Protein Recognition of Immobilized Ligands: Promotion of Selective Adsorption

T. William Hutchens¹ and Jerker O. Porath²

We are using simple immobilized ligands to evaluate the biochemistry and mechanisms of selective, high-affinity, protein adsorption events. Several specific means have recently been developed to more selectively utilize the favorable entropy changes associated with the displacement of protein-bound water during the formation and stabilization of protein–ligand recognition events. For protein and peptide immobilization these include, besides hydrophobic interaction, for example, metal ion, π-electron-mediated, and thiophilic interactions. This latter type of protein–ligand recognition process represents a previously unrecognized interaction mechanism of considerable selectivity, affinity, and utility. Specific examples of the above-mentioned principles and protein fractionations include (a) thiophilic adsorption of immunoglobulins to achieve immunoglobulin-free serum for in vitro production and purification of monoclonal antibodies and (b) urea-induced binding of estrogen-receptor proteins to immobilized DNA. The interaction mechanisms are discussed in terms of the molecular architecture of protein surfaces. We present possibilities for the further utilization of these immobilized ligands and their associated proteins in the areas of clinical biochemistry and immunology.

Additional Keyphrases: thiophilic adsorption chromatography · monoclonal antibodies · immunoglobulins · estrogen receptors · hydrophobic interactions · ligand:ligate interactions · DNA · urea-promoted interaction · metal-binding proteins · salt-promoted adsorption

Understanding the chemical and physical mechanisms by which proteins recognize specific ligands—e.g., in hormone–receptor interactions, antibody–antigen recognition, and protein–DNA interactions—is one of the fundamental goals of those investigating biological chemistry. Some of these molecular recognition events readily occur in free solution and have been characterized to considerable detail in vitro. However, the essence of cellular and subcellular structure and function involves compartmentalization, with elegant designs for interfacial interactions such as those required for information and energy transfer between various mobile and stationary-phase constituents. Increasing awareness of the need to further understand and exploit the utility of selective high-affinity (as well as low-affinity) solid-phase interactions in medicine and industry has led to comprehensive studies of the individual driving force(s) behind these interactions. A primary driving force is related to the availability of suitable ligands on the stationary phase that have known affinity for select binding sites on the ligate of interest.

Ever since we were first able to chemically derivatize suitable stationary phases for the convenient, covalent coupling of biomolecules (1, 2), considerable effort has been devoted to the design of interacting as well as noninteracting stationary phases for molecular fractionations. The resulting processes referred to now as “affinity” chromatography (3, 4) and size-exclusion (gel-permeation) chromatography (5) represent outcomes near either end of this spectrum. The design of new stationary phases deserves much continued interest, but another considerable driving force influencing selectivity and affinity of ligand:ligate interactions is the nature of the solvent or aqueous environment.

Modifying the structure of water can greatly influence ligand:ligate interactions. Already, about the time size-exclusion chromatography was discovered (5), with cross-linked dextran (Sephadex), it was appreciated that the so-called inertness of these gels was a matter of solvent composition—the salt-promoted adsorption of aromatics having been observed (6, 7). Possibly the first to recognize the potential utility of certain salts for achieving adsorption separations was Tiselius, 40 years ago (8). But only after the first “pure” (electrically neutral) hydrophobic gels were introduced (9) were the salt concentrations in eluent effectively utilized to modify hydrophobic adsorption. Today, hydrophobic interaction chromatography appears to be the most widely utilized form of salt-promoted adsorption chromatography; however, salt-promoted adsorption phenomena may be more common than was earlier believed. For example, one technique with enormous, but as yet under-exploited, potential is immobilized metal ion affinity chromatography (10, 11). The specific affinity of peptides and proteins for soft and intermediate metal ions (e.g., Zn²⁺, Ni²⁺, Cu²⁺) is significantly enhanced by increased concentrations of water-structure-forming (“anti-chaotropic”) salts.

