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Peptomers: A versatile approach for the preparation of diverse combinatorial peptidomimetic bead libraries

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Summary

This report describes a versatile approach in the generation of peptidomimetic bead libraries. The method is based on the preparation of peptide–peptoid hybrids using the portioning–mixing procedure, which gives diverse peptidomimetic bead libraries composed of peptides, peptoids and peptide–peptoid hybrids. We term these peptomers, from *pep*tide–pep*to*id hybrid poly*mers*. The synthesis of the peptomers is easily accomplished by adapting the peptoid synthesis strategy, in which a primary amine reacts with bromoacetic acid, and we combine this methodology with conventional peptide synthesis. The sequence of the active compound is deduced by conventional microsequencing using Edman degradation chemistry, thus avoiding the synthesis of a coding structure or the addition of molecular tags. We demonstrate the utility of the peptomer approach by the synthesis of a bead library together with the identification of novel peptidomimetic ligands binding to the macromolecular targets streptavidin and the insulin receptor.

Introduction

The peptide bead library approach by Lam et al. [1] is attractive since the library is easily synthesized by the portioning-mixing procedure [2] and the sequence of the binding peptides is readily deduced by conventional microsequencing using Edman degradation chemistry. Furthermore, the complexity of the library can be made very large in that millions or even billions of different peptide sequences can be prepared by the synthesis of multiple peptides on a single bead – also termed 'library of libraries' [3]. In addition to the discovery of novel peptide ligands to various receptors, the peptide bead library approach has also proved valuable in the search for phosphorylation substrate motifs for kinases [4,5], enzyme substrates for endoproteases [6] and for the identification of peptides binding to or inhibiting the growth of cells [7].

However, peptides are not ideal as drug therapeutics because of relatively poor pharmacokinetics and bioavailability. Thus, the focus of combinatorial libraries has since shifted to other types of polymers and the small-molecule libraries [8]. While the soluble combinatorial libraries by Houghten and co-workers [9–12] are based on an iterative or a positional scanning strategy and can be composed of almost any type of building block, the bead library approach relies on the co-synthesis of a coding structure or the addition of tags that must be accompanied in order to deduce the active sequence/structure of the nonsequenceable library compound. The coding part must be amenable for microsequencing, DNA sequencing or other analysis, e.g. mass spectrometry or gas chromatography, and is synthesized in a parallel manner together with the library compound. This coding structure can be DNA

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[13–15] or a peptide or combinations of amino acids [16–19], but more versatile approaches based on either electrophoretic tags or the use of secondary amine tags have been reported [20–22]. Alternatively, a microchip encoded library has been employed, avoiding the use of any chemicals in the encoding step [23,24]. Otherwise, direct analysis of the library members can successfully be accomplished by mass spectrometry [25–29]; this technique may not be applicable when the library of libraries approach is used or the complexity of the library is more than ∼100 000 different compounds.

Although very efficient in the generation of small-molecule libraries, encoded library synthesis is not straightforward and can, in some cases, present problems in library synthesis. Notably, the encoded library strategy may be labor intensive and technically sophisticated for the broad academic community compared to peptides that are readily microsequenced. We report here the synthesis and screening of a versatile bead library approach which comprises *pep*tide–pep*to*id hybrid poly*mers*, which we term peptomers. The complexity and diversity of such peptidomimetic bead libraries can be very high, since not only α-amino acids but also the large low-cost collection of amines and aldehydes can be utilized. We demonstrate that this type of peptidomimetic bead library can be prepared by conventional peptide chemistry and, importantly, the sequence of the polymer can be determined by standard Edman degradation chemistry avoiding the co-synthesis of a coding structure.

Materials and Methods

Synthesis of model peptoids on resin

The synthesis of model peptoids with the general format Ala-$N_{(4-7)}$-Ala-resin, where N represents a peptoid monomer, was performed on TentaGel resin. Portions (50 mg) of TentaGel resin S amine (Rapp Polymere, Tübingen, Germany) were coupled with Fmoc-Ala-OH (5 equiv), diisopropylcarbodiimide (DIC) (5 equiv) and HOAt (5 equiv) for 60 min in syringes equipped with a polypropylene filter [30]. After Fmoc-deprotection with 25% piperidine in DMF, 10 equiv bromoacetic acid (0.5 M) and 10 equiv DIC were coupled for 30 min followed by a double coupling (>60 min). The primary amine (all purchased from Aldrich, see Table 1) was added as a 1–2 M solution in DMF and left overnight. After removal of excess amine, the procedures were repeated. Finally, Fmoc-Ala-OH (0.5 M, 20 equiv) and DIC/HOAt (20 equiv) were added and coupled overnight. After Fmoc-deprotection the resin was subjected to 95% TFA and 5% H_2O for 30 min and then washed with ethanol and dried. Peptomers on beads were then analyzed by microsequencing using Edman degradation chemistry performed on a Procise™ protein sequencer (Applied Biosystems, Perkin Elmer).

