immobilized metal ions follows the order $Cu^{2+} > Ni^{2+} > Zn^{2+}$ The adsorption capacity of the rat erythrocytes decreased in the following order $Zn^{2+} > Ni^{2+} > Cu^{2+}$ Pre-saturation of the columns with imidazole lead to the recovery of over 90% of the cells applied on the columns Enzymic removal of sialic acid residues from the surface of erythrocytes has no effect on the adsorption-elution profiles of these cells on affinity adsorbents These findings suggest that histidine residues localized on the cell surface are involved in the cell binding to the adsorbent This new separation principle could be expanded to other types of cell It could be used as a diagnostic tool and for separation, as well as for probing cell surfaces

INTRODUCTION

Different adsorption techniques, both specific and non-specific, have been developed for eucaryotic cell separation Immunomagnetic cell separation was developed [1] by coupling of monoclonal antibodies to magnetic beads to achieve specific binding of the magnetic particles to the target cells This type of adsorbent is especially useful for depleting a cell population of a specific cell type Lectins immobilized on a solid support [2] are employed to separate different types of cell Hydrophobic chromatography on alkyl agaroses [3] is used for the separation of cells from different species. These techniques are

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used for separating functionally distinct cell subpopulations The adsorbent could interact exclusively or preferentially with a cell subpopulation of interest

Conversely, adsorption of eucaryotic whole cells on a metal chelate is a logical extension of the earlier work when immobilized metal ion affinity (IMA) chromatography was introduced [4] for the fractionation of proteins IMA adsorption is a collective term that was proposed to include all kinds of adsorption by which metal atoms or ions immobilized on a polymer cause or dominate the interaction at the sorption sites [5] In contrast to biological ligands, the number and type of experimental variables (e g., ionic strength, pH, salt type) can substantially affect the ligand–ligate affinity interaction The separation process depends on differential adsorption

IMA adsorption is based on interfacial interactions between biomolecules in solution and metal ions fixed to a solid support, which is usually a hydrophilic cross-linked polymer [6] The IMA event can be understood in terms of coordination of a specific amino acid side-chains (e.g. His, Cys, Trp) which can serve as electron donors [7]

The erythrocyte surface is densely coated with carbohydrates, either as glycolipid or as glycoproteins [8] Many ligands (metal-combining sites) situated on the cell surface, which are located externally, are therefore accessible for complex formation with the metal ions fixed to the gel

The importance of histidine residues on a protein surface for recognition and binding to an immobilized metal ion has been demonstrated [4,9] In this preliminary study we propose a new development of the affinity adsorbent by exploiting the affinity that may exist between eucaryotic cells and heavy metals We used the red blood cell as a 'model' to look into the mechanism involved in cell binding to the IMA adsorbents and to explore the implication of histidine residues by pre-saturating the columns with imidazole Erythrocytes from various sources were tested under physiological conditions and without significant damage to the cells

EXPERIMENTAL

All chemicals used were commercially available and of pro analysi grade Sepharose 6B was obtained from Pharmacia (Uppsala, Sweden)

Preparation of M^{2+}-IDA-Sepharose 6B

Chelating Sepharose 6B was a gift from the Separation Research Center of the Biomedical Center of Uppsala University, Uppsala, Sweden (Lot No 317, IDA) The gels were synthesized according to published procedures [4,5]

Buffers

Phosphate-buffered saline (PBS, pH 7 4) had a composition of 137 m*M* NaCl, 2 7 m*M* KCl, 6 5 m*M* Na_2HPO_4 and 1 46 m*M* KH_2PO_4 Imidazole (pH

7 4) buffer was composed of 0 68 g of imidazole and 1 17 g of NaCl in 100 ml of water with 37 2 ml of 0 1 *M* HCl added to a final volume of 200 ml

Erythrocytes

Freshly heparinized human or rat blood was centrifuged (530 *g*, 5 min) The red cell pellet was washed three times with PBS and finally suspended in this buffer Cells were counted in triplicate with a haemocytometer The viability of the cells was measured by Trypan blue staining One volume of erythrocyte suspension was mixed with one volume of Trypan blue solution, 0 2% in saline