One purpose of this paper is to present the rationale for further investigations into the role of solvent in the promotion of specific ligand:ligate interactions. To best illustrate the significance of solvent-promoted protein recognition of immobilized ligands, we provide two examples, involving immunoglobulins and estrogen-receptor proteins, which are of considerable clinical significance. For the first example we discuss a newly discovered and highly selective type of

¹ Reproductive Research Laboratory, St. Luke’s Episcopal Hospital; and Department of Obstetrics and Gynecology, Baylor College of Medicine, Houston, TX 77030.
² Fogarty Scholar-in-Residence, Fogarty International Center, National Institutes of Health, Bethesda, MD; and Institute of Biochemistry, Biomedical Center, University of Uppsala, Box 576, S-75123 Uppsala, Sweden.

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salt-promoted protein–ligand interaction, tentatively named thiophilic adsorption (12–14). Even though the mechanism of thiophilic adsorption remains uncertain, its utility is revealed by the selective and reversible immobilization of immunoglobulins from serum, ascites fluid, and hybridoma cell-culture media. The second specific example of promoting selective protein adsorption (likely involving perturbed water structure) is illustrated by the urea-induced binding of estrogen-receptor complexes to immobilized DNA (15). The mechanism(s) of urea-promoted receptor–DNA interaction is also unknown but is discussed relative to the electron-donor/acceptor properties of urea and its influence on charge-transfer-type interactions (16–18). The maintenance of high receptor affinity for estradiol under conditions promoting quantitative binding to DNA suggests the possibility of a direct solid-phase assay for estrogen receptor.

Materials and Methods

Materials

Altaex TSK-3000SW (30-nm pore size) and Bio-Sil TSK-400 (40-nm pore size) “high-performance” size-exclusion columns (600 mm × 7.6 mm, i.d.) were purchased from Beckman Instruments, Fullerton, CA, and Bio-Rad Laboratories, Richmond, CA, respectively. Bio-Gel HT hydroxyapatite was from Bio-Rad Laboratories. 4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES buffer)8 was from Research Organics Inc., Cleveland, OH. [3H Estradiol-17β (90–100 KcI/mol) was purchased from New England Nuclear, Boston, MA. Trizma (Tris) base, diethylstilbestrol, diithreitol, double-stranded calf thymus DNA–cellulose (5–6 mg of DNA per gram of DNA–cellulose), ATP–agarose (lot nos. 115F-9635 and 54F-9645; 1.7–2.2 μmol of ATP per milliliter of packed gel), and glycerol were from Sigma Chemical Co., St. Louis, MO. Disodium EDTA, metal chlorides, and metal sulfates were from Fisher Scientific, Pittsburgh, PA. Sephadex G-25 (PD-10) columns (prepacked), Polybuffer Exchanger 94 (PBE 94), Polybuffers 96 and 74, fast-flow cleating Sepharose 4B, and Blue Dextran 2000 were obtained from Pharmacia Fine Chemicals, Uppsala, Sweden. Ultra-pure urea and calf thymus single-stranded DNA–agarose (lot nos. 41101 and 51102; 0.66 to 0.69 mg of DNA per milliliter of gel) were obtained from Bethesda Research Laboratories, Gaithersburg, MD. All buffer solutions were freshly prepared, filtered through HVLP filters (0.2-μm pore size; Millipore Corp., Bedford, MA) and degassed just before use.

Procedures

Thiophilic adsorption chromatography. The gel used for thiophilic adsorption chromatography (T-gel) consisted of agarose (80 g/L) activated with divinylsulfone and coupled with mercaptoethanol (12, 13). The T-gel ligand can be represented as follows:

\[
\text{agarose-O-CH}_2\text{-CH}_2\text{-SO}_2\text{-CH}_2\text{-CH}_2\text{-S-CH}_2\text{-CH}_2\text{-OH}
\]

