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Generation of Novel DNA-Binding Compounds by Selection and Amplification from Self-Assembled Combinatorial Libraries

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Abstract: We describe a general method for the selection of compounds from self-assembled libraries which employs an immobilized receptor (i.e., an affinity reagent) to effect the selection. Using commercially available oligo d(A•T) DNA-cellulose resin, a set of three setereoisomeric coordination complexes are identified as DNA binding compounds from an equilibrating, self-assembled library of 36 bis(salicylaldiminato)-zinc coordination complexes. © 1997 Elsevier Science Ltd.

The *in vitro* selection and amplification of oligonucleotides has proven to be an extraordinarily successful method for the generation of biopolymers with specific receptor-binding or catalytic properties.[1] The development of analogous selection and amplification methods for small molecules, where the selection and amplification criteria are based strictly on differences in binding affinity to a receptor, would be of tremendous utility for ligand generation. While this area remains largely unexplored, a few reports of kinetic[2] or thermodynamic[3,4] selection of nonbiopolymeric materials from mixtures of compounds[5,6] have recently appeared in the literature. We describe herein the first utilization of an immobilized biopolymeric receptor (DNA) to drive the selection and enrichment of the highest affinity coordination complex from a self-assembled, equilibrating combinatorial library of coordination complexes. The experimental design is shown in schematic form in Figure 1. Introduction of an excess of a transition metal salt ("M") to a pool of compounds ("monomers") capable of forming coordination complexes with M should initiate a series of equilibria among various combinations of complexes. Assuming ligand exchange is reasonably fast, this equilibration will permit all combinatorially possible coordination complexes to be populated. By adding a receptor covalently linked to a solid support (an affinity resin), however, an additional equilibration would ensue involving the binding of these coordination compounds to the receptor with varying degrees of affinity. Since some compounds might be expected to bind to the receptor with higher affinity than others, those compounds would be depleted from the pool of equilibrating complexes. By simple mass balance rules, the equilibrium would then have to shift in favor of compounds that bind to the receptor, effectively utilizing the receptor to direct the synthesis of its own ligand. Although the amount of *monomer* does not change, this also may be viewed as an amplification of a particular *complex* relative to the amount of that complex present under receptor-free conditions.

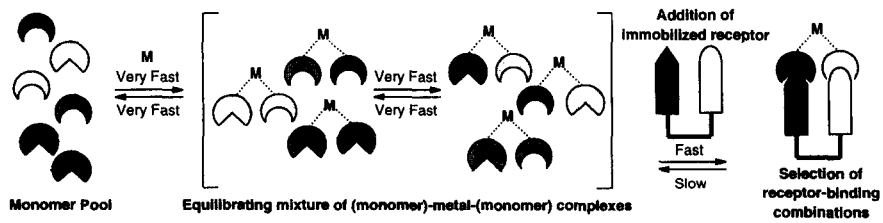

Figure 1

Double-stranded DNA seemed to be a particularly attractive target for initial tests of this concept, for several reasons. First, the design and synthesis of sequence-selective DNA-binding agents is a problem of continuing fundamental and medical importance.[7] Second, the ladder-like structure of DNA is ideally suited to the application of a modular, self-assembly strategy for ligand synthesis. Furthermore, the ability of DNA to

selectively bind single enantiomers from non-equilibrating, racemic solutions of transition metal complexes has been elegantly demonstrated by Barton and coworkers,[8] suggesting that selection from an equilibrating mixture of complexes might also be feasible. Finally, several oligonucleotide affinity resins are available commercially, allowing the experimental details of initial selection strategies to be determined without the need for costly oligonucleotide and affinity resin synthesis.

As "monomers", the readily available salicylaldimines **1** were chosen. Salicylaldimines are well known to form coordination complexes with a wide variety of transition metals,[9] and a substantial amount of structural information about these complexes is available. Because of its tetrahedral coordination geometry with most salicylaldimines[10] and compatibility with nucleic acids, divalent zinc was used as the metal for initial studies. Six salicylaldimines (**3** - **8**) displaying a variety of sidechain functionality were synthesized by the condensation of salicylaldehyde and commercially available amines (Figure 2).[11] Given that compounds **4** and **6** were synthesized as racemates, a combinatorial library formed from these six salicylaldimines would provide a maximum of 36 unique bis(salicylaldiminato)zinc complexes.