Establishing a calibration file

All model peptoids were subjected to Edman degradation using a protein sequencer and sequenced using the standard gas-phase sequencing program supplied from the manufacturer. The coupling of phenylisothiocyanate (PITC) was accomplished by a predelivery of gaseous *N*-methylpiperidine (NMP) in MeOH/H_2O for 20 s followed by PITC (large loop), a second delivery of NMP for 170 s followed by PITC, and then a third delivery of NMP for 170 s followed by PITC. A modified gradient was used in the HPLC system of the sequencer in order to obtain better resolution of the hydrophobic phenylthiohydantoin derivatives (PTH-peptoid monomers). The gradient was run from buffer A (3.5% THF in water and 11 ml premix® (Applied Biosystems) in 1.0 l) to buffer B (12% 2-propanol in AcCN) in the following steps: 5–11% buffer B (0.0–0.2 min); 11–14% buffer B (0.2–0.4 min); 14–38% buffer B (0.4–14.0 min); 38–50% buffer B (14–26 min); 50–95% buffer B (26–27 min); 95% buffer B (27–31 min). All sequencing cycles were combined and saved using the software program (model 610A, v. 2.1) supplied with Procise™. The identification of the PTH-peptoid monomers from the bead library was then determined by the retention time from that of the calibration file and, if these were close to others, the HPLC profiles were compared using the software program. The conversion time in 25% TFA of the ATZ intermediates was set to 9 min.

Synthesis and screening of the peptomer bead library OOOOX-resin

TentaGel S amine resin (1 g) was divided into two groups and portion 1 was coupled with 1.1 equiv of an equivalent mixture of amino acids (Fmoc-Pro-OH, Fmoc-Lys(Boc)-OH, Fmoc-Phe-OH, Fmoc-Asp(*t*Bu)-OH, Fmoc-Gln(Trt)-OH, all purchased from Novabiochem) and 1.1 equiv DIC/HOAt followed by a double coupling overnight. Portion 2 was coupled with bromoacetic acid (0.5 M, 10 equiv) and DIC (10 equiv) followed by a double coupling and then an equiv-

Table 1. The amino acids and amines used in the synthesis of model peptomers (columns 2 and 3) and peptomer bead library OOOOX-resin (columns 1 and 2)

Fmoc-amino acids used in library synthesis	Amines used in model peptomers and library synthesis	Amines used in model peptomers
Fmoc-L-Lys(Boc)-OH	2-Aminoethyl-2-pyridine (A2)	2-Methoxyethylamine (A1)
Fmoc-L-Asp(tBu)-OH	1-Butylamine (A4)	Isobutylamine (A3)
Fmoc-L-Gln(Trt)-OH	Benzylamine (A5)	2-Pentylamine (A8)
Fmoc-L-Phe-OH	Tryptamine (A6)	(4-Methoxy)-2-phenylethylamine (A10)
Fmoc-L-His(Trt)-OH	2,3-Dimethoxybenzylamine (A7)	3-Methylbenzylamine (A11)
Fmoc-L-Tyr(OBzl)-OH	Cyclohexylamine (A9)	4-Methyl-2-pentylamine (A13)
Fmoc-L-Pro-OH(a)	1-Aminoindane (A12)	Hexylamine (A15)
Fmoc-D-Pro-OH(b)	3,3-Dimethylbutylamine (A14)	Aminomethyl-cyclohexane (A17)
	1-Naphthalenemethylamine (A16)	6-Methyl-2-pentylamine (A19)
	2,2-Diphenylethylamine (A18)	

Numbers in parentheses denote the code of the amines as presented in the HPLC chromatogram in Figure 2.