Adsorption–elution profiles of erythrocytes on IMA adsorbents

Columns (1 cm×1 cm, bed volume, 0.8 ml) were packed with M^{2+}-IDA-Sepharose 6B on a porous polyethylene disc, which allowed free passage of the erythrocytes with no leakage of the Sepharose beads This small height ensures a homogeneous flat surface of the gel Each of the columns was equilibrated (saturated) with 10 ml of PBS containing different concentrations of imidazole Then a sample of 0 15 ml cell suspension, corresponding to $6\ 1 \cdot 10^7$ cells, was gently applied The gel was covered with 0 15 ml of PBS and allowed to incubate for 15 min The the column was gently washed with 10 ml of PBS to exclude unadsorbed cells Desorption of the cells from the column was achieved by adding 1 ml of PBS containing 10% (v/v) of a human serum albumin (HSA) solution, followed by 10 ml of imidazole buffer Underivatized Sepharose 6B was used as a control gel

Enzymic removal of sialic acid residues by neuraminidase

Washed erythrocytes ($2\ 5 \cdot 10^6$) in 50 μl were suspended in 0 1 ml of saline solution (0 9% NaCl) containing 0 1 U of *Clostridium perfringens* neuraminidase (Type V, Sigma, St Louis, MO, U S A) The suspension was incubated for 1 h at 37 °C (with occasional shaking) and the cells were then washed twice with PBS and finally suspended in PBS to a final concentration of $2\ 5 \cdot 10^6$ cells per 50 μl These suspensions were applied to each column and the experiment had proceeded as described earlier A control was performed where the cells were incubated without neuraminidase

RESULTS

The three IMA adsorbents (IDA-Cu^{2+}, -Zn^{2+} and -Ni^{2+}) are capable of adsorbing cells under isotonic conditions and at physiological pH (Fig 1) An extremely strong retention of the cells was observed on all the three types of gel, and to the greatest extent on the IDA-Cu^{2+} gel, which retained more than 80% of the applied cells The cells retained on the three absorbents were not desorbed with the imidazole eluting buffer A series of control experiments established that the interaction of cells with underivatized Sepharose 6B (not

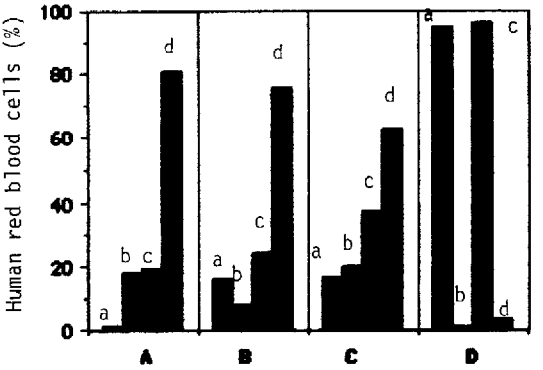

Fig 1 Adsorption-elution profiles of human erythrocytes on IDA-Cu^{2+}, -Ni^{2+} and -Zn^{2+} Samples (0 15 ml) containing 6 1 10^7 human blood red cells, blood type AB^+, were applied to each column (0 8 ml bed volume) Adsorption was allowed to proceed for 15 min at 22 °C, and then the columns were washed with 10 ml of PBS to exclude non-adsorbed cells Desorption of the cells from the column was achieved by adding 1 ml of PBS mixed with 10% (v/v) of a HSA solution, followed by 10 ml of imidazole buffer The number of excluded and eluted cells is expressed as a percentage with respect to the number of cells applied on the column (A) Sepharose-IDA-Cu^{2+}, (B) Sepharose-IDA-Ni^{2+}, (C) Sepharose-IDA-Zn^{2+}, (D) Sepharose (a) Excluded cells, (b) eluted cells, (c) total cells recovered, (d) adsorbed cells

charged with M^{2+}) was minimal for human and rat cells Around 100% of the cells applied to the control gel were excluded with PBS Therefore, the retention of cells on IDA-M^{2+} is the result of their specific interaction with the metal chelate

Effect of the pre-saturation of the columns with imidazole

The columns were first saturated with PBS containing different concentrations of imidazole, and the experiment was continued under the conditions described in Experimental When the columns were pre-saturated with concentrations of imidazole greater than 20 m*M*, almost no human or rat cells were adsorbed onto the columns (Fig 2) The number of adsorbed cells diminished when the columns were pre-saturated with increasing concentrations of imidazole (Fig. 3)

In the case of pre-saturation of the columns with 20 m*M* imidazole, the Cu^{2+}, Ni^{2+} and Zn^{2+} columns excluded 62, 65 and 80%, respectively, of the human blood cells applied to the columns

Rat blood cells displayed a different adsorption profile on IMA adsorbents The Cu^{2+}, Ni^{2+} and Zn^{2+} columns excluded 81, 70 and 60%, respectively, of an identical number of the cells applied to the columns pre-saturated with 20 m*M* imidazole (Fig 4)

