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Selectide Technology: Bead-Binding Screening

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The Selectide process is a random synthetic chemical library method based on the one-bead one-peptide (structure) concept. A "split-synthesis" method is used to generate huge random libraries (10^6–10^8). At the end of the synthesis, each bead expresses only one chemical entity (e.g., peptide). The whole library is then tested simultaneously for binding to a specific acceptor molecule of biologic interest. The ligand bead that interacts specifically with the acceptor molecule is then isolated for structure determination. Once a binding motif is identified, a secondary library (based on the motif of the primary screen) is generated and screened under a more stringent condition to identify leads of higher affinity. This process can be applied to both peptide and nonpeptide (small organic) libraries. In the case of nonsequencable structure libraries, the coding principle has to be applied for structure elucidation of positively reacting beads. Coding peptide is synthesized in parallel to the screening structure, and classical Edman degradation (one or multiple-step) is used for structural analysis. To exclude the possibility of interaction of the macromolecular target (e.g., receptor, enzyme, antibody) with the coding structure, a synthetic technique for segregation of the surface (screening structure) and the interior (coding structure) of the beads was developed. The one-bead one-structure process is invaluable in drug discovery for lead identification as well as further optimization of the initial leads. It also serves as an important research tool for molecular recognition.

Combinatorial peptide library methods have proven to be powerful in identifying ligands with binding or biological activities. Currently, there are three methods of generating huge peptide libraries: (i) the biologic libraries (e.g., filamentous phage library (1–4) and plasmid libraries (5), (ii) the combinatorial library with iterative process (6–10), and (iii) the one-bead one-structure process (11–13).

The Selectide process is a random synthetic library method based on the one-bead one-structure concept. Using the split synthesis method (8, 11, 14), each bead expresses only one chemical entity (11). The entire bead-bound peptide library can then be screened against a tagged acceptor molecule (e.g., receptor, enzyme, antibody, or even small molecules) for binding (11, 12, 15). Alternatively, using an orthogonal two-stage release process, the peptide or nonpeptide structure can be released from each bead for solution-phase assay (13, 16–18).

Individual positive beads isolated from the "bead-binding assay" or the "release-solution-phase assay" will then be loaded to a microsequencer for structure determination. Since the library is generated by synthetic chemical methods, the library need not be restricted to peptides with eukaryotic amino acids only. Instead, it could be any unnatural amino acids, structures with reduced peptide bonds, and even nonpeptide moieties (19–24). In this paper, the Selectide process using a bead-binding assay will be described in detail, and various applications of the methodology will also be discussed. The release-solution-phase assay technology will be discussed elsewhere in this issue (25).

DESCRIPTION OF METHOD

Material

Library syntheses were performed on TentaGel Resin S Amino-NH2 (Rapp Polymere, Tubingen, Germany) (26). Fmoc amino acids, with standard side chain protecting groups, were obtained from Advanced ChemTech (Louisville, KY) Bachem (Torrance, CA), or Propeptide (Vert-le-Petit, France). Benzotriazolyl-oxy-trisdimethylamino-phosphonium hexafluorophosphate (BOP), *N*-hydroxybenzotriazole (HOBt), diisopropylethylamine (DIEA), piperidine, and diisopropylcarbodiimide (DIC) were obtained from Advanced ChemTech. Commercial-grade solvents were used without further purification. 2-Bromo-3-chloro-indolyl phosphate (BCIP) was obtained from Ameresco (Solon, OH).

Sequencing by Edman degradation was performed on an ABI 4778 protein sequencer (Applied Biosystems, Foster City, CA) and a Porton PI 3010 instrument (Porton Instruments, Tarzana, CA). Both analytical and preparative HPLC were carried out on a Waters 625 LC system

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with a Waters 490E programmable multiwavelength detector using Vydac Peptide and Protein C18 analytical (0.46 × 250 mm, 5 μm, 1 ml/min) and preparative (10 × 250 mm, 10 μm, 3 ml/min) columns, respectively. UV/VIS absorption spectra were recorded on a Hewlett–Packard HP 8452A diode-array spectrophotometer using a 1-cm quartz cuvette. Amino acid analyses were carried out on a D-500 system (Durrum Corp., Palo Alto, CA) system.

