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Template-Mediated Synthesis of a Polymeric Receptor Specific to Amino Acid Sequences**

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The idea of template-mediated assembly in biological systems was first introduced by Linus Pauling to explain the working of the immune system.[1] We now know this elegant hypothesis to be incorrect but the principle of using a target molecule to create its own recognition site was fruitfully exploited by chemists in the preparation of artificial polymeric receptors by molecular imprinting.[2] The resulting materials have been shown to display specificities similar to polyclonal antibodies[3, 4] and bind ligands with a single dissociation constant.[4, 5] However, it is the recognition of oligonucleotide, -saccharide, or -peptide sequences that is often seen as the real test of the ability of "plastic antibodies" to compete with their natural counterparts. Herein we will address the latter problem by describing the preparation of imprinted polymers specific for amino acid sequences.

Several considerations were taken into account when designing a suitable oligopeptide template[6] for the purpose of this study. Firstly, it was preferable to have a relatively short target molecule such that it could be synthesized in the required quantities by conventional solution-state chemistry. A relatively small target peptide was also preferred as a consequence of the necessity for chemical modification, recovery, and characterization of the template. Secondly, a highly functionalized oligopeptide would enable us to probe the contribution of individual interactions/functional groups by substitutions in the amino acid sequence. At the same time, a net charge of zero would be advantageous to eliminate possible artefacts that arise from nonspecific "ion-exchange type" interactions between the oligopeptide and receptor. Finally, the incorporation of an aromatic amino acid residue in the sequence should facilitate the analysis. Thus, a sequence containing lysine (Lys) and aspartic acid (Asp) in the terminal positions with a bulky tryptophan (Trp) residue in the middle was selected as satisfying all of the above requirements.

The peptide Lys-Trp-Asp (**1**) was preferred to Asp-Trp-Lys for purely synthetic reasons, and the target **1** was prepared in seven steps in 13% overall yield. The synthesis was accomplished by using conventional activated-ester coupling from suitably protected amino acid precursors. A portion of the tripeptide product was then further modified to prepare the template. Carboxylic acids were the natural choice of functionality to interact with amino groups of the lysine residue in the polymer's binding sites. Hence, the tripeptide **1** was treated with 2-methacryloyloxybenzoyl chloride[7] (**2**) to obtain the template **3**. The key feature of this approach is the presence of a relatively labile ester bond between the methacrylic acid residue and the hydroxybenzamide moiety, the cleavage of which would leave the carboxy group in the precise spatial arrangement to interact with the target tripeptide as well as a small "void" in the polymer to facilitate the template removal and re-binding of **1** (Scheme 1).

This simple approach also enabled us to position carboxy groups exclusively in the recognition sites, thus minimizing nonspecific interactions between ligands and the polymeric matrix itself.[8] The carboxylic acid groups of the template **3** were targeted by noncovalent complexation with 2-vinylpyridine[9] (**4**), as depicted in Scheme 1. In order to achieve a reasonable degree of complexation **4** was used in a fourfold molar excess over the template **3**, and the polymerization reaction was carried out in acetonitrile. The choice of solvent was influenced by template solubility and the necessity to stabilize hydrogen-bonding interactions that were essential for the successful imprinting of the tripeptide template. Not only was this important to promote the formation of the complex between the template **3** and monomers **4**, but also to restrict the conformation of the *o*-hydroxybenzamide spacer groups by intramolecular hydrogen bonds. The latter should help to position the methacrylic acid residue precisely in the polymer binding site.[10] Divinylbenzene was selected as the cross-linker to ensure that no degradation of the polymer matrix would occur under the hydrolytic conditions used for template removal. The polymers were prepared photochemically at 4 °C (see the experimental section). Nonimprinted polymer was synthesized under exactly the same conditions, the template being replaced by two equivalents of methacrylic acid, to ensure that the final chemical composition of the polymers were identical.