The final ligand concentration was estimated to be 0.9–1.0 mmol per gram of dry gel, with an adsorption capacity for immunoglobulins of 65–70 μg of protein per gram of suction-dried T-gel (13). Columns of T-gel (1–3 cm, i.d.) were prepared with settled bed volumes of 5–40 mL, depending upon sample type (serum or cell-culture fluid) and volume. The column equilibration buffer consisted of, per liter, 0.5 mol of K₂SO₄, 0.5 mol of NaCl, and 20 mmol of HEPES, pH 7.4–7.6. Samples were prepared for salt-promoted thiophilic adsorption chromatography by the addition of solid K₂SO₄ to give a final concentration of 0.5 mol/L. [Ammonium sulfate, 0.75 mol/L, has also been used (13).] Samples were applied to the T-gel column at a linear flow rate between 10 and 20 cm/h. Procedures for the preparation of immunoglobulin-free serum varied slightly from the procedure used to obtain the purified monoclonal antibodies.

Preparation of immunoglobulin-free serum. Bovine or human serum containing K₂SO₄, 0.5 mol/L, was passed through a T-gel column having a bed volume equal to 0.5–1.0 volume of applied serum. The flow-through fraction was collected and desalted by chromatography on Sephadex G-25 or by dialysis against phosphate-buffered saline (pH 7.4). Sera were evaluated for immunoglobulin titers before and after thiophilic adsorption chromatography by using enzyme-linked immunosorbant assay techniques and used to grow hybridoma cells (Hutchens, Ruan, Andersson, and Porath, ms. in preparation).

Purification of monoclonal antibodies by thiophilic adsorption. Cell-culture media or other fluids containing monoclonal antibodies were collected and solid K₂SO₄ was added to give a final concentration of 0.5 mol/L. The sample (usually 10–25 mL) was pumped onto a 5-mL T-gel column (1.0 cm, i.d.) at 20 cm/h. Unbound protein was washed out with column elution buffer until no absorbance was detected at 280 nm. Monoclonal antibodies were eluted with HEPES buffer (20 mmol/L, pH 7.4) with and without NaCl, 0.5 mol/L, as described previously (13).

Protein analyses of purified immunoglobulin fractions. We monitored the protein elution profile during thiophilic adsorption chromatography by measuring absorbance at 280 nm and determined the elution position of specific immunoglobulins by using enzyme immunoassay techniques. For polyacrylamide gradient gel electrophoresis (Pharmacia PAA 4/30 gels) we followed the conditions specified by the manufacturer and used the standard reference proteins albumin (68 kDa), lactate dehydrogenase (EC 1.1.1.27; 140 kDa), catalase (EC 1.11.1.6; 230 kDa), ferritin (450 kDa), and thyroglobulin (668 kDa). Protein bands were detected by overnight staining with a 1 g/L solution of Coomassie Brilliant Blue R-250 followed by silver staining (19).

For electrophoresis of sodium dodecyl sulfate (SDS)-denatured protein fractions we used 15% SDS–polyacrylamide gels as outlined by Blobel and Dobberstein (20). Protein bands in the SDS–polyacrylamide gels were detected directly by silver staining. Reference proteins used to calibrate these electrophoretic patterns were α-lactalbumin (14.4 kDa), soybean-trypsin inhibitor (21 kDa), carbonic anhydrase (EC 4.2.1.1; 30 kDa), ovalbumin (45 kDa), bovine serum albumin (68 kDa), and phosphorylase b (EC 2.7.1.37; 94 kDa), available in the low-molecular-mass calibration kit from Pharmacia.

Preparation of estrogen receptor. Uteri from small, immature calves, obtained from a local abattoir, were rinsed immediately after removal in ice-cold saline, cut into 1-g pieces, frozen in liquid nitrogen (or on solid CO₂), and stored frozen at −85 °C.