Preparation of Peptide Library

Based on the split synthesis methodology (8, 11, 14), each bead is exposed to only a single activated amino acid at each coupling cycle, and the coupling reaction is driven to completion. Therefore, at the completion of the library synthesis, each resin bead expresses only one peptide entity (11). Standard solid-phase peptide synthesis chemistry (Fmoc chemistry) was used in the preparation of our libraries (27, 28). In our standard peptide libraries, polyethylene glycol-grafted polystyrene beads or TentaGel-S were used. Alternatively, polydimethylacrylamide beads or Pepsyn Gel Resin (Cambridge Research Biochemicals, Northwitch, UK) can also be used. In fact, any resin bead that is compatible with peptide synthesis (organic solvents) and screening under aqueous conditions is adequate. Depending on the application, additional noncleavable linkers such as Fmoc–aminocaproic acid, Fmoc–aminobutyric acid, and/or Fmoc–β-alanine, may first be added onto the resin prior to the amino acid randomization steps. In our standard library synthesis, the resins were first divided into 19 aliquots contained in 19 polypropylene vials. Nineteen Fmoc-protected eukaryotic amino acids (all but cysteine) were then added separately into each of the resin aliquot. A minimal amount of dimethylformamide (DMF) was used. The amino acids were added in threefold excess, and coupling was initiated by adding threefold excess of BOP, DIEA, and HOBt. In some experiments DIC and HOBt were used instead. A trace amount of bromophenol blue was added into the reaction mixture. The vials were capped tightly and rocked gently for approximately 30 min at room temperature or until all beads turned from blue to colorless (29). Completion of the coupling was then confirmed by a ninhydrin test (30). For the aliquots in which coupling reactions were incomplete, the beads were allowed to settle, and the supernatant was gently removed. Fresh Fmoc amino acid was then added to that vial followed by BOP, DIEA, and HOBt, and the reaction was allowed to proceed for another hour. In general, most randomization cycles required only one coupling, and only on rare occasions was double coupling needed. The resins were then mixed in a siliconized cylindrical glass vessel fitted with a frit at the bottom. Dried N_2 was bubbled through for mixing of the resin. After washing (8×) with DMF, 20% piperidine (in DMF) was added. After 10 min of bubbling with N_2, the piperidine was removed, and the resins were washed 10 times with DMF. The amount of released fulvene–piperidine adduct was quantitated by UV spectrometry (302 nm). A stable level of substitution determined in this manner throughout the library synthesis served as one of the quality control measures. The resins were then divided into aliquots for another cycle of coupling. During the past 2 years we have synthesized more than 300 peptide libraries up to 18 amino acids long. After all the randomization steps were completed, the Fmoc group was removed with 20% piperidine (v/v) in DMF, and the side chain protecting groups were removed with a mixture of trifluoroacetic acid–phenol–anisole–ethanedithiole (94:2:2:2, v/w/v/v) or reagent K (TFA–phenol–water–thiophenol–ethanedithiole, 82.5:5:5:5:2.5, v/w/v/w/v) (31). The resin was then washed thoroughly in DMF, neutralized with 10% DIEA (in DMF), thoroughly washed again, and stored in DMF at 4°C. In the cases of synthesis of larger batches of the library (up to 80 g), the library was stored in protected form in 0.2% HOBt in DMF at 4°C.

To verify the quality of the library, several randomly chosen beads were submitted for sequencing, and the average amount of the peptide per bead was determined. This value was confirmed by quantitative amino acid analysis of a random sample from the library (cca 1 mg). Amino acid analysis, as well as sequence analysis of pooled sample of beads (~50 beads) must confirm random distribution of all amino acids (in the case of Edman degradation, distributions in particular positions).

Synthesis of Library of Small Organic Molecules

Fmoc–βAla–Gly–βAla–Gly linker was assembled first on the resin (TentaGel S NH_2, 1 g) using DIC and HOBt. After cleavage of the Fmoc group, the peptide resin was divided into 18 equal portions, and 18 (excluding cysteine and proline) Fmoc-protected L-amino acids (one in each reaction vessel, 3 eqs each) were coupled using DIC and HOBt in DMF. Fmoc protection of the α-amino group was removed by piperidine in DMF (20%, 10 min) and after washing with DMF (5×) and DMF containing 2% of acetic acid, the resin was divided into 20 reaction vessels. The free amino groups were exposed to the solution of aldehydes in methanol/dichloromethane/1% AcOH (2 to 30 eqs according to the aldehyde reactivity). After a 20-min preincubation, cyanoborohydride (1 mmol/ml DMF, 2 to 30 eqs) was added, and the reaction proceeded for 40 min. Resin was washed by DMF (5×), and the ninhydrin test was performed to check the completeness of the primary amino group transformation to the secondary amino group. In the case of a positive test, the coupling was repeated. Resin was pooled and redistributed to 20 reaction vessels, and 20 carboxylic acids were coupled either by the DIC/HOBt method or by the application of symmetrical anhydride. After thorough washing, the side

chain protecting groups were removed with reagent K (defined above).