alent mixture of primary amines (0.5 M, 50 equiv, Table 1) was added and coupled overnight. The two portions were washed with DMF, combined and Fmoc-deprotected with 25% piperidine to give H-X-resin, which was divided into 18 portions. To portions 1–10, bromoacetic acid (0.5 M, 10 equiv) and DIC (10 equiv) were added followed by a double coupling. After washing with DMF, solutions of amines in DMF (1–2 M) were added separately to the portions and reacted overnight. To portions 11–18, the Fmoc-aa-OH (8 equiv) were activated with DIC (8 equiv) and HOAt (8 equiv) and added separately. Each portion coupled overnight. After coupling, portions 11–18 were combined and washed with DMF and then combined with portions 1–10, washed with DMF and then Fmoc-deprotected with 25% piperidine and redivided into 18 portions as previously described. Fmoc-L-Pro-OH and Fmoc-D-Pro-OH were coupled with the addition of a small amount (10%) of Fmoc-norleucine-OH (index residue) and Fmoc-norvaline-OH, respectively. The split synthesis procedure was repeated until the desired library OOOOX-resin was obtained. The resin was then deprotected using 85% TFA, 5% H_2O, 5% ethanedithiol (EDT) and 5% phenol for 1 h. The resin was washed with dichloromethane (DCM) and tetrahydrofuran (THF) followed by 5% HOAc and lyophilized. The library was screened against streptavidin alkaline phosphatase (1:20 000) in Tris-HCl (25 mM, pH = 7.4), NaCl (0.2 M), Tween 20 (0.05%), gelatin (0.1%) and NaN_3 (0.05%) for 60 min. After washing, the library was incubated with BCIP in staining buffer, Tris-HCl (0.1 M, pH = 9.3), NaCl (0.1 M), for ca. 30 min and washed with Tris buffer.

Screening with the insulin receptor

The OOOOX-resin library was initially washed with 8 M GuCl, pH = 2.0, for 15 min followed by extensive washing with Tris washing buffer. The library was then incubated with streptavidin alkaline phosphatase diluted 1:10 000 for 30 min and washed with Tris washing buffer. Stained brown beads were developed by the addition of BCIP and INT in staining buffer for 40 min. The library was then washed in 8 M GuCl, pH = 2.0, for 20 min followed by extensive washing with Tris-HCl washing buffer. The soluble biotinylated insulin receptor ($\sim$10 μg/ml) was then added to the library and incubated for $\sim$60 min. After removal of excess receptor, streptavidin alkaline phosphatase was added diluted (1:40 000) and incubated for 30 min. Stained blue beads were developed by the addition of BCIP in staining buffer for $\sim$30 min. Blue beads were easily observed among colorless and brown beads (specific for streptavidin) and only blue beads were removed for microsequencing.

Synthesis of A5-P-A5-A6-S-S-S-NH_2

The synthesis of the ligand A5-P-A5-A6-S-S-S-NH_2 was accomplished on TentaGel resin with Rink amide linker (*p*-[(*R,S*)-α-[1-(9*H*-fluoren-9-yl)-methoxyformamido]-2,4-dimethoxy]-phenoxyacetic acid) essentially as described for the library synthesis, with the exception that bromoacetic acid was coupled three times to the proline residue and the proline residue was coupled with 10 equiv followed by a double coupling. The peptomer was cleaved from the resin using 85% TFA, 5% H_2O, 5% EDT and 5% phenol for 1 h and precipitated in diethyl ether. After washing

with diethyl ether the product was dissolved in glacial acetic acid and lyophilized. Analytical HPLC was performed on C_{18} columns using Waters 600E equipped with a Waters 996 photodiode array detector. Mass spectrometry was performed on an MS-MALDI-TOF spectrometer (Fisons Instruments, VG Tofspec E).

Competition ELISA

Biotinamidocaproate *N*-hydroxysuccinimide ester (Sigma), 1 equiv, coupled to bovine serum albumin (BSA) was immobilized to microtiter wells (Maxisorb, Nunc, Denmark) overnight in $NaHCO_3$ (0.1 M, pH = 9.5). Serial dilutions of peptomer A5-P-A5-A6-S-S-S-NH_2 and peptide FSHPQNT were incubated with streptavidin horseradish peroxidase conjugate (1:8000) for 30 min and then transferred to wells coated with BSA-biotin for 60 min. The substrate tetramethylbenzidine (TMB tablets, Sigma) in citrate buffer (0.1 M, pH = 5.0) was added to the wells and the absorbance was read in an ELISA reader (Bio-Tek Instruments EL 340).

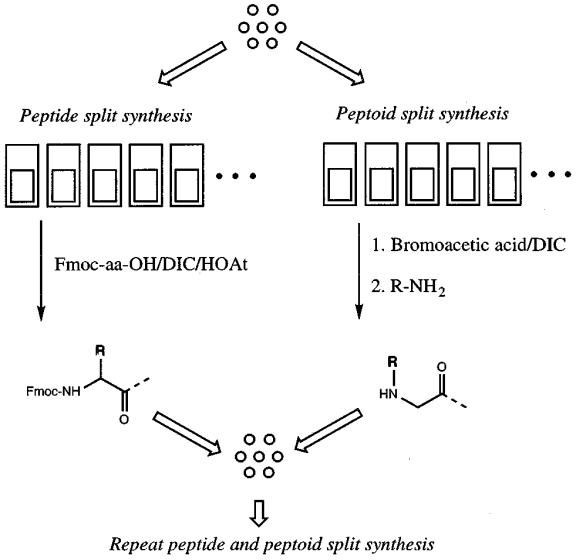

Figure 1. A schematic representation of the synthesis of the peptide–peptoid hybrid library by the portioning-mixing procedure.