The quantitative recovery of the cells (Table I) indicates that during the adsorption–desorption procedure there is very little lysis and there is essentially no entrapment of cells in the columns

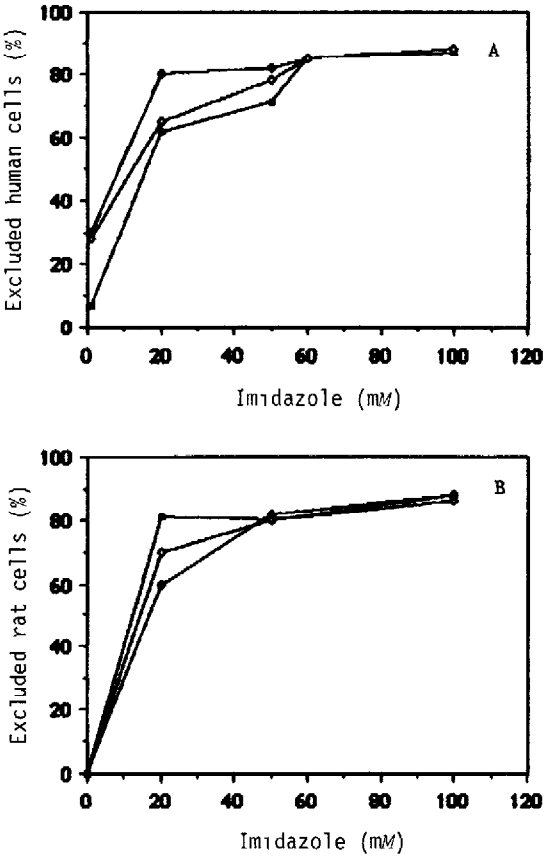

Fig 2 Effect of pre-saturation of the columns with imidazole on excluded cells Columns were first saturated with PBS containing different concentrations of imidazole Samples of cell suspension (0 15 ml) were then applied (numbers of applied cells are shown in Table I) The experiment was continued as described in the legend to Fig 1 (■) IDA-Cu^{2+}, (◇) IDA-Ni^{2+}, (♦) IDA-Zn^{2+}

Comparison of the adsorption–elution profiles of intact and neuraminidase-treated human erythrocytes on IMA adsorbents

The adsorption–elution profiles of cells before and after treatment with neuraminidase were similar (not illustrated).

Delayed desorption

The phenomenon of delayed desorption observed in the case of proteins [5] was also observed with eucaryotic whole cells. The desorption process is time-dependent We noticed that if elution is stopped after no further desorption of cells is observed, and then continued after 15 min, we obtained additional desorbed cells. This 'pulse elution' was repeated at different intervals of time (30 and 60 min) After each time interval, additional desorbed cells were observed

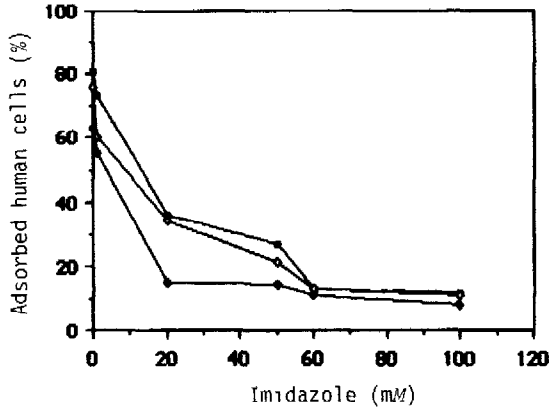

Fig 3 Effect of pre-saturation of the columns on the adsorbed human cells The columns were first saturated with PBS containing different concentrations of imidazole The number of applied cells in 0 15 ml is shown in Table I The experiment was continued as described in the legend to Fig 1 The number of adsorbed cells was evaluated from the number of applied cells minus the total number of cells recovered (expressed as a percentage with respect to the number of applied cells) (■) IDA-Cu^{2+}, (◇) IDA-Ni^{2+}, (♦) IDA-Zn^{2+}

