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BIOAFFINITY CHROMATOGRAPHY: SYNERGY BETWEEN INTERACTIVE CHROMATOGRAPHY AND MOLECULAR RECOGNITION FOR THE SEPARATION AND ANALYSIS OF MACROMOLECULES

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SUMMARY

Affinity chromatography, commonly regarded as an integral tool in macromolecular separation sciences, also provides an analytical method to study structure–function relationships of macromolecular interaction processes and to design recognition molecules. The latter, as found recently for the case of antisense peptides, may be useful as affinity agents in immobilized forms to effect new types of biomolecular separation.

INTRODUCTION: PROTEIN SURFACES, BIOAFFINITY CHROMATOGRAPHY, AND MACROMOLECULAR SEPARATION

The success of bioaffinity chromatography for the separation of biological macromolecules is rooted, as all interactive chromatography, in complementarity between molecular surfaces of the biomolecules and of the chromatographic supports. Considering macromolecular surfaces generally (Fig. 1), the distribution and nature of surface and polarity alone are responsible for an important array of chromatographic separation methods including ion-exchange, hydrophobic interaction and reversed-phase chromatography. Here, as shown by many examples elsewhere in this volume, sufficient variation exists just in these properties on the surfaces of different molecules that they can be distinguished by differential interaction with insoluble charged or apolar chromatographic supports. For macromolecules, though, the separation problem intensifies, since their increased sizes lead to more complex surfaces, blurred differences of surface charge and polarity features, and thus incomplete separation.

However, biological macromolecules have another surface recognition feature useful for chromatographic separation, namely localized binding sites (Fig. 1).

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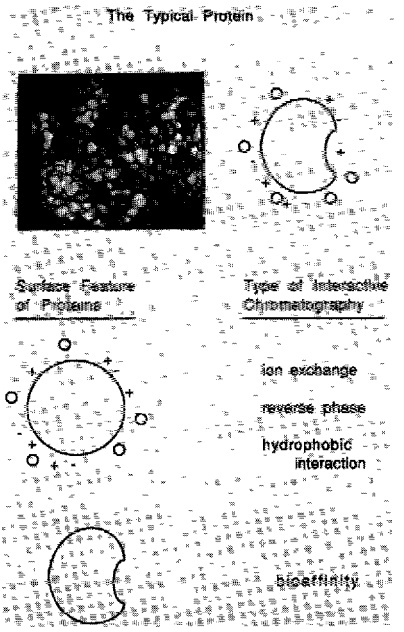


Fig. 1. Surface characteristics of proteins and their exploitation in interactive chromatography. Top: views of a typical protein surface: left, by computer graphics for bovine pancreatic ribonuclease S, with different intensity spheres depicting varying hydropathic properties of atoms — with black and darkest grey being negatively and positively charged, respectively, and lightest being non-polar (courtesy of Richard Feldmann, National Institutes of Health); right, schematically as a mosaic of charged (+, −) and hydrophobic (○) elements and molecular binding sites most often associated with biological activity. Bottom: correlation of surface features with interactive chromatographic modes which use these features. Charged and hydrophobic elements are the basis for such chromatographic modes as ion exchange, reversed phase, and hydrophobic interaction, while affinity sites are the basis for affinity chromatography.

These sites, often the origins of biological activity, have specificities and definable affinities for complementary molecules including macromolecular ligands, macromolecular assemblies and cells. These are the surface features used in bioaffinity chromatography, and their exploitation has made this chromatographic method a powerful separation science of wide-ranging impact for biochemists. As understanding of selective macromolecular recognition has improved, so have increasing opportunities to develop bioaffinity chromatography as both a preparative and analytical tool in biochemistry and biotechnology.

The basic approach in bioaffinity chromatography is to immobilize an interactive ligand or other substance complementary to a binding site of a macromolecule (Fig. 2A) under conditions in which the immobilized substance remains accessible to bind to soluble macromolecule and on insoluble supports which do not interact non-specifically with the macromolecule. When these basic conditions are met, efficient purifications can be attained by two-step elutions first under binding conditions, in which the target molecule is effectively retained while non-binding molecules elute, and then by a second, chaotropic elution step to remove the selectively bound macromolecule (Fig. 2A). An elegant example of this is shown in Fig. 2B for the pioneering purification of staphylococcal nuclease