Results

An N-substituted glycine polymer, also termed peptoid [31], can be synthesized on solid phase by stepwise acylation with bromoacetic acid and subsequent reaction with a primary amine [32]. Since the synthesis occurs in the N- to the C-terminus direction, we assumed that solid-phase peptoid and peptide synthesis could be combined, giving peptide–peptoid hybrid polymers, which we term peptomers. The synthesis of a peptomer bead library is readily accomplished by performing two parallel portioning-mixing procedures as shown in Figure 1.

The coupling of an Fmoc-amino acid to a secondary amine is often very slow and should generally be performed using high concentrations and extended reaction times. We experienced unsatisfactory results with HOBt as the coupling additive. However, HOAt as the additive has been reported to increase the coupling yields in solid-phase peptide synthesis [33] and DIC/HOAt proved to be efficient coupling reagents in the synthesis of peptide–peptoid hybrids. During the synthesis of the model peptomers, high concentrations (0.5 M) and large excesses (20 equiv) of Fmoc-amino acids were used. Coupling of the three Fmoc-amino acids, alanine, phenylalanine and asparagine, to immobilized Bzl-peptoid monomer resulted in coupling yields >99% and were obtained in less than 60 min as measured by preview analysis performed on the microsequencer. Using the more sterically hindered Fmoc-isoleucine only ∼80% coupling yield was obtained, but on increasing the reaction time to overnight almost quantitative incorporation was obtained. The coupling of bromoacetic acid to a peptoid monomer also proceeds efficiently as reported by Zuckermann et al., and we were not able to detect any side reaction when bromoacetic acid was coupled on an α-amino acid. In both cases the preactivation of the bromoacetic acid with DIC is allowed for ca. 3–5 min before adding to the resin. A double coupling is performed since the activation of the bromoacetic acid with DIC introduces the risk that the intermediate *O*-acylisourea may be transformed into the unreactive *N*-acylurea. We encountered that the synthesis of peptoids incorporating aniline and other anilines like 4-aminoacetophenone and 4-aminobiphenyl was unsatisfactory and, consequently, we focused only on the use of primary aliphatic amines. Generally, these were added at a concentration of 1–2 M and reacted overnight.

During Edman degradation the cleavage and conversion of the PTC intermediates to the PTH derivatives, which are detected in the HPLC system of the microsequencer, occur via the anilinothiazolinone (ATZ) intermediate, which is a rate-limiting step. Thus, a fraction of the ATZ intermediate may be present when the PTH derivatives are analyzed in the HPLC sys-

tem and will be observed as two peaks in the HPLC chromatograms; however, the conversion of the PTC peptoid intermediates seems to be as rapid as for the PTC amino acid intermediates since the presence of ATZ intermediates was not observed. We observed that the sequence of peptoids and peptide–peptoid hybrids is readily deduced by standard microsequencing without any difficulties, e.g. low coupling yields of phenylisothiocyanate (PITC) or inefficient cleavage and conversion of the PTC and ATZ intermediates leading to low repetitive yields. By calculating the yield of the N- and C-terminal alanine residues in model peptomers, we found repetitive yields in the range of 85–93%. Typically, the repetitive yields for synthetic peptides on resin beads are in the same range.

All of the analyzed PTH-peptoid monomers elute later than leucine in the HPLC chromatogram, except for the PTH-peptoids 2-methoxyethylamine (A1) and 2-(2-aminoethyl)-pyridine (A2), as shown in Figure 2. For simplicity, the peptoid monomers are named according to the amine attached to the bromoacetic acid. The retention time of the PTH-peptoid monomers was then used to determine the identity of the residues. As an example, the PTH benzylamine peptoid monomer (A5) elutes at 22.22 min with a standard deviation (SD) of ± 0.046 min. Alternatively, the residues were identified by an overlay of the HPLC profiles with that from a calibration file stored in the computer.