Once prepared, the polymers were tested with solutions of **1** in aqueous acetonitrile to evaluate their binding properties. Specific binding was seen at all ratios of aqueous/organic solvent (inset in Figure 1) but the binding of **1** to the polymer dropped significantly from 47% in acetonitrile/water 4:1 to 28% in pure water, where the tripeptide was noticeably more soluble. Binding studies were therefore made at 80% acetonitrile where high specific binding and a reasonable range of ligand concentration could be used. Isotherms were then determined for two polymer concentrations (15 and 90 mg mL^{-1}; Figure 1). It is evident from these data that the imprinted polymer shows considerable binding of the peptide, even at concentrations as low as 0.02 mM, while the nonimprinted and the unhydrolysed imprinted polymer (not

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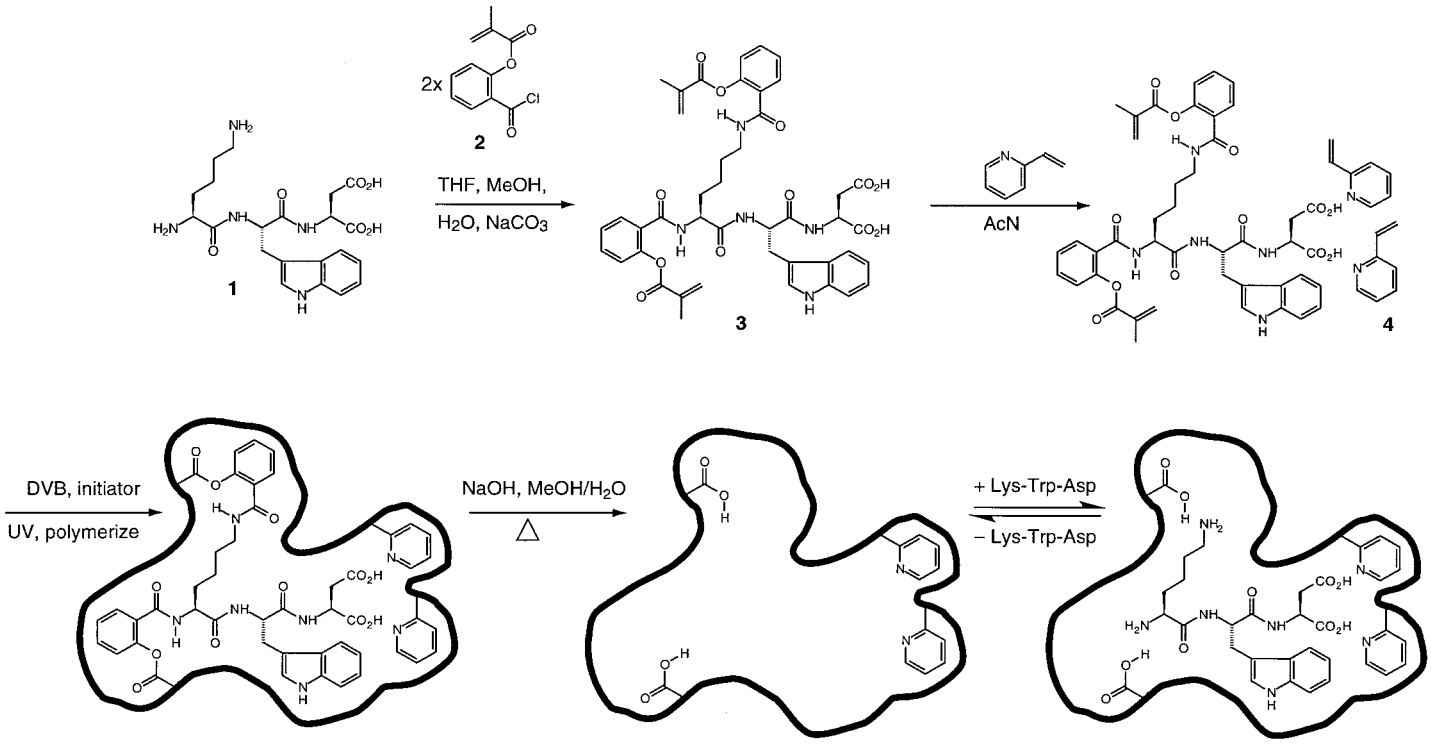

Scheme 1. Covalent modification of Lys-Trp-Asp with 2-methacryloyloxybenzoyl chloride and preparation of the imprinted polymer.

