Solvent Effects on the Screening of Parallel Combinatorial Libraries for Selectors for Chiral Chromatography

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Abstract

The correlation between the resin-swelling property and the outcome of the crucial equilibration assay used in our parallel library screening method is investigated. It is found that the incorporation of $CHCl_3$ (an effective swelling solvent for both the polystyrene and TentaGel resins) into the equilibration solvent leads to faster equilibration and thus shorter library screening time. The outcome of the equilibration experiment is also found to depend on the chemical nature of the solid base resins. It appears that polystyrene resin, which has a relatively inert surface, provides higher enantioselectivity than the polar TentaGel resin. The importance of a thoroughly swelled resin for the direct assay of functional groups on the resin is demonstrated.

Introduction

In recent years, the use of combinatorial techniques has evolved considerably for the development of selective binders for a given target molecule (1–3). In these techniques, a large number of compounds (the library) can be screened for a desired property. In a short period of time, combinatorial methods have found applications in pharmaceutical, material sciences, and more recently in separation science (4).

There are two major classes of combinatorial methods: one based on the synthesis of mixtures and the other on the parallel synthesis of pure library components (also called the high-throughput approach) (5–6). In the former approach, a mixture of library components is synthesized and screened for the desired property. In a parallel library, individual library components are synthesized and screened (one-by-one approach). The parallel library approach is similar to a traditional chemistry method in which a compound in its pure form is synthesized and evaluated, except that a large number of compounds are designed to be studied quickly.

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Several research groups (including ours) have investigated the application of both classes of combinatorial libraries to the development of chiral selectors (examples can be found in the works cited in reference 7). One of our screening methods, the development of chiral selectors from parallel combinatorial libraries (8,9), is rather straightforward. In this procedure, the parallel library is prepared by synthesizing all potential chiral selectors (library members) onto a solid-phase polymeric synthesis resin individually. In order to determine which potential chiral selector (library member) is capable of discriminating between the two enantiomers, the racemic analyte of interest in the proper solvent is allowed to equilibrate with each potential selector on the resin. The enantiomeric ratio of the analyte in the supernatant is then analyzed by circular dichroism after the equilibration period. A change in this enantiomeric ratio after equilibration implies a selective adsorption of one of the two enantiomers to the resin and thus the presence of a chiral selector. This library member on the resin could then be washed and recovered for future experiments.

The crucial step in this parallel library method is the equilibration assay of the racemic analyte with the potential chiral selector on the resin. During preliminary studies, it was observed that both the equilibration time and resulting enantiomeric excess depend on the equilibration solvents used. Because of the importance of this equilibration assay step, we recently performed an in-depth investigation of the solvent dependence of this resin equilibration experiment. Our results demonstrated a strong correlation of the swelling of the resin with the kinetic and outcome of the resin equilibration experiment, which will be presented and discussed.

Experimental

General supplies and equipment

Solid-phase synthesis resins and amino acid derivatives were purchased from NovaBiochem (San Diego, CA). All other chemicals and solvents were purchased from either Aldrich (Milwaukee,

WI), Fluka (Ronkonkoma, NY), or Fisher Scientific (Pittsburgh, PA). Circular dichroism was measured with a JASCO J-720 spectropolarimeter (0.40-mL cell volume, 1-mm cell pass-length), and the UV spectra were obtained with a Shimadzu (Kyoto, Japan) UV 201 spectrometer (3-mL cell volume, 10-mm cell pass-length).

Preparation of 3,5-dinitrobenzoyl-L-leucyl-4-aminobutyric acid and 3,5-dinitrobenzoyl-L-alanyl-4-aminobutyric acid-aminomethylpolystyrene resins

3,5-Dinitrobenzoyl (Dnb)-L-leucyl (Leu)-4-aminobutyric acid (Abu)-aminomethylpolystyrene (AmPS) and Dnb-L-alanyl (Ala)-Abu-AmPS resins were prepared following procedures previously reported (8). Surface coverage of the AmPS resin used to prepare these chiral selectors was reported by the manufacturer as 0.41 mmol/g.

Preparation of Dnb-L-Leu-Abu and Dnb-L-Ala-Abu-amino-TentaGel resins

Dnb-L-Leu-Abu-amino-TentaGel (AmTG) and Dnb-L-Ala-Abu-AmTG resins were prepared following procedures previously reported (8). Surface coverage of the TentaGel resin used to prepare these chiral selectors was reported by the manufacturer as 0.29 mmol/g.