Screening of the Library

Various detection schemes could be applied for the screening of the libraries. In general, a tagged acceptor molecule was used. The tag could be an enzyme (e.g., alkaline phosphatase or horse radish peroxidase), a fluorescent probe (e.g., FITC), or a radionuclide (e.g., ^{125}I). Alternatively, a two-step process using the biotin/streptavidin system or the primary and secondary antibody system could be used. In this case, the library is first probed with a biotinylated acceptor molecule or the primary antibody, followed by a tagged streptavidin probe or the secondary antibody. The method for the enzyme-link detection scheme is described below (11, 12, 15).

The random library of peptides (structures) on beads was gently mixed with an incremental increase of double-distilled water to removed all the DMF. The beads were thoroughly washed with double-distilled water. Gelatin (0.1% w/v) was then used to block any nonspecific binding. Alternatively, bovine serum albumin may be used. The beads were then mixed with the acceptor–alkaline phosphatase complex (as diluted as possible to minimize any nonspecific binding) in PBS with 0.1% gelatin and 0.1% Tween 20 with gentle mixing for 1 to 24 h. The beads were then thoroughly washed with PBS with 0.1% Tween 20 followed by TBS (Tris-buffered saline: 8 g NaCl, 0.2 g KCl, and 3 g Tris base in 1 liter water, pH 8.0). Standard substrate BCIP (5-bromo-4-chloro-3-indolyl-phosphate) in alkaline phosphatase buffer (5.85 g NaCl, 12.1 g Tris base, 0.476 g $MgCl_2$, pH 8.5) was then added, and the beads were transferred to 10–20 polystyrene petri dishes (100 × 20 mm). The reaction was carried out for up to 2 h. The positive beads turned turquoise while the majority of the beads remained colorless.

Positively reacting beads were treated with 8 M guanidine hydrochloride, pH 2.0, for 20 min and destained by an intensive wash with DMF, and the above described experiment was repeated with the competing ligand or competing unlabeled acceptor added to the incubation medium. Colorless beads were selected, stripped by guanidine hydrochloride, and reincubated with the acceptor–alkaline phosphatase complex in the absence of competitor. Stained beads (true positive) were then isolated for sequencing.

