Communications

Chemical Evolution: A Model System That Selects and Amplifies a Receptor for the Tripeptide (D)Pro(L)Val(D)Val

Hideaki Hioki and W. Clark Still*

Department of Chemistry, Columbia University, New York, New York 10027

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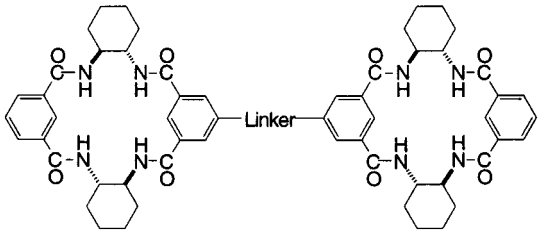
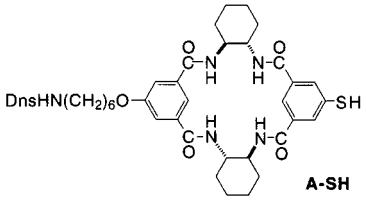
One of the most remarkable advances in recent years is the development of in vitro selection systems that evolve to enrich mixtures of chemical compounds in those components having selected properties. Indeed, mixtures of RNA aptamers can evolve to yield highly effective receptors or catalysts by an in vitro process involving selection and amplification.[1] Using such an approach more generally to effect evolution in simple chemical systems, however, is difficult because few other classes of molecules can be efficiently copied to effect the amplification step (which in the case of RNA aptamers is accomplished enzymatically using reverse transcription to DNA, PCR amplification, and retranscription to RNA).[2] In recent years, an alternative approach to chemical evolution has emerged in which a reversible equilibrium between different chemical compounds is set up and the desired compounds are somehow selected and removed from the equilibrating mixture.[3] In such an approach, enrichment in the selected compound (i.e. amplification) results from the equilibration process that carries out a preferential destruction and recycling of *unselected* compounds rather than from an explicit copying of *selected* compounds as in the aptamer system. Several simple systems have been described in recent years that employ such equilibrium shifting to effect chemical evolution.[4] Some of the first examples operated by equilibrating a mixture of stereoisomeric molecules in the presence of a cationic substrate (barium ion,[4a] methylguanidinium ion[4d]) to select and amplify those molecules that bind the substrate most tightly. Others equilibrated systems of imine-linked synthons to evolve DNA-binding DNA analogues[4b] and small molecule ligands for a protein.[4e,f] In this communication, we describe a simple study that shows how equilibrium shifting can be used to select and amplify a hostlike receptor for a complex substrate, here the tripeptide sequence (D)Pro(L)Val(D)Val. In this work, the receptor molecule is formed by a disulfide exchange reaction, and amplification is driven thermodynamically by binding of the selected receptor to the cognate tripeptidic substrate on a solid support.

The experiments we carried out involved first finding a disulfide-linked small molecule receptor (**X-SS-X**) that binds a particular peptide. The question we then asked was: given an equilibrating mixture of disulfides that includes **X-SS-X** (i.e. **X-SS-X**, **X-SS−Y**, **Y−SS−Y**, **Z-SS-Z**...), can the position of the equilibrium be significantly shifted to favor **X-SS-X** by introduction of the peptide it binds? Or more generally, can a system of equilibrating disulfides evolve in the presence of a given substate to significantly increase the amount of (i.e. to amplify) the particular disulfide that best binds the substrate?

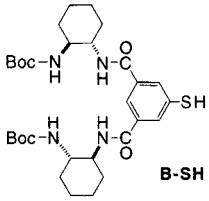
To find a suitable disulfide-linked receptor, we drew on previous work indicating that many molecules containing linked oligomers of isophthalic acids and *trans*-1,2-diamines (e.g. the two-armed receptor below) are highly sequence-selective receptors for peptides.[5,6]



Although most previous studies of such receptors employed 1,2-diamine linkers, considerable variation in the linker structure is tolerated by the receptors and we thought it likely that an analogous receptor employing a disulfide linker might also have the properties we sought. Thus we prepared the dansyl-labeled, fluorescent mercaptan **A-SH** and oxidized (I_2) it to the receptor-like disulfide **A-SS-A**. Using fluorescence microscopy to detect binding to an encoded combinatorial library of 3375 different *N*-acetyl tripeptides on polystyrene (PS) beads,[7] we found that **A-SS-A** does indeed selectively bind peptides. In particular, **A-SS-A** in $CHCl_3$ preferentially bound the following polystyrene-supported tripeptide sequences: Ac(D)Pro(L)Val(D)-Val-PS, Ac(L)Asn(L)Pro(D)Xxx-PS, and Ac(L)Pro(L)Pro(D)Xxx-PS. Though we were unable to accurately measure the binding constants for these peptides, the 8 μM equilibrium concentration of **A-SS-A** from which the beads were picked implies binding constants on the order of 10^4−10^5.



A-SH



B-SH

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(3) The approach has precedents in classical synthetic organic chemistry. For example, in his total synthesis of steroids, Woodward was able to set up an equilibrium between the cis and trans Diels−Alder adducts of 1,3-butadiene and 2-methoxy-5-methyl-*p*-quinone and drive that equilibrium to the less stable trans adduct in 90% yield by crystallization. Woodward, R. B.; Sondheimer, F.; Taub, D.; Heusler, K.; McLamore, W. M. *J. Am. Chem. Soc.* **1952**, *74*, 4223.