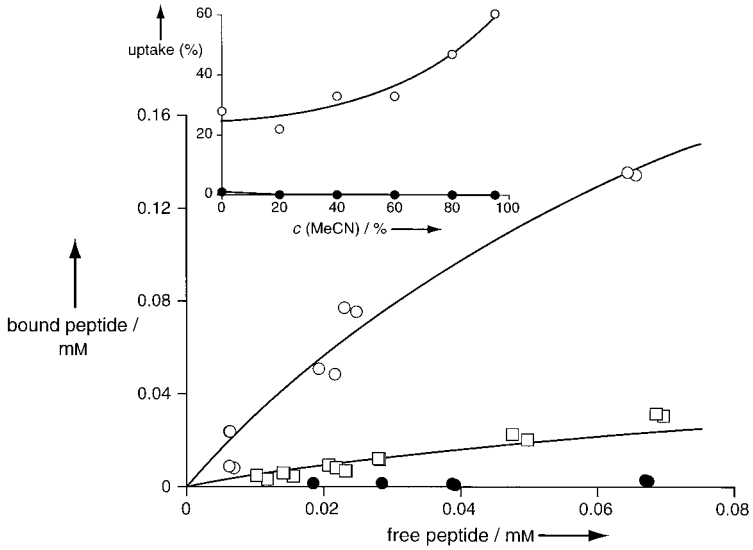

Figure 1. Polymer binding characteristics in acetonitrile/water mixtures. Main figure: Isotherms for the binding of Lys-Trp-Asp to imprinted (15 and 90 mg mL^{-1}, □ and ○, respectively) and nonimprinted (15 mg mL^{-1}, ●) polymers in acetonitrile/water 4/1. Fitted curves are calculated using the following parameters: $K_d = 113\,\mu M$ and capacity = 4.1 μmol g^{-1}. Inset: Dependence of binding on solvent composition in acetonitrile/water mixtures for Lys-Trp-Asp (0.1 mM) incubated with imprinted (○) and nonimprinted (●) polymer at 90 mg mL^{-1}. (Binding experiments were carried out as described in the experimental section.)

shown) have negligible affinity for **1**. In fact nonimprinted polymer showed a slightly "negative uptake" at 90 mg mL^{-1}, which was attributed to polymer swelling in the incubation mixture.[5] Binding parameters, calculated by curve-fitting the isotherm data, were determined as $K_d = 113 \pm 47\,\mu M$ at a capacity of 4.1 ± 1.2 μmol g^{-1}.

We then proceeded to probe the polymer selectivity with structural analogues of the tripeptide **1**, which were prepared by conventional solid-state peptide synthesis, purified by HPLC, and characterized by high resolution mass spectrometry. Initially, the three tripeptides **5**–**7** were designed with the N-terminus lysine exchanged for arginine, leucine and glutamine (Table 1). The binding of these tripeptides relative to **1** would provide information on the importance (and relative contribution) of the two carboxy groups that were introduced in the recognition site by the covalent method. Leucine and glutamine were chosen because leucine has a nonfunctionalized side chain of similar overall size to lysine and the γ-amide group of glutamine is capable of hydrogen bonding with carboxylic acids. Binding

Table 1. Sequence-specific binding of peptides to the Lys-Trp-Asp-imprinted polymer.

Peptide	Sequence	Percentage bound[a] imprinted polymer	nonimprinted polymer
1	Lys-Trp-Asp	43%	11%
5	Arg-Trp-Asp	24%	9%
6	Leu-Trp-Asp	<2%	6%
7	Gln-Trp-Asp	<2%	<2%
8	Lys-Phe-Asp	4%	<2%
9	Lys-Trp-Glu	5%	3%
10	Lys-Trp[b]	35%	17%
11	Lys-Phe[c]	9%	5%
12	Lys-Val[c]	15%	4%

[a] Peptides (1 mM in acetonitrile/water 4:1) were incubated with polymer (30 mg mL^{-1}) as described in the experimental section. [b] Obtained from Bachem. [c] Obtained from Sigma.