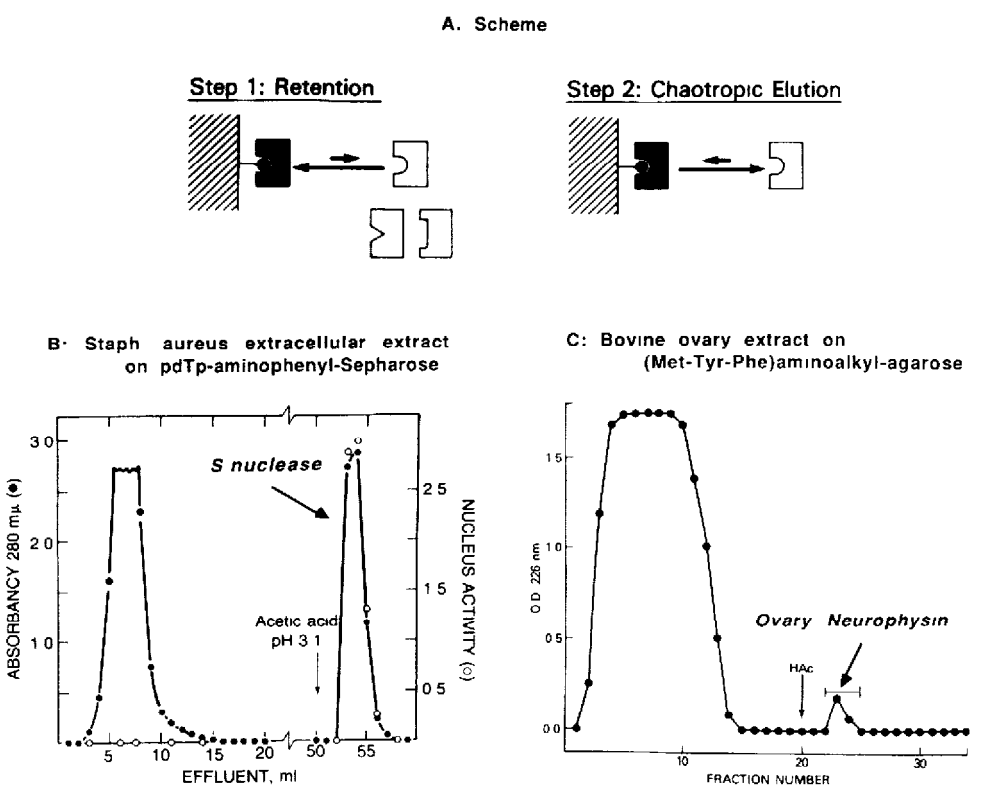


Fig. 2. Fundamental approach and examples of preparative affinity chromatography. (A) Scheme of two-step retention/chaotropic elution. (B) Purification of staphylococcal nuclease on immobilized thymidine diphosphate (adapted from ref. 1). (C) Use of immobilized Met-Tyr-Phe [2], a known pituitary neurophysin ligand, to purify ovarian neurophysin [3]. The ovary neurophysin fraction denoted was verified to contain bovine neurophysin by reversed-phase HPLC as performed before for pituitary proteins [4].

[1] using the cyanogen bromide method [5] to attach the active site ligand 5′-aminophenylphosphorylthymidine 3′-phosphate to agarose covalently. Numerous purification successes [6–8] have followed this early experiment, for example the ovary neurophysin separation on Met-Tyr-Phe-aminohexyl-agarose shown in Fig. 2C. These use essentially similar two-step elutions based on hydrophilic supports like agarose and covalent attachment methods designed to retain molecular recognition between mobile macromolecules and immobilized ligands. New linkage methods as well as rigid chromatographic supports have led to an expanding number of macromolecular separation applications by high-performance liquid affinity chromatography [9,10]. In all these cases, the key advantage is macromolecular recognition — the ability to identify substances which interact selectively with binding sites of biological macromolecules and to immobilize these under conditions which retain their selective binding characteristics.

DEVELOPMENT OF BIOAFFINITY CHROMATOGRAPHY FOR THE ANALYSIS OF MACROMOLECULAR RECOGNITION

The success of affinity chromatography as a purification method in turn has stimulated the analytical development of this chromatographic mode [10–12]. Selective separation of an ever-increasing number of macromolecules emphasizes a basic point, that macromolecular recognition properties in solution can be retained on the solid phase at least sufficiently to achieve preparative separation of macromolecules from complex mixtures based on binding site characteristics. This biospecificity predicts that macromolecules interact with affinity supports directly through these specific binding sites and thus that the extent of retardation during chromatographic elution should be a direct, quantitative reflection of the binding affinity between mobile and immobilized components. Thus, in principle, the quantitative elution characteristics of affinity chromatography should be usable to determine quantitative binding characteristics of macromolecules for their ligands.

The primary requirement here is that the macromolecular elution occur in real time, so that it can be measured, using buffers which allow binding, not the chaotropic elution used in preparative chromatography. What is sought is retardation (Fig. 3A), not retention (Fig. 2A). Such non-chaotropic, isocratic elution usually can be achieved on the same types of matrices used preparatively, simply by decreasing the density of immobilized binding sites. Alternatively, retardation instead of retention can be achieved using (i) buffer conditions (for example, slightly altered pH, ionic strength, or temperature) favoring weaker binding but not fully chaotropic elution, (ii) weaker-binding ligands as immobilized species, or (iii) competitive elution.

As it happens, isocratic elution normally is achievable and elution volumes can be measured. The experimental retardation volumes, V, can be used to calculate the dissociation constants for matrix interaction, $K_{M/P}$. In addition, competitive elution also can be done by including a molecule in the elution buffer which competes with immobilized molecule for binding to mobile macromolecule (Fig. 3A). When this latter occurs, V varies inversely with competitor concentration $[L]_T$ and the variation can be used to determine $K_{L/P}$, the dissociation constant for the solution L–P complex, in addition to $K_{M/P}$. Analytical elutions have been carried out both zonally (with peak position analysis to determine V) and continuously (with V determination using frontal analysis) [10–13]. In general, our own studies have stressed the usefulness of zonal elutions for analysis of micro amounts of macromolecules necessitated by the limited amounts of the latter often available to the biochemist. In contrast, continuous elution also has advantages, including the more rigorous analysis of stoichiometry of binding interaction, functional capacity of matrix, and concentration dependence of binding across a broad range of mobile macromolecule concentration including high concentrations. Examples of zonal elution are shown in Fig. 3B and C, respectively, for staphylococcal nuclease and neurophysin on the same matrices, pdTp-aminophenyl-Sepharose and Met-Tyr-Phe-aminohexylagarose, respectively, used preparatively (Fig. 2).