Sequencing of the Peptide Screening or Coding Structure

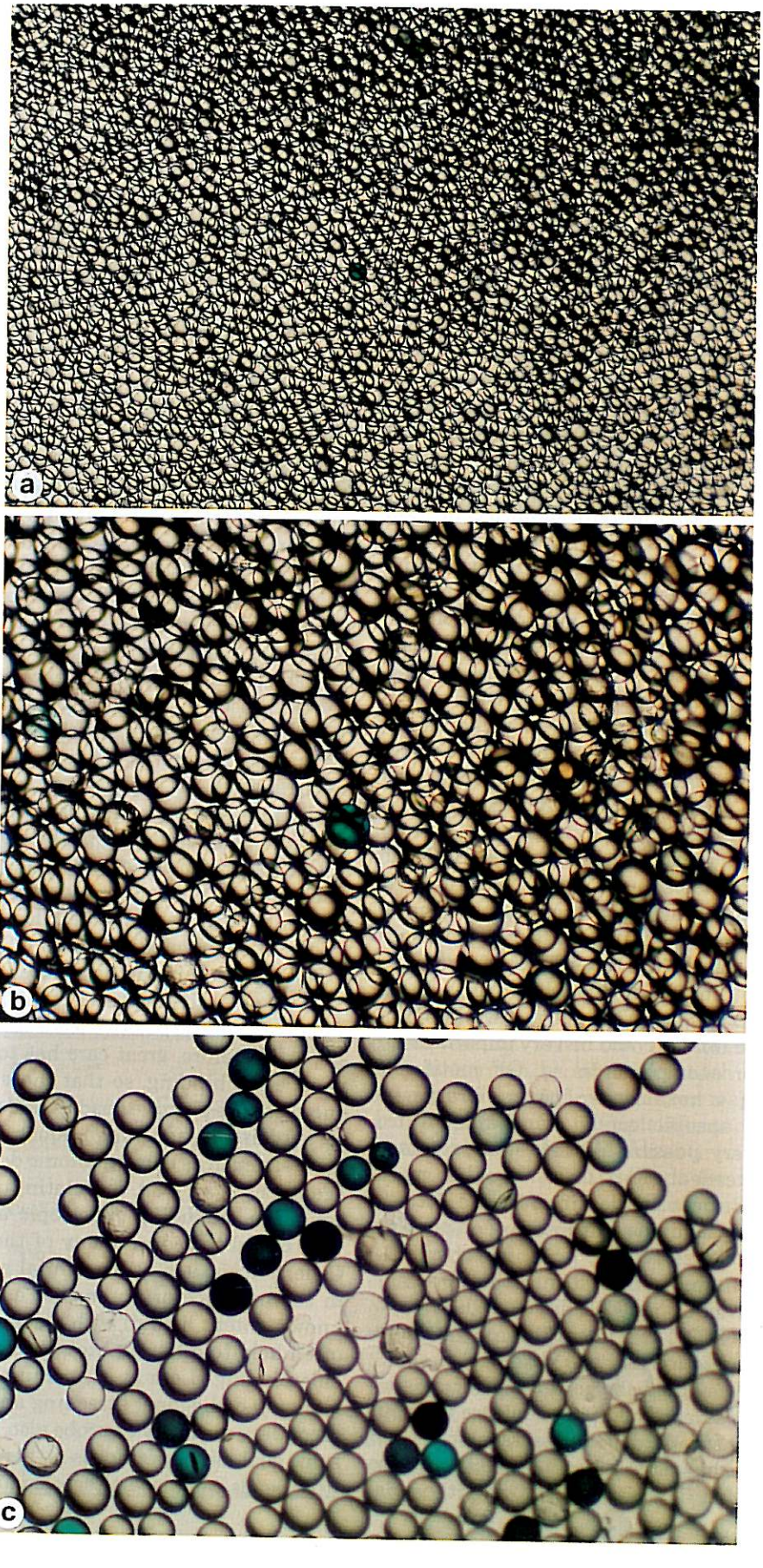
The positive beads were physically removed with a micropipette and treated with 8 M guanidine hydrochloride, pH 2.0, for 20 min to 1 h. The beads were then washed with double-distilled water. Individual beads were then placed on a glass filter and inserted into the cartridge of a microsequencer (e.g., ABI Model 477A, Applied Biosystem) for sequencing as described (11, 12, 15).

RESULTS AND DISCUSSION

As indicated earlier, there are three general methods for the generation and screening of huge random peptide libraries: (i) the biological libraries (1–5), (ii) the combinatorial library with iterative process (6–9), and (iii) the one-bead one-structure (Selectide) process (11–13). There are advantages and disadvantages to each of these general methods. The biological libraries approach has the potential of generating up to 10^9 peptides, and the libraries are not limited by the length/size of the peptides. However, because it is a biological method, the library can consist of only genetically encoded amino acids. In addition, there is a biological bias in the synthesis and screening (multiple rounds) of the biological library. In contrast, the other two synthetic chemical methods have a practical limit on the size as well as the length of the peptide libraries. The synthetic approach, however, does offer an opportunity for the incorporation of unnatural amino acids, disulfide and nondisulfide cyclic structures, various scaffoldings, reduced peptide bond, or even non-peptide moieties (19–21, 32–34) into the libraries. The major difference between the iterative approach and the Selectide approach is that the former approach relies on the multiple sequential synthetic steps to arrive at one of the several possible motifs (sequential approach), whereas the latter approach is a one-step process in which all the peptides to be tested are screened concurrently and often result in multiple different motifs (parallel approach). At the completion of the iterative approach screening (the alternative of which is the concept of positional scanning libraries (35)), the complete structure of the positive ligand is known (derived from the synthetic algorithm), whereas the structure of the positive beads isolated in a Selectide screen remains unknown until they are sequenced.

In general, the screening of the biological library relies on a binding assay. That is, the positive phages or protein-bound plasmids are isolated by affinity binding, and often multiple cycles of binding are needed to enrich the positive leads. The iterative approach works best in a solution-phase screen (8–10), although in principle should also

FIG. 1. Representative color micrograph of a bead-binding library screen. The peptide beads that interact with the acceptor–alkaline phosphatase complex turn turquoise upon incubation with the BCIP substrate (see Materials and Methods for details). (a and b) Easy identification of one color bead in a background of thousands of colorless beads (a, lower power; b, higher power). (c) A collection of positive beads with different color intensity.