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studies were carried out at tripeptide concentrations of 1 mM as all the ligands were soluble in 80% acetonitrile at this concentration.

It is evident from the results presented in Table 1 that the replacement of lysine with arginine led to a significant decrease in binding from 43 to 24%, although the polymer still recognized the latter tripeptide reasonably well. This is not too dissimilar to the specificity of enzymes and antibodies, which often do not discriminate well between sequences containing these two amino acids. However, when the polymer was challenged with leucine- and glutamine-containing peptides, **6** and **7**, respectively, with the aim of eliminating the interaction between one of the carboxy groups and the side chain amino group of lysine, the binding was negligible. This result confirmed that the tight binding of **1** to the imprinted polymer was a result, at least in part, of the cooperative interaction between the lysine amino groups and the two carboxylic acids in the recognition sites. Ligands capable of participating in only one of these interactions exhibited a significantly lower affinity to the polymer.

We then replaced the tryptophan residue with phenylalanine (→**8**), and, once again, the binding was dramatically reduced (Table 1). The amplitude of the effect was somewhat surprising however, as the central amino acid residue was not expected to be strongly involved in any of the functional group interactions and hence a high degree of selectivity at the second position was not predicted. Perhaps the smaller phenylalanine residue of **8** cannot adopt a suitable conformation to allow it to occupy the "space" created by the apparently much bulkier side chain of **1**. As the indole group of tryptophan is connected through the pyrrole ring, it is possible that the void created in the polymer simply cannot accommodate a phenyl group. Although such a "tight fit" seems unlikely, it would explain the observed selectivity. In this case an interaction between the indole N proton and vinylpyridine could be responsible. Such interactions have been reported between 4-vinylpyridine and indole templates in the preparation of imprinted polymer catalysts for the benzisoxazole isomerization reaction.[11]

Substitution of the C-terminus aspartic acid with the structurally very similar glutamic acid (→**9**) also resulted in dramatically reduced binding. Two explanations can be advanced to account for the difference in binding between the template **1** and the glutamic acid containing tripeptide **9**. Firstly, the poor binding of **9** could simply arise from the loss of the hydrogen bond between the side chain carboxyl group and the corresponding polymer-bound pyridine residue.[12] Alternatively some other more subtle effects may be at play. For example, the binding of the slightly larger glutamate residue in the aspartate binding pocket could prevent the lysine or tryptophan residues (or both) occupying their parts of the imprint site because the peptide as a whole cannot adopt a conformation that satisfies all of the binding interactions. In order to distinguish between these possibilities the polymer was tested with the dipeptide Lys-Trp (**10**). We reasoned that if the loss of affinity occurred primarily because of the disruption of hydrogen bonding **10** should be as poor a ligand for the polymer as **9**. However, if the overall size and/or conformation of the glutamic acid residue was responsible for the reduced binding of **9** then the dipeptide should bind noticeably better than **9**, but not as well as **1**. It was found that uptake of Lys-Trp (**10**) was only slightly weaker than that of **1**. This observation confirmed that complexation by vinylpyridine groups contributed a significant but somewhat lesser part of the overall energy of binding and highlights the advantage of the covalent "sacrificial spacer" method.

Finally, we determined the uptake of two more dipeptides, Lys-Phe (**11**) and Lys-Val (**12**), which were chosen to probe the fit of the polymer binding site in the "hydrophobic pocket". In accordance with our previous result the replacement of tryptophan with phenylalanine led to a significant decrease in binding, however the uptake increased slightly in the case of **12**, presumably as the side chain of valine is less sterically demanding than that of phenylalanine and therefore **12** fits somewhat loosely in the site.