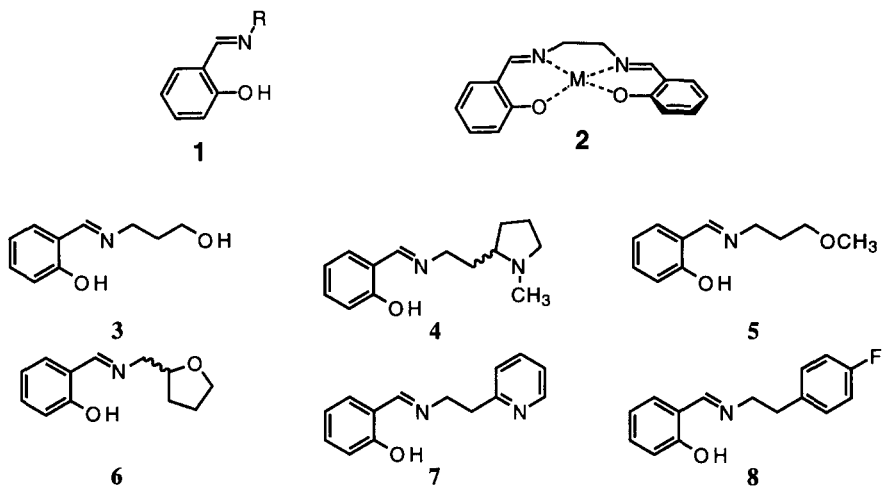

Figure 2: Salicylaldimines Synthesized for a Trial Self-Assembled Library

Although the ability of bis(salicylaldiminato)-zinc[12] and -nickel[13] complexes to self-assemble in halogenated solvents is well known, we felt it was important to verify that this could also occur in aqueous solution before attempting to construct a combinatorial library. Therefore, the dependence of the proton NMR spectrum for **5** on $ZnCl_2$ concentration in D_2O was examined. Characteristic of slow-exchange phenomena, substantial line broadening of resonances is observable on addition of substoichiometric quantities of $ZnCl_2$. In addition, chemical shifts change markedly. As might be expected, some imine hydrolysis is also observed in the presence or absence of zinc; however, imino protons are still observable even after 24 hours in aqueous solution.[14] These observations indicate that complexation occurs between **5** and Zn^{2+}, although they do not provide high-resolution structural information.[15]

With a series of monomers and verification of self-assembly in aqueous solution in hand, the experimental tools were in place to conduct an affinity selection (and amplification) experiment. A standard solution of 0.5 mM **3** - **8** (total concentration 3 mM in "monomer") was prepared in 10 mM Tris•HCl, 100 mM KCl, 1% DMSO, pH 7.5. To determine the effect of zinc on the observed selection, samples were prepared, in triplicate, either with or without 26 mM $ZnCl_2$. Samples were allowed to incubate for one hour to allow an equilibrium mixture of complexes to form, and the latter were then individually added to affinity columns

prepared from 30 mg of poly(dT)-cellulose resin preincubated with one A_{260} unit (approximately 7 nmol) of oligo $(dA)_{12\text{-}18}$[16] in 10 mM Tris•HCl, 100 mM KCl, pH 7.5 for formation of double-stranded DNA (giving an approximate complex:base pair ratio of 85:1). Following a two-hour incubation of the libraries on the resin, solutions were eluted and lyophilized. Analysis of these initial elutions was used to provide information about what components of the library did *not* bind to the DNA or the underlying affinity matrix. By comparing relative amounts of compounds retained on the resin (i.e., depleted from the mixture) in the presence and absence of zinc, this allowed for determination of which complexes remained bound. Analysis of the mixtures was carried out by hydrolysis of the complexes with trifluoroacetic acid, followed by derivatization of the amines with excess 2-naphthoyl chloride (to allow for UV detection) and separation by reverse-phase HPLC.