The model peptomer bead library has the format OOOOX-resin, where X is either a mixture of amino acids or peptoid monomers and O represents the defined residues, and is synthesized by two parallel split synthesis procedures according to Figure 1 using eight amino acids and 10 of the amines listed in Table 1. This format is an adoption of the library-of-libraries concept [3], which involves the generation of several combinations of defined and mixture positions for a peptide. In the presented model peptomer library, only one motif (OOOOX-resin) was constructed. However, this format ensured that the library contained at least two streptavidin binding ligands: the well-known L-amino acid motif, H-P-Q, and the nonproteinogenic peptide F-p-YBzl (YBzl = *O*-benzyl-L-tyrosine and p = D-proline) previously found in a nonproteinogenic motif library [34]. In order to evaluate the library, two beads were randomly removed from the library and subjected to microsequencing prior to the screening of the library against streptavidin. The sequences could be determined unambiguously as shown in Figure 3. The macromolecular target streptavidin alkaline phosphatase was then added to the library and stained beads were observed within 10 min by the use of an ELISA-type assay. A dual color screening assay, which differentiates between streptavidin binders and binders to alkaline phosphatase, is not necessary since the binders to streptavidin are clearly visible before any binding to the enzyme is observed. In fact, so far we have never detected any binders to the enzyme alone. Among approximately 200–300 colored beads, 15 beads were removed and subjected to microsequencing, and the result is listed in Table 2. The nonproteinogenic peptide motif F-p-YBzl is identified but it is not the dominating motif. Instead the peptomer ligand ψ_1-α-ψ_2-ψ_3 is found, where ψ is an aromatic peptoid monomer and α is L-proline. Notably, ψ_1 and ψ_2 are aminoindane (A12) and benzylamine (A5), respectively. One of the ligands, A5-P-A5-A6-S-S-S-NH_2 (Table 2), was synthesized and tested in solution (Ser-Ser-Ser replaced the mixture X in order to enhance the solubility of the peptomer and A6 denotes tryptamine). The peptomer ligand, shown in Figure 4, binds in or near the biotin binding site as measured by competition ELISA and can displace biotin with an IC_{50} = 25 μm. The affinity of the peptomer ligand is significantly higher than the peptide FS*HPQ*NT found in an L-amino acid peptide library [35,36], which under the same conditions displaces biotin with an IC_{50} >400 μm.

The X position in the peptomer motif A5-P-A5-A6-X-resin was replaced by three serine residues. By doing so, the possibility that some building blocks may

Table 2. Peptide–peptoid hybrids interacting with *streptavidin* found in peptomer bead library OOOOX-resin

ψ-P-ψ-ψ[a]	F-p-YBzl-	Other motifs
A5-P-A5-A6-X (2)	F-p-YBzl-p-X	YBzl-p-YBzl-p-X
A5-P-A5-A5-X	F-p-YBzl-A2-X	YBzl-A5-F-p-X
A12-P-A5-A5-X (2)		
A12-P-A5-A2-X		H-A18-K-F-X
A12-P-A5-A6-X		
A12-P-A9-A18-X		
A12-P-A9-A5-X		
A14-P-A5-A6-X		

[a] ψ represents a peptoid monomer. All peptoid monomers are underlined and named according to the amine attached to the bromoacetic acid: A2 = 2-(2-aminoethyl)-pyridine; A5 = benzylamine; A6 = tryptamine; A9 = cyclohexylamine; A12 = 1-aminoindane; A14 = 3,3-dimethylbutylamine; A18 = 2,2-diphenylethylamine. YBzl = *O*-benzyl-L-tyrosine. Letters in lower case are D-amino acids. Numbers in parentheses indicate the number of beads sequenced. X = 5 different amino acids and X = 10 different peptoid monomers.