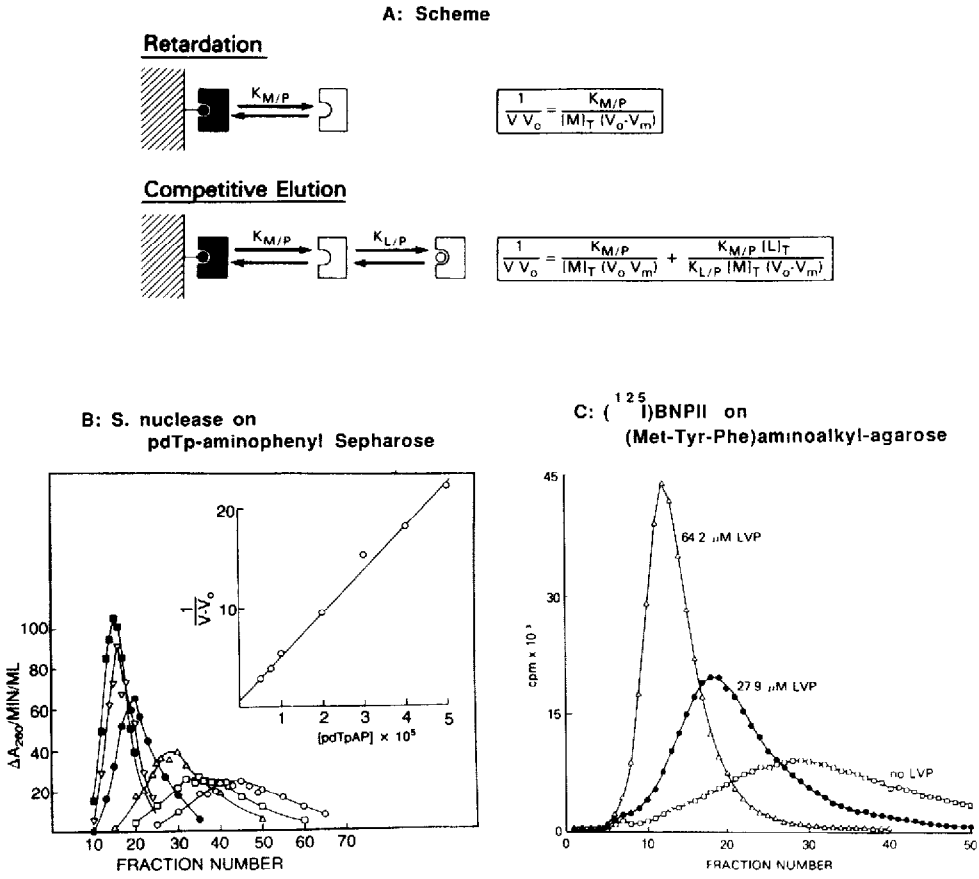


Fig. 3. Fundamental approach and examples of analytical affinity chromatography. (A) Scheme of zonal elution, including competitive elution, and the equations which describe the relationship of experimental elution volume V to dissociation constants $K_{M/P}$ for matrix (M)-mobile protein (P) complex and $K_{L/P}$ for solution complex of P with competitor L [13]. V_0 and V_m are unretarded elution volume and volume outside pores of affinity matrix, respectively; $[M]_T$ is the concentration of matrix-bound ligand, ideally defined as concentration of functional sites; $[L]_T$ is the concentration of competitor. (Figure adapted from ref. 14.) (B) Analytical elutions of staphylococcal nuclease on thymidine diphosphate-Sepharose, with values of V determined from position of retarded peaks in elution profiles obtained at different concentrations of competitor pdTp-aminophenyl and replotted as $1/(V-V_0)$ versus $[L]_T$ according to the lower equation of part A of this figure. Dissociation contants determined with these data are given in Fig. 4. (Figure adapted from ref. 15.) (C) Analytical elution profiles of iodinated bovine neurophysin II on immobilized tripeptide Met-Tyr-Phe at different concentrations of the competitor lysine vasopressin (LVP). (Figure adapted from ref. 16.)

The results of analytical elutions for staphylococcal nuclease, neurophysin, and a variety of other protein systems show that the chromatographic dissociation constants obtained are reliable descriptions of macromolecular interaction. The results for nuclease show that $K_{M/P}$ and $K_{L/P}$ are very similar when the same ligand, pdTp-aminophenyl, is both the immobilized species and the competitor (Fig. 4). When other competitors are used, $K_{L/P}$ varies as expected depending on the nature of the competitor. Several other macromolecular interacting systems