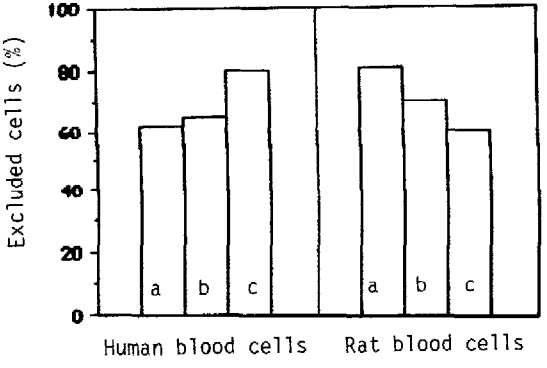

Fig 4 Discrimination between human red cells and rat red cells by IMA adsorbents Columns were first saturated with PBS containing 20 m*M* imidazole Samples (0 15 ml) containing 6 2 10^7 human or rat cells were applied on 0 8 ml of each of the columns The experiment was continued as described in the legend to Fig 1 (a) IDA-Cu^{2+}, (b) IDA-Ni^{2+}, (c) IDA-Zn^{2+}

TABLE I

ADSORPTION–ELUTION PROFILES OF RED BLOOD CELLS ON IDA-Cu^{2+}, -Ni^{2+} AND -Zn^{2+}

Columns were first saturated with PBS containing different concentrations of imidazole, and 0 15 ml of cell suspension was then applied on the columns and the experiment was continued as described in Experimental

Imidazole (m*M*)	Number of applied cells ($\times 10^7$)	Excluded cells (%)			Eluted cells (%)			Total cells recovered (%)			Adsorbed cells (%)		
		Cu	Ni	Zn	Cu	Ni	Zn	Cu	Ni	Zn	Cu	Ni	Zn
Human red blood cells													
0	6 1	1	16	17	18	8	20	19	24	37	81	76	63
1	6 2	7	28	30	20	12	15	27	40	45	73	60	55
20	6 0	62	65	80	2	1	5	64	66	85	36	34	15
50	6 2	71	78	82	2	1	4	73	79	86	27	21	14
60	6 3	85	85	85	2	2	4	87	87	89	13	13	11
100	6 2	87	88	88	1	1	4	88	89	92	12	11	8
Rat red blood cells													
20	6 0	81	70	60	3	1	1	84	71	61	16	29	39
50	6 3	80	80	82	1	0	0	81	80	82	19	20	18
100	6 2	86	88	88	0	1	1	88	89	89	12	11	11

Accordingly we can increase the capacity of cell desorption This phenomenon of delayed desorption could be due to the changing of orientation in space favouring its detachment from the solid matrix

DISCUSSION

There are at least two, usually three, free coordination sites on IDA gel [5] In this case chelate structures involving more than two ligands on the cell surface may be formed Adsorption could be selective according to the spatial distribution of various ligands on the cell surface The cells are strongly retained on all the tested IMA adsorbents, and to a greater extent on IDA-Cu^{2+} in the case of human erythrocytes. The retained cells were not desorbed by the eluting imidazole buffer This phenomenon suggests that, after the cells have been specifically adsorbed on the IDA-M^{2+}, the cell membrane may interact with the surface of the adsorbent. In order to 'tame' the IDA-M^{2+} surface, and to reduce the strength of the cell surface–adsorbent interaction, the adsorbent surface was pre-saturated with a competing ligand (imidazole) The latter is an efficient electron donor capable of forming strong coordination bonds with M^{2+} ions This pre-saturation will avoid strong multi-point attachment of the cell to the adsorbent surface and will give a partial explanation for the mechanism of the cell binding to the IMA adsorbent, i e if the binding involves the histidine residues localized on the cell surface The tested IMA adsorbents are known to interact via histidine residues on proteins surfaces [4,9–11] The high percentage of the excluded cells (more than 80%) when columns are pre-

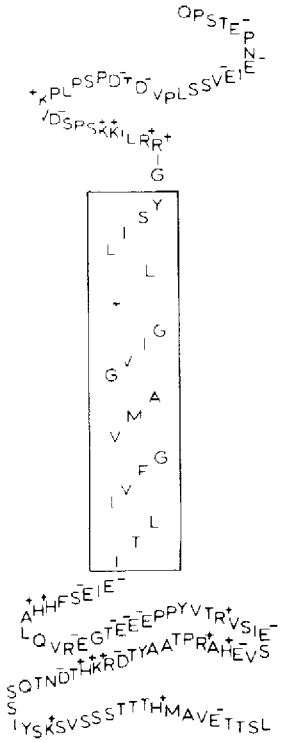

Fig 5 Amino acid sequence of human glycophorin Residues are drawn in the one-letter code as follows A, alanine, C, cysteine, D, aspartic acid, E, glutamic acid, F, phenylalanine, G, glycine, H, histidine, I, isoleucine, K, lysine, L, leusine, M, methionine, N, asparagine, P, proline, Q, glutamine, R, arginine, S, serine, T, threonine, V, valine, W, tryptophan, Y, tyrosine Written in the box is the sequence of residues within the apolar interior of the membrane (Reproduced from ref 12 with permission)

saturated with imidazole (Fig. 2) gave an indication of the specificity in the interaction between the immobilized M^{2+} ions and exposed histidine residues on the cell surface, and in particular of the high affinity of histidine towards Cu^{2+} ions It was not possible to test the effect of histidine on desorption owing to the instability of the copper ions toward exposure to the eluting buffer containing histidine

