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Affinity Chromatography of Complexation Hosts

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Abstract. Chromatographic separation of complexation hosts based on their complexation interactions with a bonded aminopropyl silica support is described. Unlike common separations based on simple physical properties such as size or polarity, this procedure takes advantage of the chemical properties which were specifically designed into the complexation hosts.

Key words. Affinity chromatography, macrocycles, purification.

1. Introduction

A myriad of molecules of varying degrees of complexity and sophistication have been designed and prepared in efforts to complex chosen classes of molecules or ions [1–4]. Often, these molecular recognition 'hosts' are macrocylic compounds. The macrocyclization steps in the syntheses of these hosts, in general, yield varying amounts of oligomeric or polymeric impurities which must be separated from the desired monomeric macrocycles. Though a variety of techniques have been brought to bear on this purification problem, one common method is the use of gel permeation ('size exclusion') chromatography. The comparatively low molecular weight monomeric 'hosts' are preferentially retained within the porous support while the larger oligomers are washed through.

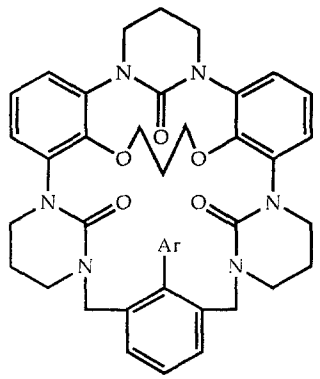
This popularity of size exclusion chromatographic purification is rather surprising, as when one considers the various and sundry properties that have been carefully designed and built into the myriad of known complexation hosts, their *size* is perhaps one of the *least* significant. Consideration of the techniques of affinity chromatography, long applied to the purification of biochemical materials [5], has permitted chromatographic separation of macrocylic hosts based on their predesigned affinity for complexation of primary ammonium groups, as reported herein.

2. Experimental

Compound **1**, as a mixture of diastereomers **1a** and **1b**, was prepared as described. Methylene chloride was refluxed with and twice distilled from $CaCl_2$. Other materials and reagents were used as obtained from commercial sources.

2.1. GEL PERMEATION CHROMATOGRAPHY OF 1

A 1.5 g sample of crude **1** was dissolved in 24 mL CH_2Cl_2, filtered, and chromatographed in eight equal fractions through a 24 ft × 0.25″ Styragel 100-A column,



1(Ar = 3,5-$(CH_3)_2C_6H_3$)

eluting with CH_2Cl_2 at a flow rate of 3 mL min^{-1} and a back pressure of 400–600 psi, monitoring with a refractive index detector. Two bands of unidentified oligomeric materials eluted first, followed by diastereomers **1a** and **1b**, which co-eluted. Evaporation of solvent afforded ca. 1.1 g of the mixed diastereomers.

2.2. REVERSED PHASE CHROMATOGRAPHY OF 1

The mixture of **1a** and **1b** was chromatographed in eight equal portions on a Waters HPLC (254 nm UV detector), using a 20 mm × 250 mm bonded C-18 reversed phase silica column (Whatman) and eluting with 75% CH_3OH/25% H_2O/1% NaBr at a flow rate of 2 mL min^{-1}. Under these conditions, **1a** eluted first (retention time ca. 10 min), with **1b** appearing next (retention time ca. 15 min) and several impurities emerging last. (On a 4.6 mm × 250 mm analytical column under otherwise identical conditions, elution times were ca. 3 min and 4.5 min for **1a** and **1b**, respectively.) Evaporation of solvent afforded the separated diastereomers as their NaBr complexes, which were decomplexed by washing their solutions in CH_2Cl_2 four times with deionized H_2O. Free hosts **1a** (0.459 g) and **1b** (0.442 g) were thereby obtained in crystalline, analytically pure form. Anal. Calcd. for $C_{43}H_{46}N_6O_5$: C, 71.05; H, 6.38; N, 11.56. Found (**1a**): C, 71.24; H, 6.49; N, 11.53. Found (**1b**): C, 71.17; H, 6.34; N, 11.51.