In conclusion, we have shown the feasibility of preparing polymeric materials that can discriminate between very similar amino acid sequences with a specificity approaching that of antibodies and other biological receptors. Polymers of this type are expected to find applications in affinity separations for the purification of biologically active oligopeptides from natural sources or as sensing elements in biomedical devices.[13]

Experimental Section

Template synthesis: Lys-Trp-Asp·2 $CF_3COOH \cdot H_2O$ (520 mg, 0.75 mmol) was dissolved in aqueous methanol (15 mL, 30%) containing Na_2CO_3 (200 mg) and cooled to 0 °C. The aqueous mixture was added to a solution of 2-methacryolyloxybenzoyl chloride (505 mg, 2.25 mmol) in THF (15 mL), also at 0 °C. The reaction mixture was stirred at room temperature overnight. The organic solvent was removed by rotary evaporation, and HCl (0.1N) was added to precipitate the product. The solid was collected and dissolved in ethyl acetate and the solution combined with an ethyl acetate extract of the filtrate. The combined organic extracts were washed with water (2 ×), HCl (0.1N, 3 ×), and water (3 ×), dried over Na_2SO_4, and evaporated. The crude product was purified by preparative HPLC to give the pure product in 49% yield.

Polymer synthesis: Template (116.4 mg), 2-vinylpyridine (74.3 mg), divinylbenzene (technical grade, 80%, 809.3 mg) and azobiscyclohexanecarbonitrile (29.8 mg) were dissolved in acetonitrile (2 mL) in a test tube fitted with a stopcock. The polymerization mixture was degassed by a series of three freeze – thaw cycles and polymerized photochemically for 48 h at 4 °C with 366 nm radiation. The polymer was ground to an average particle size of 30 μm using a mechanical mortar and the template removed by treatment with refluxing NaOH (7.5N) in methanol (template removal 58%, by amino acid analysis). The control polymer was prepared in exactly the same manner, except that the template was replaced by methacrylic acid (27.4 mg).

Binding experiments: The polymer (10 mg) was shaken overnight at room temperature with peptide solution (1 mM acetonitrile/water (4/1), 0.333 mL). The solutions were filtered through a 13 mm diameter, 0.2 μm PTFE membrane syringe filter (HPLC Technology, Macclesfield, UK) fitted to a disposable syringe before analysis by HPLC (LiChroshper C18 column (Merck), isocratic elution with 20% methanol and UV detection.

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A New Platinum Anticancer Drug Forms a Highly Stereoselective Adduct with Duplex DNA**

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Much attention is currently focused on the design of new generations of platinum anticancer complexes that circumvent cisplatin resistance. Such resistance often involves the recognition of platinated DNA adducts by proteins and enzymes in the excision – repair systems in cells.[1] It is therefore vital to understand how ligand design influences the nature of platinated DNA lesions. A complex with high activity against cisplatin-resistant cell lines is the 2-picoline (2-Pic, 2-methylpyridine) complex *cis*-[$PtCl_2(NH_3)$(2-Pic)] (**1**, ZD0473), a new anticancer Pt^{II} drug currently in phase I clinical trials.[2, 3] The steric effect of 2-picoline plays an important role in determining the rates of hydrolysis and substitution reactions with the nucleotide guanosine-5′-monophosphate (5′-GMP).[4, 5] As a result of slow rotation about the Pt–N(2-Pic) bond (0.62 s^{-1}, 296 K), together with the non-C_2-symmetrical structure of complex **1**, four isomers of the bis(GMP) adduct are possible, and these are formed in equal amounts.[5] We show here, in dramatic contrast, that reactions of **1** with the 14mer DNA duplex **2** give predominantly a single stereoisomer, whereas relatively little stereoselectivity is observed for reactions with the deoxydinucleotide d(GpG) or the 14-mer single strand **2a**. The structural basis for this unusually high stereoselectivity has been revealed by NMR studies.

5′d($A_1T_2A_3C_4A_5T_6G_7^*G_8^*T_9A_{10}C_{11}A_{12}T_{13}A_{14}$) **2a**

3′d($T_{28}A_{27}T_{26}G_{25}T_{24}A_{23}C_{22}C_{21}A_{20}T_{19}G_{18}T_{17}A_{16}T_{15}$) **2b**

2a + **2b** = **2**

Reactions of complex **1** with both d(GpG) and 14-mer single strand **2a** at a 1:1 molar ratio (1 mM, pH 6.0, 9 mM phosphate, 100 mM $NaClO_4$, H_2O/D_2O 90/10, 298 K) were followed by 1D ^{1}H and 2D [^{1}H,^{15}N] HSQC NMR spectro-

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