Figure 3 shows the results of affinity selection for our initial self-assembled combinatorial library. With data from triplicate experiments averaged and normalized to amounts observed in the absence of zinc, it is clear that monomers **4** and **6** are most strongly retained on the affinity column in the presence of zinc (i.e., less of these monomers is observed following elution and derivatization). These results would then require deconvolution of three possibilities for the strongest binding complex: **4-Zn-4**, **6-Zn-6**, and **4-Zn-6** (including all stereoisomers). However, results of control experiments rendered this question moot. The observation that twice as much of derivatized monomer **7** is eluted from the affinity column in the presence of zinc as in its absence suggests that this free monomer participates in some kind of binding interaction with double-stranded poly d(A•T) that is not available to either the **7-Zn-7** complex, or other complexes incorporating monomer **7** (possibly including Zn^{2+} coordination to the pyridine side chain).

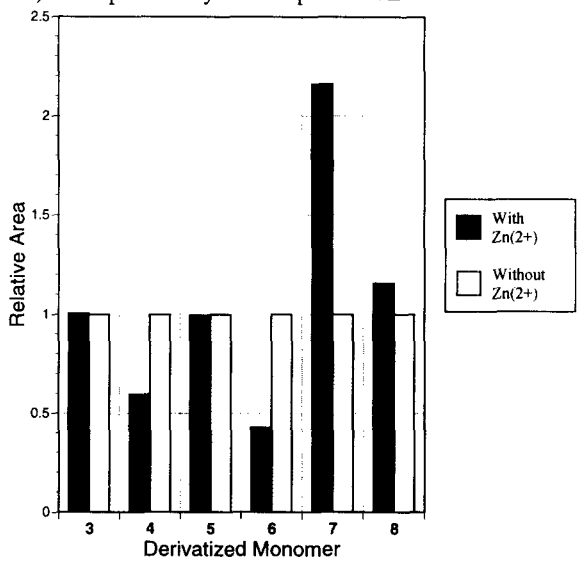

Figure 3

To ensure that selections observed were based on differential affinity for double-stranded DNA, rather than interactions with the underlying cellulose support, control experiments were conducted using cellulose alone under conditions identical to those for the double-stranded experiment. Monomer **4** was found to be retained on cellulose to the same extent in the presence or absence of zinc. In contrast, **6** was more strongly retained on cellulose in the presence of zinc, indicating that the retention of complexes formed from **6** on the DNA affinity resin was probably due to cellulose-binding rather than to DNA-binding. This suggests that the mixture of stereoisomeric complexes **4-Zn-4** is likely the subset of the library with the highest affinity for DNA.

In order to verify that the results of the affinity selection experiment accurately reflected differences in binding affinities, UV binding titrations[17] were conducted for complexes **5-Zn-5** (a presumed weak- or non-binding complex based on library results) and **4-Zn-4**. Using commercially available poly d(A•T), saturable binding was observable in each case. While any analysis of binding constants for these complexes is complicated by the homopolymeric, variable-length nature of the DNA used (providing multiple, identical binding sites), **4-Zn-4** bound to DNA with an apparent K_D of 1.1 micromolar, significantly stronger than **5-Zn-5** (27 micromolar). We also measured the binding constant for a mixture of **4** and **6** (providing **4-Zn-6** as well as homodimers); as expected from the control studies, this mixture had very weak affinity (approximately 100 micromolar) for DNA. Concerns about potential complications arising from imine hydrolysis led us to also attempt to measure binding constants for salicylaldehyde and 2-(2-aminoethyl)-1-methylpyrrolidine; however,

we saw no saturable binding between either of these compounds and poly d(A•T) in the presence or absence of zinc. A simple affinity selection experiment conducted as described above, but utilizing only monomers **4** and **5** (forming a 6-compound "library"), further corroborates these observations; the ratio of derivatized **5**:**4** eluted following incubation on poly d(A•T)-cellulose doubles on addition of zinc.