All procedures were performed in a cold room at 0–6 °C. Pieces of frozen uteri were minced, then homogenized in two volumes of Tris HCl buffer (10 mmol/L, pH 7.4 at 0 °C) containing 1.5 mmol of Na₂EDTA, 1 mmol of dithiothreitol,

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and 200 mL of glycerol per liter. We then subjected the homogenate to high-speed centrifugation (>100 000 x g, 60 min) and the cytosol thus obtained (18-22 mg of protein per milliliter) was labeled at 0 °C with [3H]estradiol-17β, 5-10 nmol/L, in the presence (nonspecific binding) and absence (total binding) of a 100-fold molar excess of the radioinert competitor diethylstilbestrol. Radiolabeled steroid–protein complexes were quickly (<5 min) separated from excess free steroid and equilibrated into different buffers by rapid chromatography on small (9-mL) columns of G-25 Sephadex (Pharmacia PD-10 columns). Concentrations of cytosol protein were estimated by the method of Bradford (21).

Buffers for estrogen-receptor analyses. We equilibrated the cytosolic receptor preparations into various buffers for ligand-binding evaluations (22, 23), size-exclusion chromatography, and DNA-affinity chromatography. These buffers are identified as follows: control buffer, 50 nmol of potassium phosphate (pH 7.4 at 0 °C), 1 nmol of dithiothreitol, 1.5 mmol of Na2EDTA, and 100 mL glycerol per liter; KCl buffer, control buffer plus KCl, 0.4 mol/L; urea buffer, control buffer plus urea, 6 mol/L; urea/KCl buffer, control buffer containing 6 mol of urea and 0.4 mol of KCl per liter. The compositions of the polyampholyte and nonpolyampholyte chromatofocusing buffers used for determining surface-charge heterogeneity of the estrogen receptors are outlined separately and discussed in detail elsewhere (24-30).

Physicochemical characterization of estrogen-receptor proteins. We characterized estrogen-receptor proteins by size, surface charge, and affinity for estradiol, exactly as described previously (23, 25, 31).

DNA-binding of estrogen-receptor complexes. All procedures were performed in a cold room at 0-6 °C. Both DNA-cellulose (32) and DNA–agarose were routinely used to monitor the DNA-binding status of transformed (DNA-binding) receptors. We packed 0.5-1.5 cm (i.d.) columns with affinity gel to a bed volume of 2-3 mL and applied 0.5- to 2-mL receptor samples to DNA-affinity columns equilibrated in either control buffer or urea buffer. After washing away the unbound sample with four to six column volumes of control or urea buffer, we eluted the bound receptor forms with urea/KCl buffer.

Results and Discussion
Molecular adsorption onto a fixed surface is the result of ligate (e.g., peptide or protein) interaction with an immobilized ligand. Unlike with free-solution chemistry, a detailed description of the thermodynamics involved requires an understanding that the restricted mobility of ligate (and bound water) at the interacting (available) surface creates a gradient of solute concentration that depends, in part, on the ligate:ligand affinity constant (Kq). This equilibrium constant is related, but not identical, to the effective partition coefficient (K) of the interacting species in the mobile and stationary phases. In chromatography, the partition coefficient is related to the ligate elution volume (Ve) according to the equation

\[ V_e = V_0 + K \cdot V_s \]

where the volume of the stationary phase (V0) is the solute-accessible or effective interacting surface area and V0 is the void (noninteractive) volume. As an approximation, this partition or distribution coefficient can be related to the Gibb's free energy of the interaction by the equation

\[ \Delta G = -RT \ln K \]

where \( \Delta G \) is composed of two familiar terms, namely, entropy (\( \Delta S \)) and enthalpy (\( \Delta H \)):

\[ \Delta G = \Delta H - T\Delta S \]

The displacement of nonpolar-solute-bound water that occurs when two hydrophobic surfaces interact is believed to create an increase in the overall disorder (\( \Delta S > 0 \)) of water. Thus, hydrophobic interactions in solution are thought to be entropically driven; that is, \( \Delta H \) is small and \( \Delta S > 0 \), making \(-T\Delta S < 0\) and overall, \( \Delta G < 0 \) (e.g., see pp 92-5 of ref. 33). How does the presence of certain salts (e.g., phosphates and sulfates) promote hydrophobic-type interactions? To our knowledge, the mechanism(s) of salt-promoted adsorption has never been explained.