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(7) The library had 3375 (15^3) members having the structure Ac-AA1-AA2-AA3-polystyrene where AAn = Gly, (D and L) Ala, Ser, Val, Pro, Asn, Gln, Lys. See ref 5 for details.

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Table 1. Disproportionation of A-SS-Ph in the Presence of Ac(D)Pro(L)Val(D)Val-PS

	(2)A-SS-Ph ↔	Ph-SS-Ph +	A-SS-A	K_{eq}
Absence of tripeptide-PS:	35%	65%		3.8
Presence of tripeptide-PS:	5%	95%		360
Solution phase	5%	95%	5%	
Resin phase	0%	0%	90%	

Table 2. Disproportionation of A-ss-b in the Presence of Ac(D)Pro(L)Val(D)Val-PS

	(2)A-SS-B ↔	B-SS-B +	A-SS-A	K_{eq}
Absence of tripeptide-PS:	43%	57%		1.8
Presence of tripeptide-PS:	15%	85%		32
Solution phase	13%	85%	10%	
Resin phase	2%	0%	75%	

To test the ability of simple mixtures of disulfides to evolve in the presence of a peptidic substrate to enrich the mixture in the best substrate-binding disulfide, we prepared the unsymmetrical disulfide **A-SS−Ph** (from **A-SH** and excess PhSH) and one of the aforementioned tripeptides (Ac(D)Pro-(L)Val(D)Val-PS). When an initial concentration of 9 mM **A-SS−Ph** in $CHCl_3$ was stirred with 2 mol % PhSH/Et_3N for 24 h at 23 °C, the starting disulfide disproportionated to give an equilibrium mixture containing 35 mol % **A-SS−Ph** and 65 mol % **A-SS-A** + Ph-SS-Ph.[8] These results correspond to an equilibrium constant of 3.8. We then added a 3.7-fold excess of the polymer bead-supported tripeptide Ac(D)Pro(L)Val(D)Val-PS (50 mg resin/mL) and allowed the system to reequilibrate with wrist-action shaking for 36 h. The results are given in Table 1.

In the presence of cognate peptide, the equilibrium was indeed shifted to give significant enrichment (95 mol %) in the peptide-binding disulfide **A-SS-A** and a new equilibrium constant of 360. As in Eliseev's system,[4d] we were able to isolate the best-binding receptor conveniently from the substrate-carrying beads by washing them first with $CHCl_3$ to eliminate unbound material and then with DMF to extract any peptide-binding compounds. In this way we directly isolated **A-SS-A** in 99.5% purity. Thus we see that the binding to a peptide substrate can significantly shift a simple equilibrium of receptor-like molecules to enrich the mixture in the best substrate-binding receptor.[9]

To test the evolution of a system of disulfides including other peptide-binding molecules, we prepared the mixed disulfide **A-SS−B** from **A-SH** and **B−SH**. The peptide-binding properties of **A-SS−B** were evaluated using the solid-phase tripeptide binding assay described above for **A-SS-A**. This screen showed that 20 μM **A-SS−B** in $CHCl_3$ weakly bound various *N*-Ac-tripeptides, but we could not detect binding to **A-SS-A**'s preferred substrate Ac(D)Pro(L)-Val(D)Val-PS under these conditions. As summarized in Table 2, when 10 mM **A-SS−B** was equilibrated using 10 mol % **B−SH**/Et_3N in $CHCl_3$, the equilibrium constant for the system was 1.8.[8] Upon addition of 3 equiv of Ac(D)Pro-(L)Val(D)Val-PS, we again observed an equilibrium shift to favor the peptide-binding **A-SS-A** side of the equation (K_{eq} = 32). As before we could also isolated the best binding component (97.5% purity) conveniently by extraction of the peptide-carrying polymer beads. While we had hoped to shift the equilbrium in the other direction to favor **A-SS−B** using one of its preferred peptides, **A-SS−B**'s binding to the peptides in our library was too weak to permit such an experiment.

This work shows that effective molecular receptors can be formed, selected, and amplified in response to the presence of complex tripeptidic substrate. Though these model experiments employ shifting of a very simple equilibrium, they demonstrate that contemporary host−guest binding energies are sufficient to induce 10−100 fold changes in an equilibrium constant. This result suggests that significantly more complex systems may be amenable to chemical evolution by equilibrium shifting. Just how many components can be equilibrated and effectively selected in practice, however, remains to be seen. One constraint is that large pools of equilibrating compounds translate to low concentrations of individual components and necessitate even larger binding constants for the molecular complexes that are to drive the shift.

The process we describe here corresponds to a rare "molding" event in Lehn's terminology[4e] in which discrete chemical fragments are assembled to form a binding site for a given substrate. Given recent advances in the synthesis of complex, receptor-like molecules by reversible fragment assembly,[10] equilibrium shifting driven by complexation with a desired substrate has potential to become an important method for the preparation of substrate-selective small molecule receptors.

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Supporting Information Available: Experimental procedures and characterization of **A-SS−Ph** and **A-SS-A** (5 pages).

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(8) Similar equilibrium constants were obtained with control equilibrations in the presence of acetylated aminomethylpolystyrene (same quantity of resin used in the equilibrations in the presence of Ac(D)Pro(L)Val(D)Val-PS).

(9) **A-SS-Ph** does not bind Ac(D)Pro(L)Val(D)Val-PS detectably at a 10 μM concentration in $CHCl_3$, though we can detect weak binding at 10-fold higher concentrations.

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