In their pioneering work, Porath et al [4] postulated that histidine, cysteine and, to some extent, tryptophan residues of side-chains of proteins are responsible for the adsorption of proteins on immobilized metal ions at neutral pH This original proposal has been extended by Sulkowski [9] to elucidate the importance of the topographical distribution of histidine residues on the surface of proteins with adsorption

The most abundant protein molecules exposed on the surface of the human erythrocyte membrane are band 3 protein, glycophorin A and band 4 5 pro-

teins, of which there are ca 1 2, 1 0 and 0.7 million copies each per cell, respectively [8]. The amino acid sequence of glycophorin includes five His residues located in the hydrophilic extra cellular region (N-terminus) (Fig 5) The amino acid sequence of band 3 protein in not known, band 4 5 proteins are poorly characterized and the arrangement of the peptides is not known [8] The fact that there is no difference in the adsorption pattern between the native cells and the neuraminidase-treated ones excludes the binding mechanism through to the sialic acid residues on the cell surface The topographical distribution and the number of His residues on the erythrocyte surface suggest their implication in the strong binding of the cells to the IDA-M^{2+} adsorbents Our results compare well with Sulkowski's conclusions in the case of proteins

The human cells are more strongly retained on IDA-Cu^{2+} than on IDA-Zn^{2+} This difference in selectivity towards His residues exposed on the surface of the human erythrocyte membrane could be explained according to the hypothesis of Sulkowski [9] for proteins, that a single His residue present on the surface of a protein is sufficient for the retention of the latter on IDA-Cu^{2+} at neutral pH. The presence of multiply exposed His residues gives rise to a multi-point attachment to IDA-Cu^{2+}, which results in a stronger retention On the other hand it is the proximity of His residues, and not simply their multiplicity, which constitutes the condition for Zn^{2+} recognition

To our knowledge, there exists no reference that mentions whether glycophorin is present in the rat erythrocyte membrane This information could explain the phenomenon we observed, that rat erythrocytes exhibit a different affinity towards the adsorbents

CONCLUSIONS

These preliminary experiments suggest that erythrocytes bind strongly immobilized metal(II) ions mainly via accessible His residues localized on the cell surface The affinity of human erythrocytes to the tested metal(II) ions follows an order different from that of rat erythrocytes

This study is based on the principle of adsorption by pseudo-affinity of biomolecules on immobilized metal ions This new approach to the testing of eucaryotic cells may provide an additional technique for cell separation or for probing their cell surfaces, as well as in applied areas such as clinical diagnosis and treatment

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REFERENCES

1 J Ugelstad, A Berge, T Ellınsen, L Kılaas, O Aune, T N Nılsen, R Schmıd, P Stenstad, S Funderund and K Nustad, Bıol Chem Hoppe-Seyler, 368 (1987) 779
2 G M Edelman, U Rutıshauer and C F Mıllette, Proc Natl Acad Scı U S A , 68 (1971) 2153
3 G Halperın, M Tauber-Fınkelstein and S Shaltıel, J Chromatogr , 317 (1984) 103
4 J Porath, J Carlsson, I Olsson and G Belfrage, Nature (London), 258 (1975) 598
5 J Porath and B Olın, Bıochemıstry, 22 (1983) 1621
6 J Porath, Trends Anal Chem , 7 (1988) 254
7 L Andersson, E Sulkowskı and J Porath, Cancer Res , 47 (1987) 3624
8 J Vıntala and J Jornefelt, Trends Bıochem Scı , 10 (1985) 392
9 E Sulkowskı, Trends Bıotechnol , 3 (1985) 1
10 M Belew, T T Yıp, L Andersson and J Porath, J Chromatogr , 403 (1987) 197
11 E S Hemdan and J Porath, J Chromatogr , 323 (1985) 255
12 R A Capaldı, Trends Bıochem Scı , 7 (1982) 292