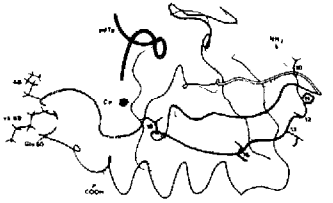
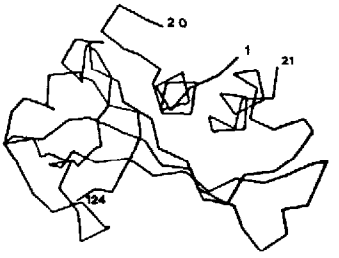
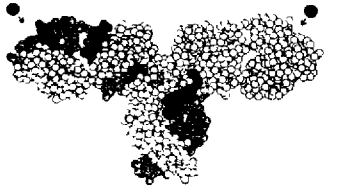
	Interactors			
	Matrix (M)	Mobile (P)	Competitor (L)	Chromatographic K_d (molar)
	pdTpAP	SNase	pdTpAP	$K_{L/P} = 2.3 \times 10^{6}$
				$K_{M/P} = 1.1 \times 10^{6}$
			NPpdTp	$K_{L/P} = 1.1 \times 10^{5}$
Enzyme/Active Site Ligands (Staph nuclease/nucleotides)				
	SPeptide (1-20)	SProtein (21-124)	SPeptide	$K_{L/P} = 1.9 \times 10^{7}$
				$K_{M/P} = 6.1 \times 10^{7}$
Protein Fragment Complex (Ribonuclease S)				
	Phosphoryl-choline	TEPC 15 (IgA) bivalent monomer	Phosphoryl-choline	$K_{L/P} = 1.2 - 1.5 \times 10^{6}$
				$K_{M/P} = 1.2 \times 10^{6}$ (low density matrix)
				$K_{M/P} = 1.3 \times 10^{7}$ (high density matrix)
Antibody/Antigen (TEPC 15-phosphorylcholine)	Phosphoryl-choline	TEPC 15 (IgA) monovalent Fab	Phosphoryl-choline	$K_{L/P} = 1.5 \times 10^{6}$
				$K_{M/P} = 3.9 - 4.2 \times 10^{6}$ (high density matrix)

Fig. 4. Quantitative dissociation constants of several interacting macromolecular systems determined by analytical affinity chromatography. Data are from ref. 15 for staphylococcal nuclease, ref. 17 for ribonuclease S, and ref. 18 for TEPC15-phosphorylcholine.

have been subjected to analytical affinity chromatography, as shown by the selected examples in Fig. 4. Importantly, K_d values determined chromatographically in most cases are consistent with values determined in solution [13,19]. One of the few exceptions is that for the bivalent form of an antibody denoted TEPC15, an immunoglobulin A (IgA), when the $K_{M/P}$ was determined at relatively high density of matrix-immobilized ligand, phosphorylcholine. Here, the lower value of $K_{M/P}$ than expected from solution measurement could be traced to the occurrence of bivalent binding of IgA on affinity matrix [18]. When the elution was carried out on a matrix with lower phosphorylcholine density, or on the same matrix with the monovalent binding fragment F_{ab} derived proteolytically from TEPC15 (Fig. 4), the $K_{M/P}$ was similar to the solution $K_{L/P}$ value. Thus, the deviations from solution behavior found for IgA at high phosphorylcholine density were explainable based on behavior fully predictable for divalent molecule. In fact, the results with TEPC15 emphasize the general feature of affinity chromatography, that multivalent macromolecules may bind more tightly to affinity matrices than expected from solution characteristics and that this multivalent binding can lead to the undesirable need for harsh, denaturing elution conditions in preparative chromatographic applications. For molecules like high-affinity multivalent antibodies, reversible denaturation can be minimized by carrying out preparative chromatography on matrices designed to avoid multivalency — in other words, low ligand density supports. Alternatively, multivalent interaction with affinity matrices may be a desirable feature to maximize for preparative chromatography of multivalent macromolecules with weak affinity ligand-binding properties, for example some monoclonal antibodies and lectins.

CHARACTERIZING MACROMOLECULAR RECOGNITION MECHANISMS: QUANTITATIVE STRUCTURE-FUNCTION RELATIONSHIPS OF INTERACTIONS AND AFFINITY SCREENING

Being able to use affinity chromatography to measure macromolecular interactions quantitatively has led to a variety of applications aimed at studying recognition mechanisms. Here, binding to a particular immobilized biomolecule can be used as a reporter or mechanistic sensor of the degree of functional intactness of mutants or otherwise altered structural variants of the mobile molecular interactor. Seen simply, a set of structurally related mobile molecules can be eluted analytically, their interaction affinities compared, and the structures correlated to affinity to deduce what structural elements of the mobile components are important or unimportant for binding.

Analytical affinity chromatography has been used to study molecular assembly interactions in the neuroendocrine pathways which form the neurohypophysial peptide hormones oxytocin and vasopressin from biosynthetic precursors. As shown in Fig. 5A, the hormone precursors have multiple sequence domains each with one hormone and one neurophysin, the latter a small protein in its mature (enzymatically processed) form that binds mature hormone non-covalently. While the assembly properties of the hormone–neurophysin (H–NP) complexes are known from studies by a variety of methods [21–23], we were interested to know