Determination of solvent effects on the swelling of AmPS and AmTG resins

The swelling volume of Dnb-L-Leu-Abu-AmPS and Dnb-L-Leu-Abu-AmTG resins was determined as follows (10): 500 mg of dry resin was added to a graduated 5-mL glass luer-lock syringe equipped with an inline polypropylene filter and crimped needle. The dry volume of the resin was recorded. One of four solvents (20% isopropanol (IPA)–hexanes (Hex), 20% $CHCl_3$–Hex, 100% Hex, or 100% $CHCl_3$) was added to facilitate complete swelling (5 mL). The plunger was inserted and wrapped with Teflon tape. The system was equilibrated for periods ranging from 1 to 24 h. Upon equilibration, excess solvent was removed via a second luer-lock syringe, and the volume of the swollen resin beads was recorded.

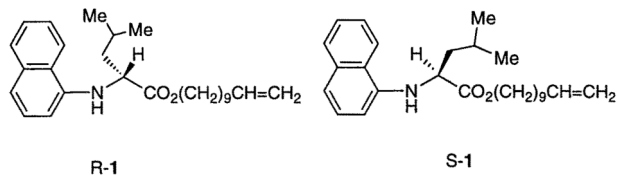

Figure 1. The R- and S-enantiomers of racemic *N*-(1-naphthyl)-Leu ester 1.

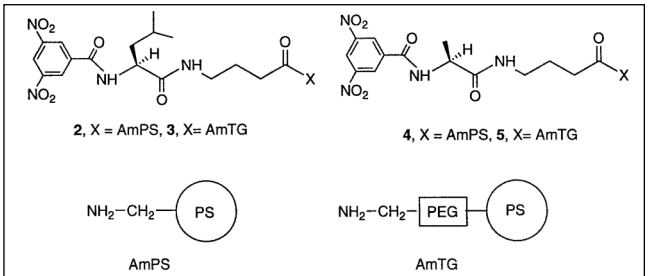

Figure 2. Structures of (2) Dnb-L-Leu-Abu-AmPS, (3) Dnb-L-Leu-Abu-AmTG, (4) Dnb-L-Ala-Abu-AmPS, and (5) Dnb-L-Ala-Abu-AmTG resins.

Screening of AmPS- and AmTG-resin-bound selectors with circular dichroism

Approximately 8 equivalents (50 mg for AmPS and 100 mg for AmTG) of each selector-bound resin was transferred to a 2-mL capacity centrifugal filter device (Millipore, Bedford, MA). The porous filter was blocked with Teflon tape to prevent solvent leaching during equilibration. To this system was introduced 1 equivalent (0.0029 mmol) of racemic *N*-(1-naphthyl)-Leu ester 1 in 1.00 mL of solvent. Solvent composition varied as follows: 100% $CHCl_3$, 100% Hex, 20% $CHCl_3$–Hex, and 20% IPA–Hex. Equilibration times varied between 15 min and 48 h. The Teflon tape was then removed and the supernatant recovered by centrifugation. With a syringe, 0.40 mL was transferred to a vial, the solvent removed by a nitrogen stream, and the sample reconstituted in 0.40 mL of ethanol. The sample was transferred to a sample cell (0.30-mL volume) of the JASCO J-720 CD spectropolarimeter, and the ellipticity at 260 nm was recorded.

Results and Discussion

Solvent dependence of this resin equilibration experiment was investigated using *N*-(1-naphthyl)-Leu ester 1 as the racemic analyte (Figure 1). This compound has been used in our previous studies, and its enantiomeric purity could be determined readily by circular dichroism (8). Two previously identified chiral selectors for this target analyte (Dnb-Leu and Dnb-Ala) were attached

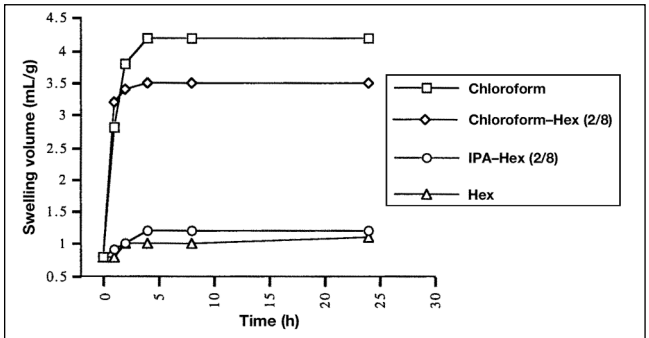

Figure 3. Swelling volume of Dnb-L-Leu-Abu-AmPS in four different solvent systems.