In conclusion, we have demonstrated the first example of the selection of DNA-binding compounds from a self-assembled, equilibrating combinatorial library of coordination complexes. While the compounds examined in this initial study, as well as the DNA sequence used for the selection, were relatively simple molecules, this method should be generally applicable to the synthesis of compounds capable of binding sequence-selectively to DNA, RNA, and proteins, since the receptor itself determines the final composition of the bound compound. Experiments designed to test this hypothesis, as well as structural characterization of the DNA-bound state of **4-Zn-4**, are in progress.

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References

1. (a) Szostak, J. W. *Trends Biochem. Sci.* **1992**, *17*, 89-93; (b) Tuerk, C.; Gold, L. *Science* **1990**, *249*, 505-510.
2. Amabilino, D. B.; Ashton, P. R.; Perez-Garcia, L; Stoddart, J. F. *Angew. Chem.* **1995**, *107*, 2569-2572; *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 2378-2380.
3. Hasenknopf, B.; Lehn, J.-M.; Kneisel, B. O.; Baum, G.; Fenske, D. *Agew. Chem.* **1996**, *108*, 1987-1990; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1838-1840.
4. Whitesides, G. M.; Simanek, E. E.; Mathias, J. P.; Seto, C. T.; Chin, D. N.; Mammen, M.; Gordon, D. M. *Acc. Chem. Res.* **1995**, *28*, 37-44.
5. A recent report has described a method for the selection of compounds capable of binding to guanidinium via a photoinduced isomerization (Eliseev, A. V.; Nelen, M. I. *J. Am. Chem. Soc.* **1997**,*119*, 1147-1148); however, selection is driven by an external energy source (light) in addition to differences in binding affinity.
6. Huc and Lehn have provided an elegant demonstration of changes in product ratios obtained in imination reactions carried out on mixtures of amines and aldehydes in the presence of carbonic anhydrase: Huc, I.; Lehn, J.-M. *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 2106-2110.
7. For example, see Trauger, J. W.; Baird, E. E.; Mrksich, M.; Dervan, P. B. *J. Am. Chem. Soc.* **1996**, *118*, 6160-6166 and references therein.
8. Mei, H.-Y.; Barton, J. K. *J. Am. Chem. Soc.* **1986**, *108*, 7414-7416.
9. Garnovskii, A. D.; Nivorozhkin, A. L.; Minkin, V. I. *Coord. Chem. Rev.* **1993**, *126*, 1-69.
10. Dreher, M.; Elias, H.; Paulus, H. *Z. Naturforsch. B.* **1987**, *42*, 707-712.
11. The synthesis and characterization of **3** and **7** have been previously described. Compound **3**: Inouye, S. *Chem. Pharm. Bull.* **1967**, *15*, 1540-1546; compound **7**: (a) Duran, M. L.; Romero, J.; Sousa, A. *Synth. React. Inorg. Met.-Org. Chem.* **1987**, *17*, 681-683. (b) Ramesh, K.; Mukherjee, R. *J. Chem. Soc. Dalton* **1992**, 83-86. All new compounds displayed satisfactory spectral data.
12. Blackborow, J. R.; Karunaratne, S.; Lockhart, J. C.; Hill, M. N. S. *J. Chem. Res.* **1978**, 119.
13. Schepartz, A.; McDevitt, J. P. *J. Am. Chem. Soc.* **1989**, *111*, 5976-5977.
14. We have also observed that the addition of $ZnCl_2$ to a solution of an amine and salicylaldehyde in water can induce imine formation followed by complexation (B.K. and B.L.M., unpublished data). This suggests that a three-stage equilibrium process could be constructed.
15. It is unlikely that the structure in solution is an addition complex (containing two salicylaldimines coordinated to $ZnCl_2$) rather than a chelate: see Bottino, F. A.; Finocchiaro, P.; Libertini. E.; Mattern, G. *Zeitschrift für Kristallographie* **1989**, *187*, 71-77.
16. Oligo $(dA)_{12\text{-}18}$ as obtained from Pharmacia Biotech is a mixture of homopolymeric, single-stranded DNA of length 12 to 18.
17. Krugh, T. R. *Proc. Natl. Acad. Sci. USA* **1972**, *69*, 1911-1914.

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