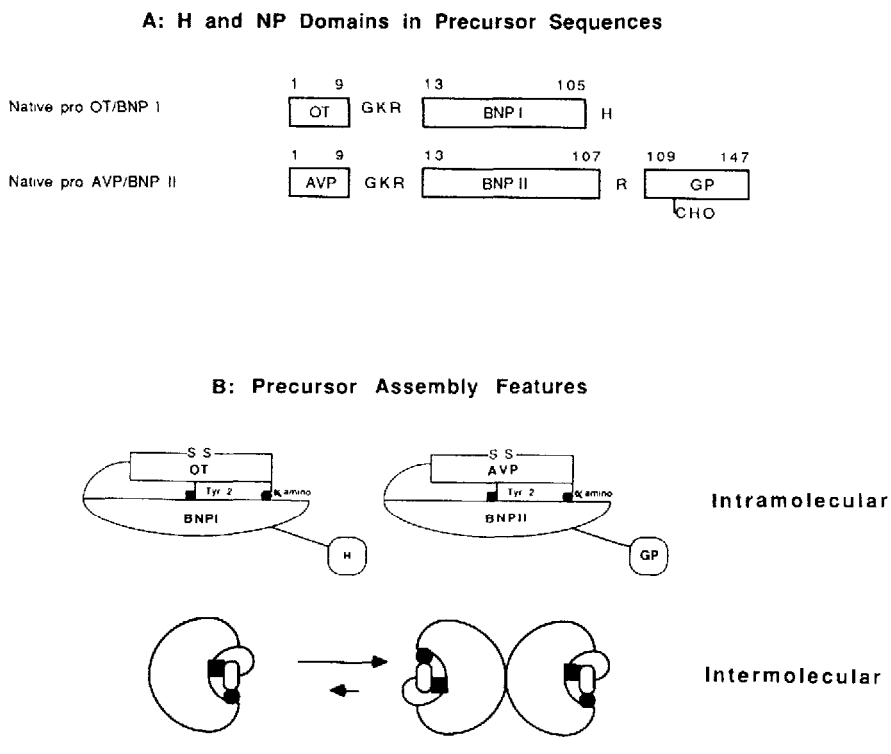


Fig. 5. Oxytocin and vasopressin precursor structure, assembly interactions, and the experimental approach to assess these interactions by analytical affinity chromatography on immobilized neurophysin. (A) Sequence domains in precursors: OT, oxytocin; AVP, arginine vasopressin; BNPI and II, bovine neurophysins I and II; GP, glycopeptide; GKR, Gly-Lys-Arg; H, His; R, Arg. (Figure adapted from ref. 20.) (B) Intramolecular properties of oxytocin and vasopressin precursors. Top: interaction of hormone (oxytocin or vasopressin) sequence domain with neurophysin sequence domain, in both precursors mediated by important stabilizing contacts with charged α-amino and Tyr 2 side-chain. Bottom: intermolecular association of intramolecularly assembled precursor monomers, with dimers more favored when domains interact than when they do not. (Figure adapted from ref. 20.). (C) Scheme depicting how immobilized neurophysin (left) can be used to distinguish assembled precursor (PRO=proAVP/BNPII or proOT/BNPI as shown in part B) from non-assembled precursor (PRO' = mutant in which one or both of contact elements is missing or altered) and NP itself by the greater retardation (and therefore lower K_d) expected of assembled precursor than of either NP alone or precursor analogue in which domains do not interact

whether, as shown in Fig. 5B, the precursors also assemble (predicted to be important in precursor packaging and processing) and the structural elements promoting the assembly process. To do this we first made the precursor semisynthetically from chemically synthesized hormone domain and tissue-derived (and in some cases chemically modified) neurophysin domains. The carboxyl terminal pieces were left out for synthetic simplicity; they eventually were found not to be required for assembly interactions. The semisynthesis was required because the precursors, being enzymatically processed and thus kinetically short-lived in vivo and also not yet cloned and expressed, were not otherwise available in amounts sufficient for structure–function analysis and subsequent sequence modification.

The assembly properties of semisynthetic precursor were defined using affinity chromatographic experiments based on the known interaction properties of the non-covalent H–NP complexes. Immobilized neurophysin can bind soluble neurophysin (Fig. 5C), with the solid phase self-association being stronger in the presence of hormone (Fig. 6B). Precursor has the same sequence components as H–NP non-covalent complex except that the H and NP components are covalently attached through the Gly-Lys-Arg linker. Thus, since non-covalently liganded neurophysin self-associates more strongly than non-liganded NP (Fig. 6B), it was thought possible that precursor also could self-associate intermolecularly with the H and NP domains interacting intramolecularly. If this were so, precursor would behave like liganded NP and associate with immobilized NP more strongly than if the domains did not interact intramolecularly (Fig. 5C). In fact, this was borne out experimentally [20,24,25], as shown in Fig. 7A, by the greater degree of retardation of precursor than NP. While this experiment measures precursor–NP association, the implication can be drawn straigthforwardly that precursor can self-associate with itself with the high affinity of liganded NP.

Based on the retardation of precursor on immobilized NP, the latter affinity matrix was used to study what structural elements of the precursor are responsible for triggering its high affinity intermolecular association. One question posed was whether domain interaction rather than simply covalent attachment through Gly-Lys-Arg was the triggering element and, if so, what sequence elements were responsible for this interaction. As shown in Fig. 5B, it was predicted from properties of the non-covalent complexes that the α-amino and Tyr 2 side-chain in precursor were important interdomain contacts. On this basis, and using the vasopressin and oxytocin semisynthetic precursors, mutants were made in which either one or both of these elements were altered. As shown by the data in Fig. 7A (lower right panel), precursors in which these elements were altered substantially interacted with immobilized NP weakly, while that with these structural elements retained but many others changed (Fig. 7A lower left panel) still interacted more strongly. The results with semisynthetic mutation and examination by the affinity screening approach have verified the triggering effect of interdomain interaction through α-amino and Tyr 2 for precursor assembly [20,25].