a
b
c

work on a solid-phase binding assay (6). Selectide approach, on the other hand, offers various possible options for screening. The most widely studied method of screening is the bead-binding assay, with which we have detected ligands for monoclonal antibodies (11, 15, 36, 37), streptavidin (11, 12, 38), avidin (12), protein G, MHC class I molecules, platelet-derived gpIIb/IIIa, thrombin and factor Xa, cytokine receptors, and even small molecules (unpublished data). The bead-binding assay has several useful features: (i) it is extremely rapid and it takes only a few hours to screen 10^7–10^8 beads, (ii) the color intensity of the bead is generally proportional to the binding affinity of the ligand (unpublished data), one may, therefore, select the bead with the appropriate color intensity for structure determination, and (iii) the library may be reused multiple times for different probes. Figures 1a and 1b show the easy identification of one color bead in a background of thousands of colorless beads. Figure 1c shows a collection of positive beads with different color intensities.

We have extensively tested the one-bead one-structure process on an anti-insulin monoclonal antibody, and the obtained data are given here as an example of the results which may be expected from this method. This murine antibody (clone AE906) recognizes a discontinuous epitope on insulin, with a binding constant of 0.01 μM. In addition to an all L-amino acid library, we have also screened two all D-amino acid libraries (hexamer and octamer), disulfide cyclic libraries (C-(X)n-C), and a type II turn library (XXXpxXXX) where "X" represents L-amino acids, "x" represents D-amino acids, and "p" represents D-proline. The results are summarized in Table 1. Several important conclusions can be drawn from this study. First, with the parallel approach employed in the Selectide process, multiple distinct motifs can sometimes be identified. This is clearly illustrated by the four motifs identified from the linear all "L" hexamer screen: _ W _ _ GF, FDW _ _ _, FNW _ _ _, and QDPR. Second, the length of the library could be very important for a specific motif. For example, _ W _ _ GF motif was identified only after a hexamer or longer library was screened. Third, a specific motif can be identified even though not every possible peptide is represented in the library screened. For instance, the motif _ _ _ _ _ W _ _ GF could be identified from a deca-library even though only a minute percentage of all possible permutations (19^{10}) was screened. Last, when specific secondary structure is incorporated into the library, a completely different motif may be discovered. For example, C _ _ _ _ HGVQC was identified only from a cyclic but not a linear octa-library.

We have recently modified the screening by using an orthogonal two-stage release method to release the peptides into solution for standard biologic assays. In certain instances we have combined the releasable-solution-phase assay with the bead-binding assay (13). This orthogonal screening approach may minimize the number of false positive beads. More recently, we screened random libraries for a potential substrate for cAMP-dependent protein kinase by covalently radiolabeling the library with [γ-^{32}P]ATP, and we were able to identify the substrate motif (39) as described in the literature. With ingenuity in designing the appropriate detection system, it is not difficult to envision that by applying the one-bead one-structure concept, chemical structures from a random chemical library with the desired physical properties, electronic properties, light absorbance properties, chemical properties, and even enzymic activities can readily be isolated. For instance, specific cofactors may be potentially incorporated into a constrained library and screened for beads with a desired enzymic activity.

In this paper we have described in length the synthesis and screening of peptide libraries using the bead-binding immunohistochemical method. As mentioned earlier, in addition to being enzyme-linked, the probe used in the screening could also be fluorescent-labeled or radiolabeled. With an appropriate fluorescent probe and a fluorescent activated cell sorter, we have demonstrated that positive ligands of biologic interest can be rapidly isolated (unpublished results). A fluorescence activated cell sorter has also been used by others (40) in the screening of nucleotide-tagged peptide libraries. The use of radiolabeled probe for screening a one-bead one-peptide library has recently been reported (41). However, from our experience, the method is often slow and insensitive and it offers no advantage over the immunohistochemical method described in this paper. On the other hand, the radiolabeled probe, when used as a labeled donor group for covalent modification, had worked very well, leading to the direct identification of the modified bead (39).

Nonspecific binding is a potential problem in library methods. Peptide sequencing and subsequent resynthesis and testing of the positive leads are often the rate-limiting steps. Therefore, great care has to be taken to minimize nonspecific binding, so that there is a reasonable assurance that the positive bead isolated is a true positive prior to submission for microsequencing. Common general methods such as using nonionic detergent, using blocking agents such as BSA or gelatin, under a basic or acidic condition, including chaotropic agents, etc., have been very useful to reduce many of the nonspecific bindings. Other tricks such as sequential screening of the library in the presence or absence of a specific blocker (e.g., a known potent ligand) with a dual-color substrate system often help to differentiate specific binding from nonspecific binding (Lam *et al.*, manuscript in preparation). A two-step orthogonal screening approach with a different secondary antibody or probe also helps to accomplish the same goal. Since peptide is rather stable on the bead, positive beads can also be recycled and restained under var-

ious conditions, and their specificity confirmed prior to microsequencing.