Various salts differentially affect protein structure. Anions of certain salts can be arranged in the so-called Hofmeister series (34) according to their effects on proteins: phosphates < sulfates < acetates < chlorides < nitrates < thiocyanates. Salts on the right side of the scale promote protein solubility (salting-in) and can induce protein denaturation, in part because of their disruptive influence on bound water. These salts are thus referred to as chaotropic (35, 36). Salts on the left side of this series can promote protein stability and precipitation (salting-out) and are said to have the opposite effect on water structure (i.e., anti-chaotropic or water-structure-forming). If a water-structuring salt (e.g., sulfate) is added to the solvent in relatively high concentration, solute (ligate) molecules may be forced out of bulk solution and accumulate at the phase boundary (mobile–stationary-phase interface). This effect delays diffusion of ligate and may sufficiently increase the equilibrium association constant so as to enhance interaction with the immobilized ligand. A much-decreased affinity would be achieved by removal of the salt. In fact, adsorption capacity as well as strength appears to increase in parallel with increasing salt concentration for all kinds of salt-promoted adsorption, including hydrophobic interactions, charge-transfer interactions, metal ion affinity interactions, and thiolphilic interactions.

High salt concentrations in the separation medium also have several important advantages in addition to increasing adsorption capacity. For example, proteins are frequently stabilized in solutions of water-structure-stabilizing salts. Bacterial growth is largely inhibited. Dilute protein solutions can be concentrated by a rapid and gentle adsorption step, followed by elution in salt-free buffers. Finally, nonspecific electrostatic interactions are eliminated during salt-promoted adsorption.

Below are two examples of promoting selective protein adsorption. The first example demonstrates the thiolphilic properties of immunoglobulins and illustrates the use of water-structure-forming salts to promote remarkably selective adsorption. The second example shows the ability of low concentrations of urea to promote the nearly quantitative adsorption of estrogen-receptor proteins onto immobilized DNA.

Salt-Promoted Thiophilic Adsorption of Immunoglobulins
Proteins attracted to the T-gel under defined conditions have been tentatively termed thiophilic (13, 14) in recognition of their affinity for the sulfur-containing thioether and sulfone groups in the ligand. Because human serum immunoglobulins are more thiophilic than most other serum proteins, buffer conditions have been developed for their selective adsorption during T-gel chromatography (12, 13).
This selective thiophilic adsorption of immunoglobulins appeared to us to have substantial utility, so we have explored thiophilic adsorption as a means of rapid and highly efficient purification of both polyclonal and monoclonal antibodies. To facilitate this goal with hybridoma cell cultures producing monoclonal antibodies and to further address immunoglobulin-related problems in serum-dependent cell cultures in general, we have also investigated the use of thiophilic adsorption to selectively eliminate immunoglobulins from human and bovine sera.

Figure 1 shows a typical T-gel elution profile of adsorbed immunoglobulins. The elution profile is similar for human and bovine serum and for hybridoma cell-culture fluid under the specified conditions, with only immunoglobulins being adsorbed. Figures 2 and 3, which illustrate the electrophoretic purity of the eluted antibodies, are representative of the purification of monoclonal antibodies from hybridoma cells grown in immunoglobulin-free bovine serum—also obtained by thiophilic adsorption. The bovine ("fetal" calf) sera with initial immunoglobulin concentrations of 10–12 g/L before thiophilic adsorption had residual concentrations ≤1–5 mg/L after a single thiophilic adsorption step. Apparently no essential growth factors were destroyed or removed from the sera during this process, because the specific growth properties of these hybridoma cells as well as other serum-sensitive cell lines were unaltered when we used T-gel-purified bovine serum. Details of this investigation are to be presented elsewhere (Hutchens, Ruan, Andersson, and Porath, ms. in preparation). We are now evaluating the use of thiophilic adsorption to remove immunoglobulins from adult bovine and equine sera for specific cell-culture applications. Similarly, we are investigating the use of T-gel-purified human sera to better understand the effects of serum immunoglobulins on in vitro fertilization and embryo development.