Immobilized ligands can be used as functional screens not just to examine one protein at a time for binding affinity but many mutants simultaneously. Immobilized NP, again as a case in point, has been used to select out binders from no binders for mutant forms of hormone [20,25,27]. We recently constructed an

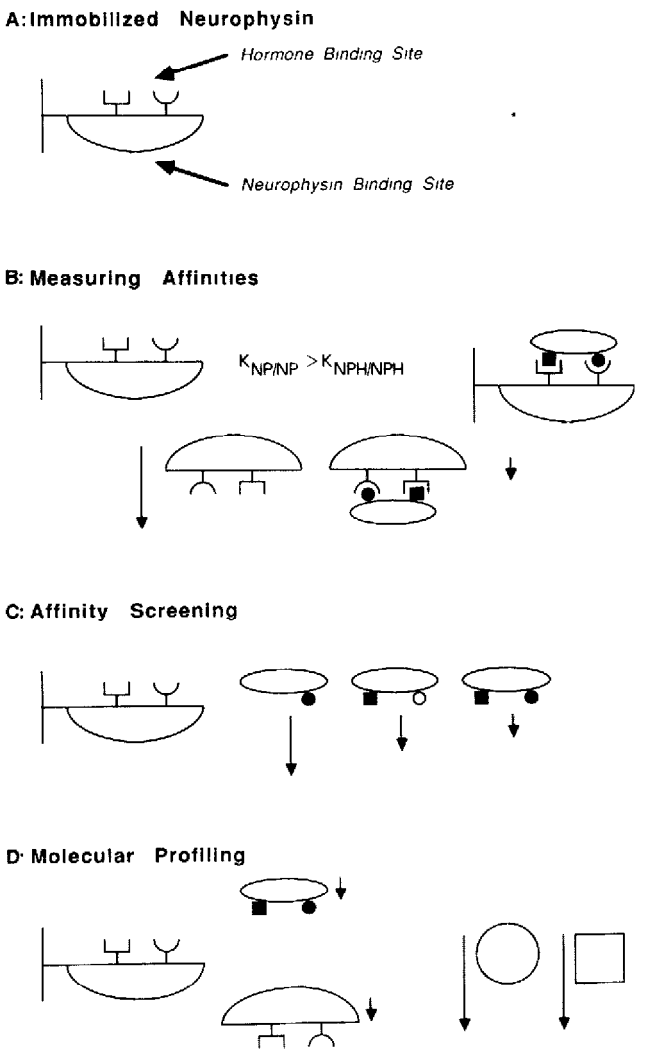


Fig. 6. Scheme of various analytical uses of chromatography on immobilized NP. (A) Scheme of immobilized NP showing presence of two binding sites, one for hormone (oxytocin or vasopressin) and the other for neurophysin self-association. (B) Comparison of binding affinities of liganded from unliganded NP by comparative elution. Liganded NP self-associates more strongly [21–23]. (C) Use of immobilized NP to screen hormone analogues which have different affinities due to different contents of contact elements. (D) Obtaining a molecular profile on interacting substances in mixtures such as extracts of biological tissues. Interacting molecules can be detected as retarded peaks and, if they bind with different affinities, distinguished from one another and perhaps identified by chromatographic K_d values.

experiment to examine how Tyr 2 functions as a contact and whether other residues could replace it [26]. We synthesized a sequence-simplified AVP-Gly-Lys-Arg analogue in which a mixture of amino acids was coupled at position 2 in addition to Tyr and the resulting mixture of solid-phase synthetic peptides was eluted through immobilized NP (Fig. 7C). The retarded peptide fraction was