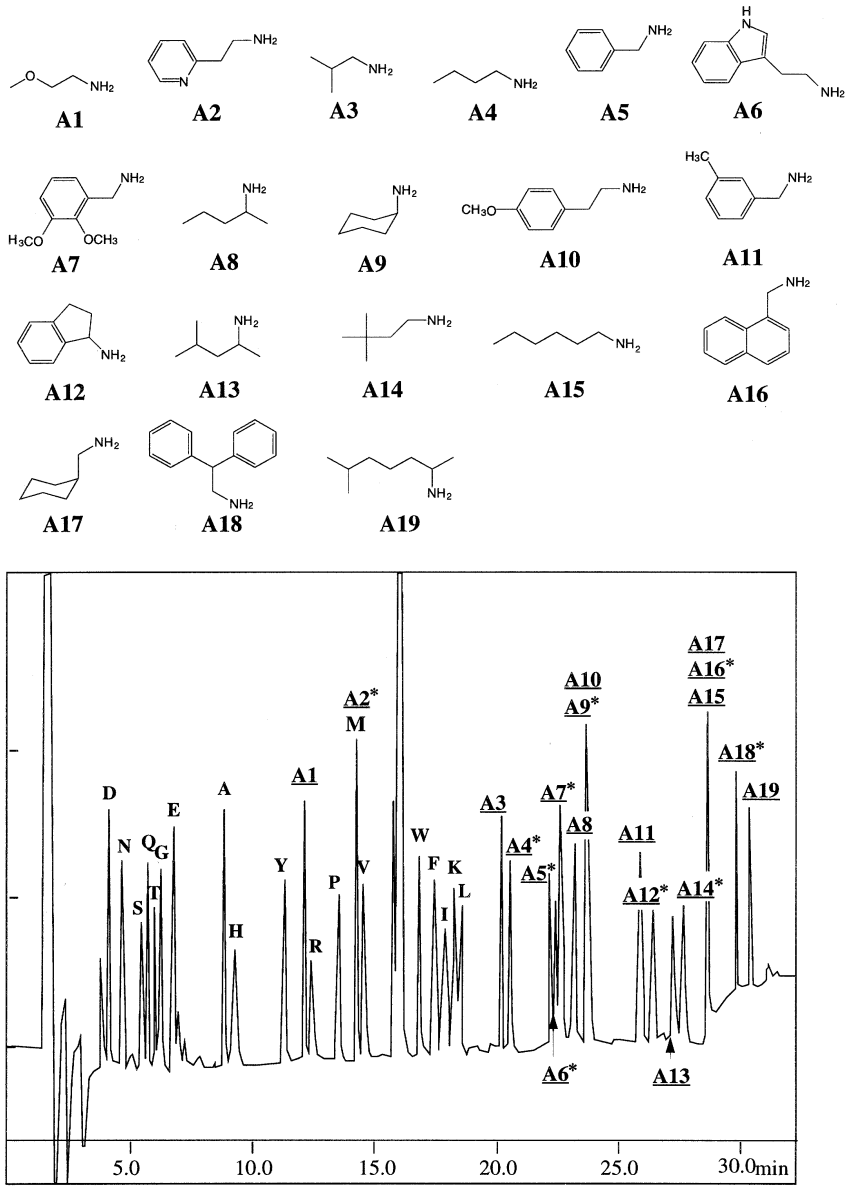

Figure 2. Amines tested in the synthesis of model peptomers for the establishment of a calibration file. An overlay of the HPLC profiles of the PTH-peptoid monomers is shown at the bottom. Amines marked with asterisks were used in the synthesis of the peptomer bead library.

be more effective than others was not explored; however, peptidomimetic ligands displaying higher affinity may be found if a sublibrary with e.g. the format A5-P-A5-A6-O-O-O-resin is constructed and screened under more stringent conditions. Such a dedicated library could easily comprise other aromatic building blocks closely related to the essential benzyl group and essentially would map the conformational space more densely. Another way would be the synthesis and screening of individual compounds with the formula A5-P-A5-A6-O-NH_2 or A5-P-A5-A6-O-O-NH_2, in which O is an amino acid or peptoid monomer.

The pharmaceutically relevant insulin receptor was also applied to the peptidomimetic library and beads specific for the receptor were identified by a dual color reaction scheme using BCIP and INT as substrates (Table 3). Alternatively, BCIP and nitro blue tetrazolium (NBT) have been used to differentiate between

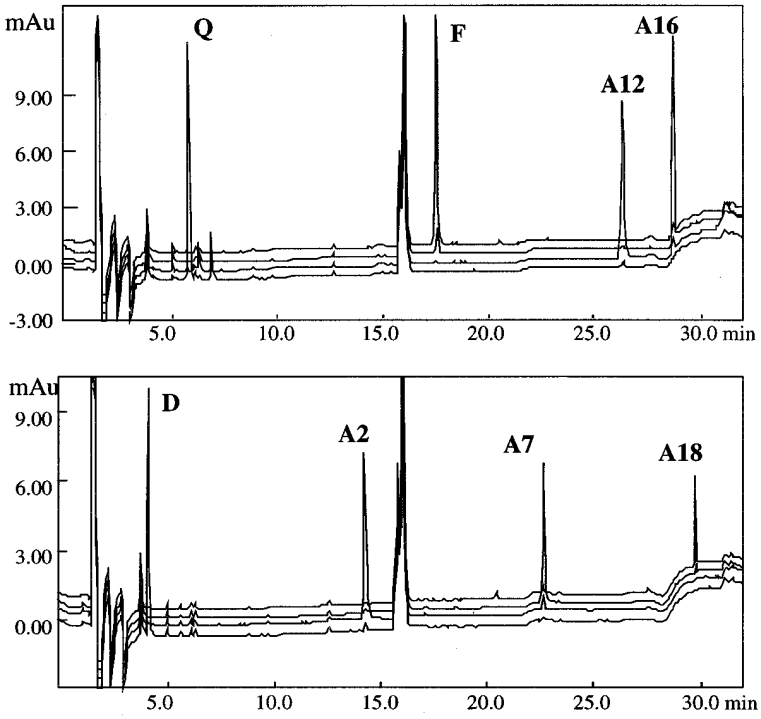

Figure 3. The HPLC chromatograms of the sequencing of two blank beads with sequences Q-A12-A16-F and D-A2-A7-A18, respectively, randomly selected from the peptomer bead library OOOOX-resin. The sequencing repetitive yield with respect to glutamine and phenylalanine (upper HPLC chromatogram) is 93%.