Selectivity, nearly total protein recovery (and preservation of antibody function) under gentle elution conditions, and the high degree of purity associated with thiophilic adsorption makes the T-gel a convenient and efficient separation tool worthy of further development. There are no theoretical limitations to its large-scale applications. Certainly, derivatization of the T-gel sulfone-thioether ligand to other surfaces (e.g., filters, membranes, nonporous beads) may further amend the geometrical or physical limitations associated with current applications.
The mechanism of thiophilic adsorption is currently unknown but may involve an electron-donor/acceptor mechanism or proton transfer between surface-accessible aromatic amino acids and the sulfone-thioether sulfur atoms in the T-gel ligand (Figure 4). Investigating the thiophilic properties of model proteins of known three-dimensional structure confirms the adsorption dependence on pH, temperature, salt type, and concentration effects previously observed with human immunoglobulins (14). Currently, however, attempts to correlate the distinguishing molecular-surface properties of thiophilic proteins with their measured binding constants at equilibrium and relative partition coefficients have not yet revealed any particular characteristics required to define the T-gel ligand acceptor site(s).

**Urea-Promoted Receptor-DNA Interaction**

The next example of promoting selective adsorption also involves an as-yet-unknown interaction mechanism. In this case urea is the mobile-phase constituent that promotes a high-affinity interaction between soluble steroid-receptor complexes and immobilized DNA.

The steroid-induced transformation of native steroid-receptor proteins to a configuration with increased affinity for DNA is currently thought to be a definitive event in the proposed mechanism of steroid hormone action (37-40). The acquisition of receptor affinity for DNA has been investigated in vitro for many years and remains a topic of considerable interest. Only surface-charge- or ionic-strength-dependent receptor-DNA interactions have been evaluated in the search for DNA-binding selectivity, capacity, and affinity (e.g., 37-40). The relative contributions of hydrophobic-type interactions between steroid-receptor proteins and DNA, with few exceptions (15, 41), have been virtually ignored. The existence of significant nuclear receptor-binding sites that are resistant to salt extraction (i.e., non-electrostatic) (42, 43) and our interest in the possibility of regulatory subunits in the quaternary structure of native receptor (26, 31, 41) have prompted a detailed investigation of urea on receptor structure/function relationships. We chose urea for these investigations because other perturbants of water/protein structure, e.g., chaotropic salts of the Hofmeister series (34), are known to disrupt not only hydrogen-bonding and hydrophobic-type interactions but also electrostatic interactions. Preliminary results from these investigations and their implications concerning the structural definition of receptor have been presented elsewhere (41).

One of the most significant findings was the discovery of urea-promoted receptor-DNA interactions. As shown in Table 1, exposure of the estrogen-receptor complex to 6 mol/L urea solution results in nearly quantitative binding to single-stranded DNA-agarose and significant binding to double-stranded DNA-cellulose. Conversely, urea (6 mol/L) had a relatively conservative effect on the following receptor characteristics: affinity for estradiol, size (i.e., Stokes radius), and apparent surface charge (Table 2). The actual concentration of urea required to promote maximum interaction of receptor with DNA-agarose was only 2-3 mol/L, even for concentrated (20-25 g/L) cytosol preparations. The time required for urea to promote the receptor-DNA binding appeared to be less than that required to perform the batch-type or column flow-through experiments (10-12 min). The “nonspecific” interactions of either free [3H]estradiol and (or) nonreceptor estradiol-binding proteins with DNA in 6 mol/L urea solution did not exceed 1-2%. Nonspecific binding values of 0.3-0.5% were typical for experiments involving calf uterus (control) and human breast or uterine tumors. There was good agreement (85-100%) between estimates of [3H]estradiol-receptor quantities determined by urea-promoted DNA binding and by the hydroxy-apatite adsorption assay (44, 45). Efforts are underway to determine the receptor-DNA affinity constant, with use of both “nonspecific” and specific DNA sequences. To date we have no evidence of urea-promoted specificity for binding DNA sequences.