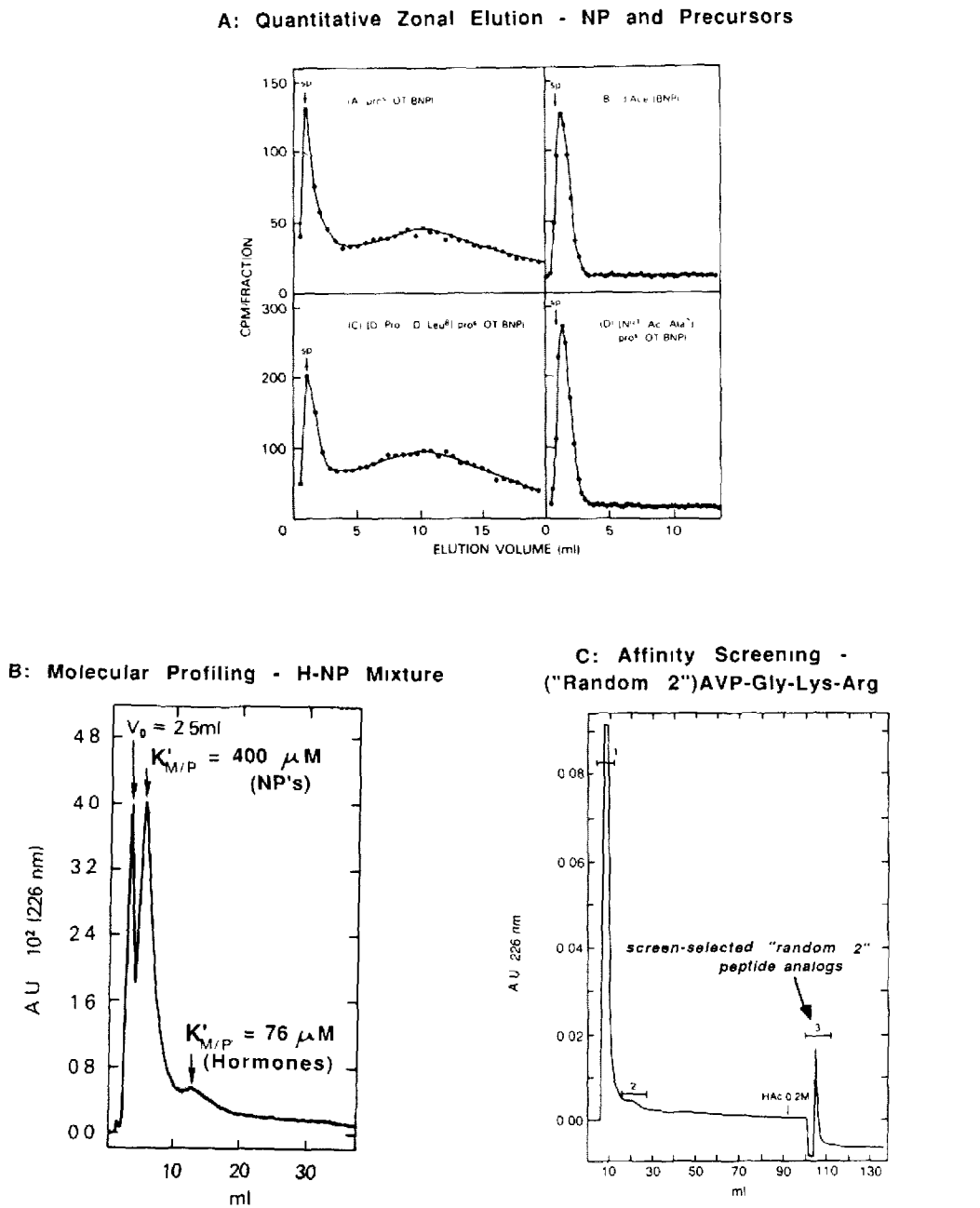


Fig. 7. Experimental examples of analytical use of affinity chromatography on immobilized neurophysin. (A) Example of measuring binding properties (Fig. 6B): differentiation of low-affinity binding neurophysin in (C) and disassembled oxytocin precursor analogue in (D) from high-affinity association of assembled oxytocin precursor and precursor analogue in (A) and (B). (Figure from ref. 25.). (B) Example of molecular profiling (Fig. 6D): separation of NPs and hormones from pituitary extracts and the distinction between these by their differential retardations. (Data taken from ref. 10.) (C) Example of affinity screening (Fig. 6C): distinguishing of binding from non-binding hormone analogues in synthetically derived ['random 2']AVP-Gly-Lys-Arg (see text). Figure adapted from ref. 26.

examined for amino acid content. Residues introduced into position 2 could be discriminated since much of the rest of the sequence was composed of Ala residues [20,26]. Only Tyr and Phe in addition to the residues expected for sequences 1/2Cys 1 and 3–12 were found after acid hydrolysis. Spectroscopic characteristics of reversed-phase chromatograms of the bound peptide mixture scanned at different UV wavelengths provided some circumstantial evidence that a Trp peptide might also be present and that Trp thus might also be a functionally acceptable replacement of Tyr. However, the latter conclusion is as yet unverified directly. Overall, the experiment reflects the way an affinity column can be used as a molecular screen for examining interacting molecules made by structural mutation, including random mutation as above. A second example of affinity screening for S-peptide synthetic design is cited below.

MOLECULAR PROFILING USING AFFINITY COLUMNS

Immobilized ligands provide tools to examine the occurrence of interacting molecules in biological sources. Both the presence of chromatographically retarded species and their quantitative affinities to immobilized interactor can be determined. Again, using the example of the H–NP system, immobilized NP can be used to detect the presence of NPs, Hs, and other interacting molecules (Fig. 6D). Moreover, NP and H have different affinities, and one can detect both simultaneously and identify them at least preliminary by their apparent quantitative affinities. For example, when neurosecretory granule extract from bovine posterior pituitary was eluted on [NP]Accell (a silica-based matrix, from Waters Chromatography Division, Milford, MA, U.S.A), several different interacting forms were detected (Fig. 7B). Based on their apparent binding affinities ($K_{M/P}$ values based on a single elution at an unknown concentration), assignments were made as NPs and Hs. These assignments were verified by correlation with chromatographic behavior of authentic molecules and reversed-phase high-performance liquid chromatographic (HPLC) examination of retarded peaks.

Quite obviously, the affinity chromatographic approach cannot be used to profile all molecules occurring in a particular biological source. Techniques like two-dimensional gel electrophoresis, as discussed elsewhere in this volume [28], are far more appropriate in that context. But, an affinity chromatography column can give a rather straightforward profile of the occurrence of molecular interactors. When the same type of profiling is done with immobilized antibodies, single or multiple antigens can be detected [29]. Importantly, analytical immunoaffinity chromatography can be seen as a way to discriminate between cross-reacting species such as metabolites versus intact forms assuming that the latter have different affinities for the antibodies.

DESIGNING NEW AFFINITY MOLECULES

Most bioaffinity chromatographic effort, both in purification and analysis, has been focussed on the use of native molecules as immobilized ligands. This approach certainly has been rewarding in yielding new methods for selective bio-

molecular separation. Yet, an impactful increase in the usefulness of the method could result from the de novo design of immobilized recognition molecules which have controlled binding affinities and specificities for macromolecules that cannot be found with naturally occurring ligands. An example of such potential can be seen in the case of triazine dyes which, though not engineered specifically for affinity chromatography, nonetheless have affinities for proteins that make them useful as immobilized ligands for protein separation. Redesign of such dyes also is being attempted, to improve specificity and affinity over that available from the accidental mimicry built into the original dyes themselves [30].