2.3. AFFINITY CHROMATOGRAPHY OF 1

A Waters HPLC equipped with a 4.6 mm × 250 mm aminopropyl derivatized silica column (Phenomenex, Spherisorb 5μ NH2) and a 254 nm UV detector was utilized. The column was eluted with methanol containing 1% NH_4Br at a flow rate of 2 mL min^{-1}. Samples of the isolated diastereomers from above and of the mixture of diastereomers after gel permeation were examined. Diastereomer **1b** appeared first (retention time ca. 3 minutes), followed by **1a** (ca. 4 minutes) and then unidentified impurities.

3. Results

Macrocycle **1** was designed and synthesized as an efficient complexation host for alkali metal and alkylammonium ions [6]. The final macrocyclization step in the synthesis proceeded in good yield, affording **1** contaminated with 15–20%

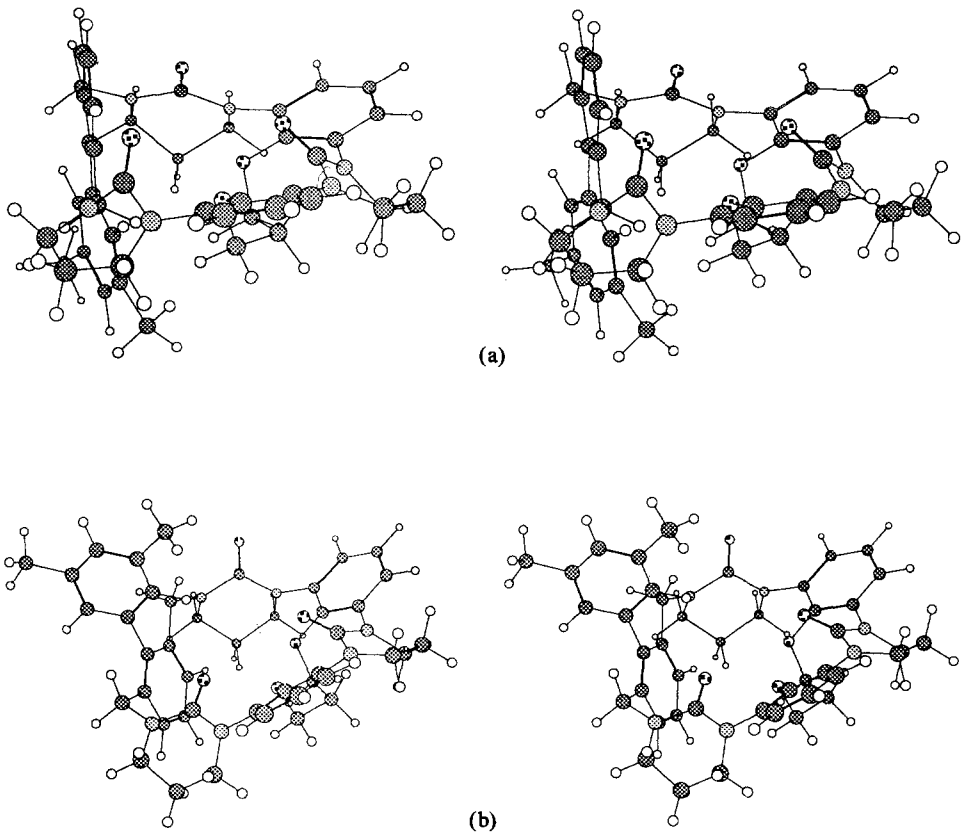


Fig. 1. Stereoscopic views (crossed eye) of the two diastereomeric forms of complexation host **1**. The dimethylbiphenyl group adopts a conformation roughly perpendicular to the plane of the macrocycle, thereby presenting the dimethylphenyl group *over* the urea carbonyl binding site in **1b**, but on the *opposite* face of the macrocycle in **1a**. Structures were generated by the 'Chem-3D' program. (Dark gray spheres = carbon, light gray = nitrogen, white = hydrogen, dotted = oxygen.) For results of X-ray diffraction analysis, see Reference 7.

oligomeric impurities. Gel permeation chromatography afforded a partial separation of **1** from these impurities, though even a lengthy column (ca. 24 ft) did not provide for complete separation. In addition, **1** exists as a pair of non-interconvertible diastereomers (atropisomers) (**1a** and **1b**, Figure 1), differing in the orientation of the biphenyl unit with respect to the urea carbonyls, yet these two diastereomers co-eluted from the gel permeation column. Reversed phase high performance liquid chromatography (HPLC) on a C-18 derivatized silica column provided a clean

separation of the diastereomers [7], with **1a**, anticipated on the basis of molecular models to be more hydrophilic (with the hydrophobic substituted phenyl group and all the $-(CH_2)_3-$ groups clustered on the same face of the molecule, leaving the carbonyl face exposed to solvent), eluting first, followed by the less hydrophilic **1b** (in which the hydrophobic groups shield both faces of the molecule from solvent). Oligomeric impurities were eluted with much longer retention times than either diastereomer.