Since peptide ligands on the surface of the beads are multivalent, the valency of probe greatly affects its affinity to the bead. The multiligand interaction phenomenon has also been observed on the filamentous peptide library where there are five copies of peptide-grafted PIII proteins on the tip of each phage. High affinity ligands were isolated only when a univalent antibody (Fab) was used in the panning of the phage library (42). In our bead-binding assay, we have evidence that streptavidin (tetramer) indeed binds to the HPQ beads with multimeric interaction (unpublished data).

The number of expected positive beads depends on the number of so-called "critical residues" in the peptide sequence (or critical pharmacophores in nonpeptidic structure), i.e., residues required for minimal observable (under the given conditions) binding. This number can be calculated according to the formula

$$n = P \times (\text{Sample}/\text{AA}^{\text{ncrit}}),$$

where n is the number of expected positive hits, P is the placement factor, i.e., the number of possible placements of the given motif in the peptidic chain, Sample is the screened number of beads, AA is number of amino acids (building blocks) used for randomization, and ncrit is the number of critical residues. Obviously, the expected number of positive hits depends on the number of tested beads, but it does not depend on the length of the library. Therefore, even very incomplete libraries, e.g., a library of decapeptides, can provide a reasonable number of positive beads if only three to four residues in the sought-after peptide are critical for the binding under the given conditions.

We can demonstrate the validity of this formula with the example of screening an octapeptide library composed from L-amino acids for binding to anti-β-endorphin antibody, streptavidin, and anti-insulin antibody. From 2 million beads screened for binding to anti-β-endorphin (0.01% of all possible combinations) we should have found $n = 1 \times 1 \times 2 \times 10^6/19^3 = 291$ positive beads since we knew that three residues are critical for binding (Tyr in position 1, Gly in position 2, and Phe in position 4) and that the motif has to be placed at the amino terminus (15). From the same number of beads screened for binding to streptavidin, we should have seen $n = 2 \times 2 \times 2 \times 10^6/19^3 = 1164$ positive beads since we know that there are two three-residue motifs (His–Pro–Gln and His–Pro–Met) and they should be placed close to the carboxy terminal end of the sequence (placement factor 2) (12). The number of beads actually observed corresponded both in the case of anti-β-endorphin (160 beads) and in the case of streptavidin binding (~800 beads). Anti-insulin binding provided ~200 positive beads. This number indicated three critical residues. This prediction was verified by sequence analysis of sequenced beads which revealed consensus Xxx–Xxx–Xxx–Trp–Xxx–Xxx–Gly–Phe (16, 37).

In certain experiments, one may face the difficulties of too many true positives (e.g., >1000). One may then elect to screen the library under a more stringent condition. There are five general methods of increasing the stringency of screening: (i) to use a monovalent probe; (ii) to use a very low concentration of probe; (iii) to add some competing ligands to the binding buffer; (iv) to incorporate some chaotropic agents into the binding buffer; or (v) to use an acidic or basic condition.

The rate-limiting step in the Selectide process is microsequencing. With the current rate, three peptides (heptamer) can be sequenced each day per protein sequencer. In certain instances, we may bypass this bottleneck by performing multiple sequencing (16). In the case where only one motif is present, we found that by combining 10 to 30 positive beads for concurrent microsequencing, valuable information can be derived rapidly. This shortcut approach has proven to be extremely useful for the identification of (i) the anchor residues for MHC class I molecules (Smith and Lam *et al.*, manuscript in preparation), (ii) the HPQ motif for streptavidin; and (iii) the YG _ F motif for anti-β-endorphin monoclonal antibody (16). Results of multiple sequencing of 56 beads identified as positively reacting in the anti-β-endorphin assay are given in Fig. 2. The absolute requirement of amino acids in positions 1, 2, and 4 is clearly visible, together with the relative unimportance of the amino acid residues in positions 3 and especially 5. Multiple se-