The design of recognition molecules to be used for affinity supports can take two routes, redesign of native or serendipitous (as dyes) molecules or de novo design. A prototype example of redesign in our own work is ribonuclease S-peptide. Redesign studies of S-peptide sequence have defined structural elements most important for binding to the complementary protein fragment S-protein [31]. These are an α-helical conformational frame and two contact elements, Phe 8 and Met 13. Once the contact elements were identified, it became possible to modulate the binding affinity of S-peptide for S-protein by replacing one or the other of the contact side-chains. Virtual elimination of binding affinity resulted from Ala replacement of either Phe 8 or Met 13 [32]. Residues also could be found which replaced Met 13 to give weaker but finite affinity. This was done by a combination of random synthesis at position 13 and affinity screening of the 'random 13' preparation on immobilized S-protein affinity matrix. Val was found to replace Met 13 with significant retention of affinity. Somewhat surprisingly, Lys also was a viable replacement even though it resulted in a peptide with a very weak affinity. S-Peptides thus were produced with a series of varying affinities to S-protein by sequence redesign of contact elements. Similar combination of contact site mutation and affinity screening could give similar sets of recognition molecules for other systems (see for example the above case of oxytocin and vasopressin sequence mutation and effects on neurophysin interaction). Variable-affinity, redesigned recognition molecules represent useful alternatives to native molecules for immobilization and use in affinity separation (e.g. use of mutant ligand of weaker affinity to effect greater ease of elution of target macromolecules without denaturing chaotropic conditions).

Recognition molecules may also be able to be designed de novo. Most would agree that, at present, we do not known enough about the rules of macromolecular recognition to design recognition mimics at will. But there are at least some contexts which may allow us to begin this process for peptides and proteins. One point of entry may be the phenomenon of antisense peptides. As shown in Fig. 8, these peptides are the sequences encoded by antisense DNA. While normally not made in cells, these peptides can be synthesized chemically. In several cases studied so far, antisense peptides have a significant affinity for the corresponding sense peptides [14,33–36]. That there is significant selectivity in this interaction, directed at particular polypeptide sequences, has been demonstrated. This suggests that antisense peptides could perhaps be made purposefully as native sequence-directed recognition molecules (Fig. 8). Some mechanistic studies of antisense–sense peptide interactions have been carried out [14,35]. Significant

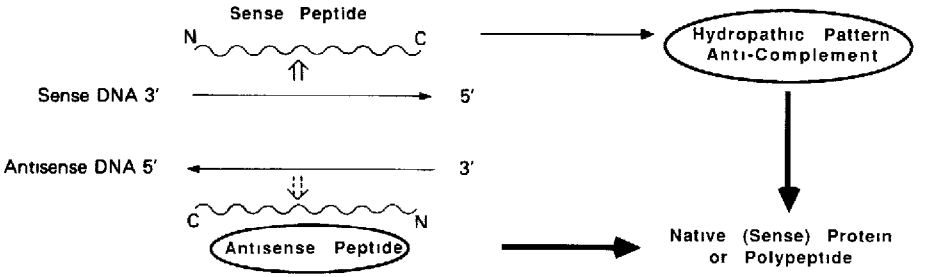


Fig. 8. Scheme showing the relationship of antisense and hydropathic pattern anti-complement peptides to native (sense) peptide sequences and the experimental observation that the former two can bind to the latter. Antisense peptides are those encoded in antisense DNA. Hydropathic pattern anti-complements (HPAs) are peptides synthesized based on sense peptide sequence directly, with residues in the 'HPAs' being hydrophilic in positions corresponding to residues in the sense peptides which are hydrophobic and vice versa. (Figure adapted from ref. 33.)

sequence and conformation degeneracy has been observed in the cases of S-peptide–antisense (S-peptide) and vasopressin–antisense (vasopressin). The data so far argue that, unlike such interaction cases as S-peptide–S-protein and H–NP, sense–antisense peptide interactions are not driven by compact conformation and limited contact surface elements of the interactors. What does appear important is the content of opposing hydrophylic and hydrophobic residues in the amino acid sequences of the component peptides. A hypothesis of hydropathic pattern recognition has been proposed [33,35,37], suggesting that antisense recognition peptides may be best considered hydropathically anti-complementary peptides when aligned with corresponding native polypeptides. In recent experiments, such anti-complementary peptides have been constructed based simply on native polypeptide sequence and ignoring DNA sequence altogether [38].

The actual mechanism controlling antisense and, more generally, hydropathically anti-complementary peptide interactions with native peptides is not yet well understood. Nonetheless, the antisense and hydropathic pattern anti-complements may well provide start points to create recognition peptides (Fig. 8) which, among other things, could be immobilized and used to separate targeted sense peptides and proteins. Recent attempts to do this with vasopressin-related antisense peptides have led to the successful separation of Arg^8-vasopressin from oxytocin and even from Lys^8-vasopressin [39].

BIOAFFINITY CHROMATOGRAPHY AS AN EVOLVING METHOD

The interplay between bioaffinity chromatography and macromolecular recognition continues to stimulate the evolution of the method for analytical and preparative separation. Surface recognition of biological macromolecules was the basis for devising affinity chromatography for purification in the first place. Ultimately, the method has been used to understand more about macromolecular recognition mechanistically. No doubt, improved understanding eventually will allow more effective chromatographic separation procedures to be devised. Affin-

ity chromatography also has proved an effective means to evaluate redesigned and newly designed recognition molecules. Again the expectation is that these may eventually be useful in immobilized form to effect new types of biomolecular separation. Thus, the interplay of affinity chromatography and recognition mechanism can be expected to provide a useful stimulus for separation science as well as macromolecular structure–function studies.

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