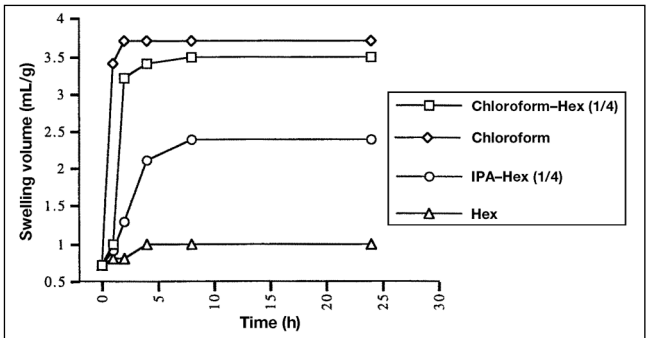

Figure 4. Swelling volume of Dnb-L-Leu-Abu-AmTG in four different solvent systems.

to both the Merrifield and TentaGel resins through an Abu linker (Figure 2). The preparation of these selector-modified resins have been reported (8). A solvent-swelling study was investigated with the two resins modified with Dnb-L-Leu, and the resin equilibration experiment was studied with all of these four resins.

The Merrifield resin consists of lightly crosslinked polystyrene, and TentaGel resin is made by crafting a layer of polyethylene glycol (PEG) onto a polystyrene core resin. Consequently, the surface of the Merrifield resin is hydrophobic and the surface of the TentaGel resin is hydrophilic.

Swelling of the resins in various solvents

Figure 3 illustrates the swelling data for Dnb-L-Leu-Abu-AmPS resin. For this resin, significant swelling was observed in both 100% $CHCl_3$ and 20% $CHCl_3$ in Hex. In both cases, saturation volume was reached in approximately 4 h. With 20% IPA in Hex, a noticeable amount of resin swelling was observed. In pure Hex, resin swelling was minimal. A comparison of these solvent-swelling behaviors pointed to the exceptional property of chloroform in swelling this polystyrene-based resin.

With Dnb-L-Leu-Abu-AmTG resin, significant swelling was achieved once again in both $CHCl_3$ and 20% $CHCl_3$ in Hex (Figure 4). In pure Hex, swelling was insignificant, as in the previous case. However, the swelling volume was significantly enhanced in 20% IPA in Hex, as compared with the polystyrene-based resin. The excellent swelling of this TentaGel-based resin in $CHCl_3$ and 20% $CHCl_3$ in Hex was not unexpected, because $CHCl_3$ proved to be an excellent solvent to swell the polystyrene resin, which was the core of the TentaGel resin. The improved swelling behavior of this TentaGel resin in 20% IPA in Hex could result from the hydrophilic character of the PEG outerlayer of the TentaGel resin.

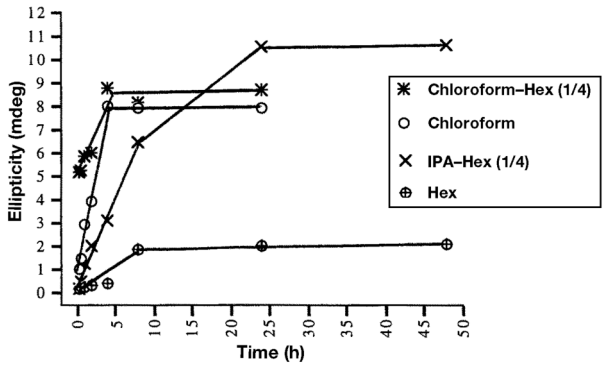

Figure 5. Equilibration of racemic analyte 1 with Dnb-L-Leu-Abu-AmPS in four different solvent systems.

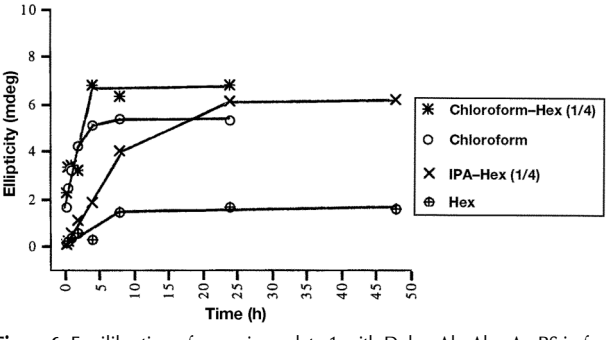

Figure 6. Equilibration of racemic analyte 1 with Dnb-L-Ala-Abu-AmPS in four different solvent systems.

Solvent dependence observed in the resin equilibration experiment

The solvent dependence of the resin equilibration experiment was followed by the ellipticity of the supernatant. As mentioned previously, the molar ellipticity of analyte 1 is quite high; therefore, the ellipticity measurement should be a sensitive method in order to monitor the enantiomeric ratio of analyte 1. Generally speaking, the higher the ellipticity is, then the higher the enantioselectivity will be.

For Dnb-Leu on AmPS resin 2, equilibration could be reached within 4 h with the chloroform-containing solvents ($CHCl_3$ or $CHCl_3$–Hex) (Figure 5). The final ellipticity reached in these two cases was quite high. In 20% IPA in Hex, a longer time (24 h) was required to reach equilibration. The final ellipticity was comparable with those achieved in chloroform-containing solvents. In Hex, the final ellipticity achieved was rather low.