TABLE 1

Peptide Motifs Identified in the Primary Screen of an Anti-Insulin MoAb

Tetra	Q N P R	All D 9-mer	_ _ _ q _ G s _ G
Penta	F N W _ _	Cyclic 9-mer	C W _ _ G F _ _ C
	F D W _ _		
	_ Q D P R	Cyclic 10-mer	C _ F _ W _ _ G G C
			C _ _ _ _ H G V Q C
Hexa	_ W _ _ G F		
	F D W _ _ _	Cyclic 11-mer	C Q D L Y _ _ _ _ _ C
	F N W _ _ _		
	_ _ Q D P R	"Turn-library"	_ F Q P _ R P _
			_ F Q P _ I P _
Octa	_ _ _ W _ _ G F		
	_ _ _ _ _ _ G F		
Nona	_ _ _ G F _ _ G F		
Deca	_ _ _ _ W _ _ G F		
15-mer	_ _ _ _ _ _ _ _ _ _ W _ _ G F		
	_ F D W _ _ _ _ _ _ _ _ _ _ _		
	_ _ _ _ _ _ W _ _ G F _ _ _ _		

quencing, originally designed for the characterization of peptides bound to MHC molecules (43), was used recently by others for library analysis (44).

Ligands isolated from the primary screen often have low to moderate activity. With our experience on several targets, particularly the anti-insulin monoclonal antibody system in which it recognizes a discontinuous epitope, we were able to generate secondary libraries based on the motif of the primary leads and isolate ligands of considerably higher affinity (16, 37). For example, during the initial primary screen, a consensus motif with three to four amino acid residues may be recognized. They could either be adjacent to each other or with intervening "nonconsensus" residues. Based on the consensus residues, a secondary library may be synthesized by fixing those consensus residues or using only several closely related amino acids at those residues while randomizing the rest of the "nonconsensus" residues and by extending the length of the peptide at the carboxyl and/or amino termini. The secondary library will then be screened under a more stringent condition to select for ligands with higher affinity. This process can be repeated for further optimization of the secondary leads.

Most of our work has been on screening peptide libraries for various targets. Over the past year, we have developed a peptide encoding method for the synthesis and screening of nonpeptide chemical libraries or small organic libraries (19, 23, 24). In this approach every synthetic step of building a nonpeptidic or nonsequenable multiplicity is accompanied by the attachment of one or several amino acids into an independent attachment point. This "peptidic tag" or coding sequence can then be sequenced by the application of standard techniques (Edman degradation or mass spectroscopy (45 and references quoted therein)), and the structure of the nonpeptidic molecule can be deciphered. Alternatively, the peptide tag is built in such a way that it can be analyzed by one-step Edman degradation and HPLC analysis (the so-called "bar coding" approach; Sepetov *et al.*, unpublished). The idea of encoding the libraries came from the phage technology, in which the structure of the peptide displayed on the phage surface is decoded by sequencing the DNA coding the phage surface protein (1–4). Coding of peptidic libraries by nucleic acid was suggested by Brenner and Lerner (46, 47) and further developed by others (40). The detailed description of this technology can be found in this issue (48). Coding by nucleic acid has the disadvantages of (i) new chemistries needed for the independent syntheses of peptide and DNA, (ii) sensitivity of DNA to a variety of chemical reagents, which are to be used during the building of nonpeptidic libraries, and (iii) extremely long coding sequences needed due to the small number of blocks available for coding. The clear advantage, however, is the exquisite sensitivity of the PCR technique that allows one to use very small beads (that is, very large libraries) in the screening (40). In contrast, even though less sensitive, the chemistry for coding by peptidic tag is straightforward and compatible with many potentially useful reactions for nonpeptide library generation. Coding by peptidic tag was suggested for the libraries containing