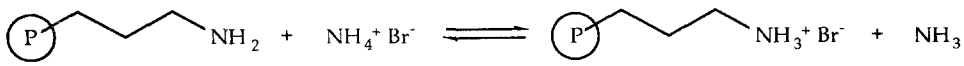
This reversed phase HPLC separation/purification clearly represents an improvement over simple gel permeation chromatography, though it is still based on a fairly nonspecific property (i.e., polarity). Since compound **1** was designed to complex alkylammonium ions, and since the two diastereomers of **1** exhibited markedly different association constants for such ions, chromatographic separation based on specific interaction with polymer-bound ammonium groups was explored. Elution of the mixed diastereomers through a commercially available aminopropyl-derivatized silica column with methanol containing 1% ammonium bromide (serving both to generate the requisite covalently linked ammonium groups from the column support and as a competitive ligand for the hosts) gave baseline separation of the two components. As anticipated, the *less* polar isomer, **1b**, (which proved to be the weaker binder of alkylammonium ions) was eluted first from this column, whereas the more polar compound, **1a**, was eluted first from the simple C-18 reversed phase column.

4. Discussion

Affinity chromatography has long been used for the purification of enzymes through their specific interaction with substrates covalently linked to chromatographic supports [5]. Molecules capable of complexing the covalently linked substrate are selectively adsorbed on the support; non-binding impurities are eluted. Elution with a solution of the free (i.e., not covalently linked to the support) substrate then removes the purified enzyme. Diastereomers **1a** and **1b** were separated under precisely the same type of conditions, in that they were eluted through a column bearing covalently attached substrates ('guests') in the form of alkylammonium ions, with their elution facilitated by the presence of free ammonium ions in the eluting solvent. Thus, it seems appropriate to describe this separation technique as an application of affinity chromatography.

The predicted and observed reversal of elution order presumably arises not only from the fact that **1a** displays higher $-\Delta G^0$ values for complexation of alkylammonium ions than does **1b**, but also from the fact that the binding site of **1b** is sterically blocked relative to the quite open site of **1a.** The chromatography support used has only a three-carbon tether between the amino group and the silica; it is possible that this amino group simply cannot reach the binding site of **1b** at all. Longer tethers might be expected to moderate the separation by allowing **1b** some additional measure of complexation. Thus, the length of the tether could serve as a fine-tuning mechanism for optimization of separations.

Quantitative analysis of the relative affinities of the two diastereomers for the bonded amino (or ammonium) groups of the support is complicated in these studies by use of ammonium bromide as the proton source; depending on the position (and



establishment) of the following equilibrium, one expects greater or lesser interaction with the support. A qualitative application of Chaiken's analysis of binding affinities as determined by affinity chromatography [8] suggests an association constant of **1a** for the support of only *ca.* $10\ M^{-1}$, whereas **1a** displays a K_a of *ca.* 10^{10} for methylammonium ion in water-saturated chloroform. This difference is ascribable to a combination of factors, including steric inhibition of complexation to the supported amino groups, solvent polarity effects, and the relatively small number of protonated amino groups present on the support under the conditions of the experiment. (A K_a of 10 is calculated assuming full protonation of the support; correction for the small number of groups actually protonated increases this number toward the observed homogeneous solution K_a.) We are currently investigating a more quantitative analysis of macrocycle-support interactions and use of the Chaiken analysis to determine complexation agent binding affinities chromatographically.

Use of affinity chromatography to separate complexation agent diastereomers based on their differential chemical properties rather than simple physical properties provided clean, rapid, and predictable separation. As a great many host compounds do complex ammonium ions, use of amino-derivatized chromatography supports should prove of quite general applicability. A number of packings are commercially available, differing with regards to length of the tether connecting the amino group to the silica, pH sensitivity, etc., so chances are good that a suitable support will be available.

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