The role of urea in promotion of receptor-DNA interaction is unclear. Urea may affect the higher order of structure of both proteins and nucleic acids because bound water
stabilizes both of these macromolecules (46–49). However, urea may also participate in the receptor–DNA binding event via its electron-donor/acceptor properties (16–18). The influence of salt on urea-promoted receptor–DNA binding is dramatic. If urea is removed from the DNA-immobilized receptor, increased ionic strengths (e.g., KCl up to 2 mol/L) do not initiate receptor elution. Yet, in the continued presence of urea, inclusion of 0.4 mol of KCl per liter resulted in quantitative recovery of the once-immobilized receptor. This effect was independent of the actual concentration of urea involved (e.g., 2–6 mol/L).

A possible clue to the precise mechanism of receptor–DNA interaction(s) and the involvement of urea (or water) in this process has recently been provided. Kumar et al. (40) have identified a linear sequence of amino acids derived from the cloned steroid-binding subunit of the estrogen receptor by using point-mutation insertions in the DNA-binding region. This putative DNA-binding region has a high degree of sequence homology with other cloned steroid-receptor proteins (50, 51) as well as with transcription factor IIIA from Xenopus oocytes (52). Bound zinc is involved in the interaction of transcription factor IIIA with DNA, and bound metals appear to be integral components of other DNA-binding proteins. Berg (53, 54) has recently summarized data suggesting the general existence of metal binding domains or "fingers" in DNA-binding proteins. Circumstantial experimental evidence suggests that the estrogen-receptor protein is possibly a metal-binding protein (55–57); however, we are not aware of any direct experimental evidence supporting this. Because the putative DNA-binding regions of the estrogen-receptor proteins cloned from humans and chickens appear to have several conserved aromatic amino acids in addition to positively charged residues, we have undertaken a direct evaluation of the metal-binding properties of the estrogen receptor, the involvement of urea in exposure of these sites, and the possible relationship of these sites to the urea-promoted DNA-binding region of receptor.

Using iminodiacetic acid gels with immobilized Ni\(^{2+}\), Zn\(^{2+}\), and Cu\(^{2+}\) ions, we have established the metal-binding potential of uterine estrogen-receptor proteins. Cytosolic estrogen receptors labeled with \(^{3}H\)estradiol were strongly adsorbed to each of the immobilized metals in hypertonic buffers at neutral pH. Affinity elution (imidazole) and pH elution techniques were used to differentially resolve various receptor forms. At least three receptor isoforms were separated by using iminodiacetic acid–Zn\(^{2+}\). We also investigated, in the presence and absence of 3 mol/L urea solution, the metal-binding properties of receptor purified by DNA-affinity chromatography. We used high ionic strengths to mask possible nonspecific electrostatic interactions. Two DNA-binding receptor forms were resolved by imidazole elution from iminodiacetic acid–Zn\(^{2+}\). In all cases, our recovery of receptor from the immobilized metal ion columns routinely exceeded 85%. Details of these studies are to be reported elsewhere (Hutchens and Li, ms. in preparation). Our results lead to recognition of estrogen-receptor proteins as metal-binding proteins and suggest new and potentially powerful regimes for receptor immobilization and purification heretofore unexplored.

In conclusion, insoluble matrices with simple, chemically defined structures as ligands can be utilized with suitable ligates as model systems to evaluate the structural and chemical properties that account for such definitive ligand–receptor interactions. The study of these matrices could contribute to an understanding of metal-ion–receptor binding and the role of metal ions in receptor function. The results of such studies may provide a basis for the design of metal-binding peptide fragments that can be used as probes of receptor–DNA interactions.


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