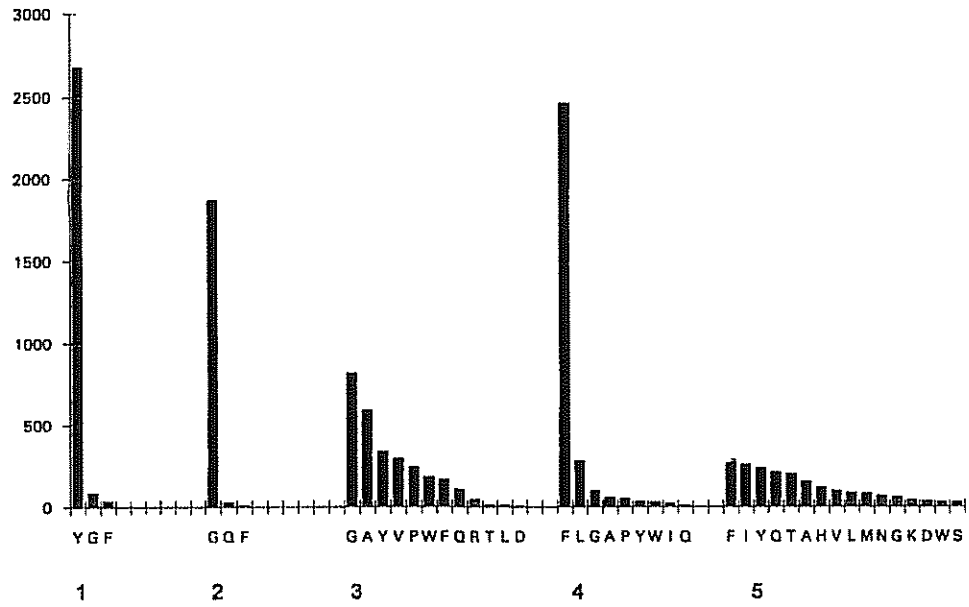


FIG. 2. Result of simultaneous multiple sequencing of 56 beads identified as positively reacting with anti-β-endorphin antibody. X-axis, amino acids identified in five cycles of sequencing; Y-axis, amount (in pmol) of amino acid residues identified.

unnatural amino acids (32) or nonpeptidic structures (19). More recently, tagging by halogenated derivatives of carboxylic acids followed by gas chromatographical analysis was described (49).

All techniques using coding, in which the coding sequence is available for interaction with the macromolecular acceptor of interest, suffer from the uncertainty of whether the acceptor molecule recognizes the testing sequence, the coding sequence, or the combination of testing and coding structures. In any case, it is always advisable to resynthesize both coding and testing structures independently and determine their binding with the acceptor.

Arrangement of the coding and screening structures, in which only the screening structure is available for interaction with the acceptor would be advantageous. We have used the properties of one of our polymeric carriers, polyoxyethylene-grafted polystyrene TentaGel, to create this arrangement. The interior of TentaGel beads is not available to the macromolecular target due to the microporous structure of this polymer. Therefore we can modify selectively the surface of this carrier and build the screening structure only on the bead surface and the coding structure within the interior of the bead. We have employed enzymatic "shaving" for selective surface modification (50), using chymotrypsin as the shaving agent and sequence Boc-Phe-Gly-Ala-Gly-TG as the substrate.

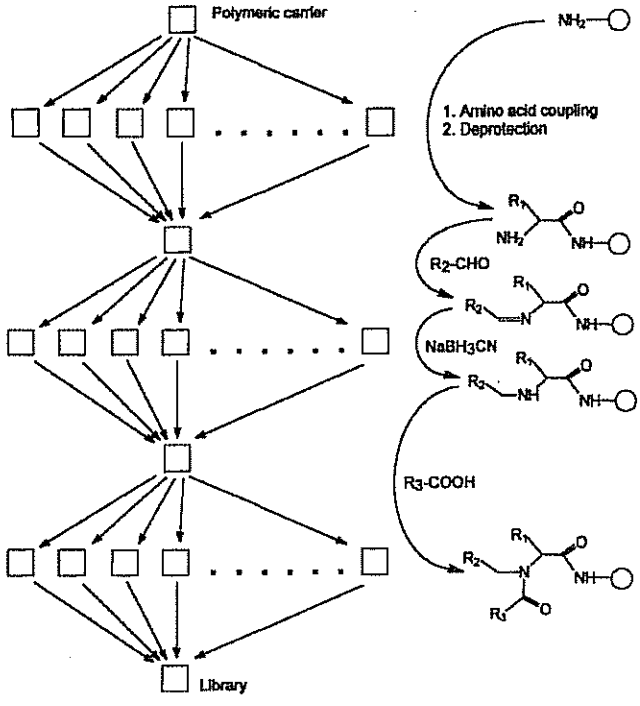


FIG. 3. Synthetic scheme for the library of small organic molecules.

The potential for small organic libraries is enormous. Thousands of building blocks are now available. We are no longer limited to the 50 or so amino acids. For instance, a small organic trimer with 100 possible subunits in each coupling step will generate a diversity of 10^6 and certainly is feasible experimentally. Ligands isolated from such libraries may have a much better chance of being orally active as well as being able to penetrate a living cell, resulting in a better drug candidate. Figure 3 shows the construction of a simple nonpeptidic library using some readily available amino acids, aldehydes, and carboxylic acids as building blocks. Despite the low number of structural permutations in this library, specific ligands were already identified in some of our screening projects.

CONCLUSION

In addition to facilitating the drug discovery process, we feel that the Selectide process can generate very useful information for the fundamental understanding of molecular recognition. In addition, it also provides a powerful research tool for various scientific disciplines.

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