Table 3. Peptomer sequences interacting with the soluble insulin receptor

A9-A12-A18-K-X[a]	(2)
A9-A4-A18-K-X	(2)
A9-A9-A18-K-X	
K-A16-A18-K-X	(2)
A2-A9-A18-K-X	
A9-A16-F-K-X	(2)

[a] Peptoid monomers are named according to the amine attached to bromoacetic acid: A2 = 2-(2-aminoethyl)-pyridine; A4 = butylamine; A9 = cyclohexylamine; A12 = 1-aminoindane; A16 = 1-naphthalenemethylamine; A18 = 2,2-diphenylethylamine. Numbers in parentheses indicate the number of beads sequenced.

unspecific binding to streptavidin alkaline phosphatase and specific binding to the target receptor [37]. Again, as is also found with the streptavidin target, the essential residues are hydrophobic peptoid monomers. Notably, the diphenylethylamine (A18) and cyclohexylamine (A9) are dominating together with lysine.

Discussion

In order to replace peptides with peptidomimetics displaying improved pharmacokinetics, a number of novel unnatural polymers have been described. Polymers in which the entire peptide bonds are replaced by a novel synthetic repeating unit have been reported and are based on the vinylogous [38] or oligocarbamate backbone [39]. Other approaches include a global chemical transformation of peptide libraries, which are turned into amide alkylated or reduced libraries comprising peptidomimetics which are resistant to proteolytic degradation [12,40,41]. Other more peptide-like polymers, the azatides [42] and the peptoid polymer [31], have been reported. The peptoid approach is especially attractive since the solid-phase synthesis can easily be performed by the acylation of bromoacetic acid followed by the condensation of a primary amine [32], leading to peptoids which essentially are N-substituted glycine polymers (NSGs) and are stable towards prote-

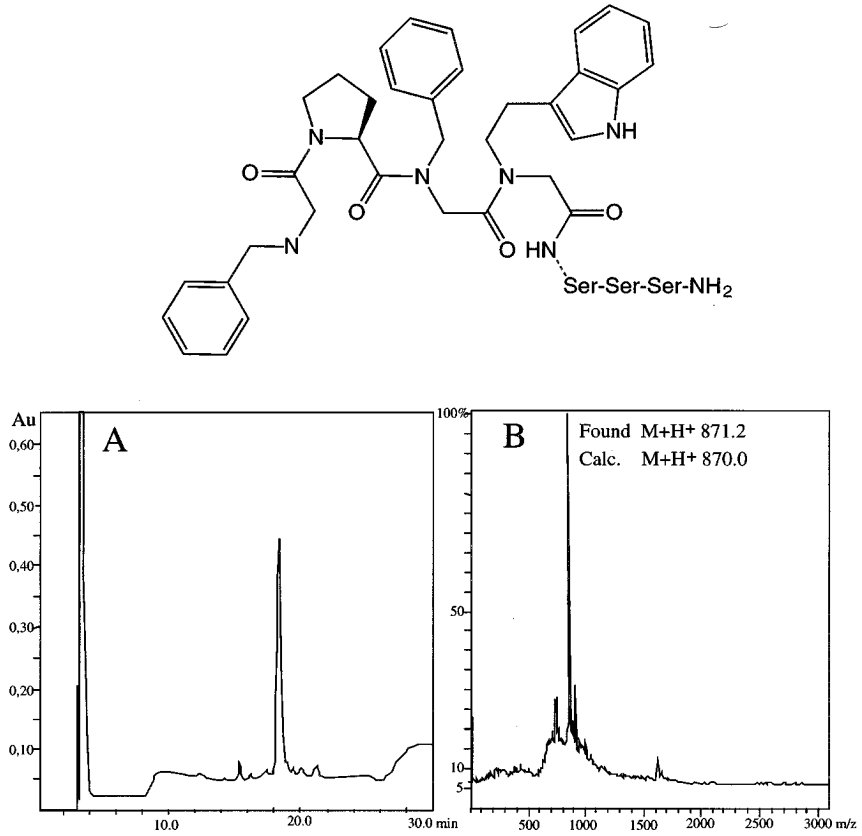

Figure 4. The peptomer ligand A5-P-A5-A6-S-S-S-NH_2 interacting with streptavidin: (A) the HPLC profile and (B) the corresponding MALDI-TOF-MS of the crude product.

olytic degradation. The construction of small nonpeptidic compounds from amino acids, bromoacids, primary amines and carboxylic acids giving compounds related to the peptoid and peptomer structure has been reported [19]. Structure determination of these compounds was accomplished by a peptide encoding strategy.