These equilibration behaviors correlated very well with resin-swelling experiments. In chloroform-containing solvents, thorough resin swelling was achieved within 4 h. Therefore, the racemic analyte could diffuse into the resin and interact thor-

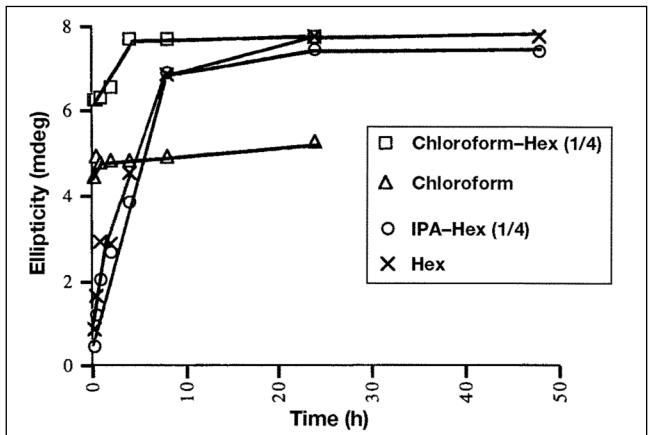

Figure 7. Equilibration of racemic analyte 1 with Dnb-L-Leu-Abu-TentaGel in four different solvent systems.

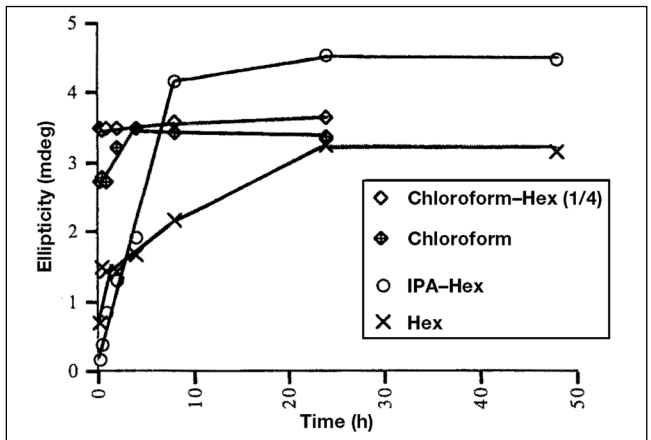

Figure 8. Equilibration of racemic analyte 1 with Dnb-L-Ala-Abu-TentaGel in four different solvent systems.

oughly with the chiral selectors attached to the resin. With 20% IPA in Hex (in which only modest resin swelling was observed) slower diffusion of the analyte into the resin was expected, which could be directly responsible for the longer equilibration time. In Hex (in which the resin barely swelled), some of the chiral selectors on the resin may not be available to interact with the analyte.

With Dnb-L-Ala-Abu-AmPS resin, almost identical behavior was observed (Figure 6), which indicates that the degree of resin swelling (not the chemical nature of the chiral selectors) dictates the kinetics of the resin equilibration experiment.

Generally speaking, when the selectors are bound to AmTG resin, screening with the same racemic analyte produces faster equilibration times but lower maximum response (when compared with the same experiments on AmPS resins). Again, when the equilibration system contained chloroform, faster equilibration was observed (Figures 7 and 8). With 20% IPA in Hex, a longer equilibration time was once again required. However, unlike the previous experiment using the AmPS resins, a respectable ellipiticity value was observed in Hex.

The relative equilibration kinetics on these AmTG resins could again be correlated with the swelling behaviors of these resins in various solvents, with faster kinetics in better swelled resins. The detailed kinetics, however, seems to be more complicated than those observed on the AmPS resins. One source of complication could come from its lower surface ligand concentration, which is approximately 60% of the corresponding AmPS resins. The lower surface ligand concentration could make the selectors on the surface more accessible to interact with the racemic analyte, thus leading to overall faster kinetics. Lower surface coverage could also make more resin surface available to interact with the racemic analyte nonselectively, which could lead to lower enantioselectivity. The relatively high ellipiticity achieved in Hex is puzzling, which defies a simple explanation at this moment.

Conclusion

We have demonstrated a close correlation between the resin-swelling property and the outcome of the crucial equilibration assay used in our parallel library screening method. Generally speaking, the incorporation of $CHCl_3$ (an effective swelling solvent for both the polystyrene and TentaGel resins) into the equilibration solvent leads to faster equilibration and thus shorter library screening time. The outcome of the equilibration experiment also depends on the chemical nature of the solid base resins. It appears that PS resin, which has a relatively inert surface, provides higher enantioselectivity than the polar TentaGel resin under the equilibration condition. These results provide guidance in the choice of suitable equilibration solvents for the selection of chiral selectors from parallel combinatorial libraries. The importance of a thoroughly swelled resin for the direct assay of functional groups on the resin is apparent from this study.

Acknowledgments

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