Libraries of either the peptide or peptoid structures can be very complex in that they can comprise millions or even billions of different sequences, but neither of the libraries are very diverse since the only changing parameter is the side chain. A simple way to increase the diversity of a peptide library would be the inclusion of both L- and D-amino acids, which create a peptide library of diastereomers by orienting the side-chain functionalities differently. Even though the L- and D-amino acids or other residues co-elute in the HPLC system of the microsequencer, the correct residue can be determined by the addition of an index residue, e.g. 10% norleucine and 10% norvaline coding for the L- and D-amino acids, respectively [34]. Furthermore, if the side chain is moved to another position in the peptide backbone, such as the nitrogen atom in a peptoid structure, an even greater diversity should be expected. Thus, by combining peptide and peptoid synthesis very diverse bead libraries can be created (Figure 5). The model peptomer bead library described in this report comprises peptide and peptoid structures as well as peptide–peptoid hybrids or peptomers (peptide–peptoid polymers). Besides making a library more diverse, the peptomer approach may also generate compounds that are more stable towards enzymatic degradation and also display improved pharmacokinetics and bioavailability compared to peptides. The approach may also be used in the conversion of biologically active peptide ligands, e.g. peptide hormones, into an active peptomeric version by ensuring that the essential amino acids comprising the lead motif are included in the synthesis. The peptomer library generated would then be composed of various peptomeric

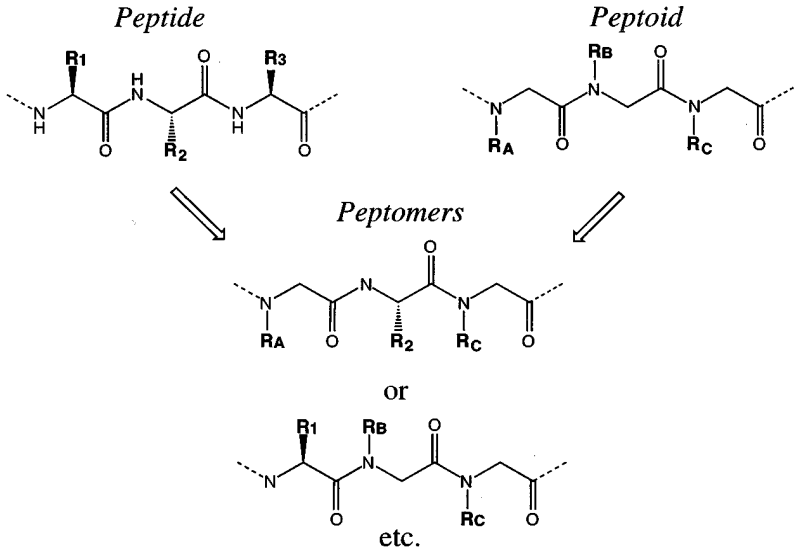

Figure 5. The synthesis of a peptide–peptoid hybrid bead library leads to greater diversity since the relative spacing between the side-chain functionalities changes.

versions of the peptide ligand, among which ligands with enhanced pharmacokinetics and affinity and/or selectivity may be found.

Due to the availability of a very large number of primary amines, a large repertoire of building blocks is at hand. The synthesis of a small soluble peptoid library and the subsequent identification of high-affinity ligands to the opioid and α-adrenergic receptor demonstrated the usefulness of even a moderate complex combinatorial peptoid library [43]. Reductive alkylation of support-bound amines with aldehydes has been used in library synthesis [44] and thus, in principle, peptoids can also be synthesized by the condensation of an aldehyde with an immobilized glycine, forming an imine which is subsequently reduced by e.g. triacetoxyborohydride. Thereby the number of available building blocks increases considerably and diverse and very complex peptomer bead libraries can be prepared.

Conclusions

Today, microsequencing of peptides using Edman degradation chemistry performed on a protein sequencer is routine work. The advantage of the peptomer bead library approach to a broader academic community is the observation that the peptoid polymer and the peptomers are also amenable for direct microsequencing using standard Edman degradation chemistry. Thus, the synthesis of an encoded library is avoided. Peptoids can also be sequenced determined by mass spectrometry [26]; this analytical approach, however, is not feasible using the library of libraries approach [3] since hundreds to thousands of different compounds are present on a single bead.

We believe that the peptidomimetic bead library approach using peptide–peptoid hybrids, peptomers, is attractive and versatile since the peptomer bead library can be made very diverse and still be relatively easy to synthesize and evaluate. Thus, the chance that a lead compound with improved pharmaceutical properties can be identified is increased and should facilitate the drug discovery process.

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