Chapter XX

Mixtures of molecules vs mixtures of pure compounds on polymeric beads

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Two methods of screening, one-bead-one-compound synthetic strategy followed by on bead binding assay, and iterative screening in solution, were compared in assays designed for finding inhibitors of an enzyme of the coagulation cascade, factor Xa. It was found hat the results of iterative screening depend on the starting point of iteration, and may miss the best ligand contained in the library. The lead found by the iterative technique was structurally different from the inhibitors discovered by the one-bead-one-compound technique.

There are basically two methods for screening to exploit molecular diversity -- screening of individual compounds or screening of synthetic mixtures (for recent reviews on library techniques see e.g. (1-3), or the dynamic database on Internet (4)). Library technology based on the one-bead-one-compound method (5, 6) utilizes the first method for screening in the case of bead binding based screening. In this case each bead represents an individual compound and the screening result is independent of the remaining beads present in the mixture. Screening of one-bead-one-compound (OBOC) libraries for activities in solution requires partial release of the compound from the polymeric support, and is best performed with mixtures of gradually decreasing complexity (7, 8). Sreening in solution is most commonly an iterative approach (9, 10). In this case mixtures with defined features (one or two conserved building blocks, where the building blocks are amino acids for peptide libraries) are synthesized and building blocks leading to biological activity of the mixture are used as the starting point for the synthesis of the next generation library After several steps of iteration, the structure of the most active compound is defined. Advantages of mixture screening include: (i) independence of the analytical techniques, which are critical for successful one-bead-one-compound technique, (ii) no limitation in the number of compounds screened -- the one-bead-one-compound technique is clearly limited by the number of beads used for synthesis and screening, and, most importantly, (iii) independence of the biological test. In the list of disadvantages of screening of mixtures we can name: (i)

their higher synthetic demands, (ii) the fact that independent leads will seldom be discovered, and (iii) the fact that a positive response may be the result of the synergy of several structures. One-bead-one-compound techniques are synthetically very simple, and all independent leads are revealed in a single screening step. However, not all biological tests can be modified for on-bead screening. In this manuscript we compare the results of two different approaches, for the screening on a target of real value -- factor Xa.

We have screened a number of one-bead-one-peptide libraries for ligands of factor Xa, and we have found a number of potent inhibitors of this important enzyme of the coagulation cascade (11). In addition we prepared an iterative library of tetrapeptides and acylated tripeptides (structure O$XX) on hydroxy-TentaGel, in which the first building block (O) was "defined" -- selected from 9 amino acids, 38 carboxylic acids and hydrogen. The second position ($) was "semi-defined" - - we have used 8 groups of five amino acids which were coupled as mixtures. The third and fourth position (X) was completely randomized -- by 21 and 23 amino acids respectively. We have therefore synthesized 384 pools of 2,415 compounds in each pool (927,360 compounds in total). The compound mixtures were cleaved from the carrier by 0.1 M NaOH, solution was neutralized by acetic acid, and the mixtures lyophilized. The pools were dissolved in 1 ml of screening buffer, generating a solution totaling 5 mM of screened compounds, in which each individual component was present at a concentration of 2.1 μM. At the same time we synthesized several mixtures containing peptide ligands defined earlier in a mixture format identical to the format of the iterative library as positive controls for the screening experiment.

Results of the screening of the library are given in Figure 1. The first column is the activity of the buffer with no added compound. Listed in columns 2 through 4 are the activities of synthetic mixtures containing three different inhibitors in the library format -- the positive controls for the screening experiment. The remaining columns present the results of seven groups (out of 48 groups defined by the last position) of mixtures. The groups shown here are those in which the mixture synthesized as positive controls should be included, together with those in which significant activities were found. For example, the third column (subgroup containing tyrosine, aspartic acid, lysine, arginine, and D-isoleucine) in group 11 (group defined by coupling acetyl as the last randomization building block), should contain the known lead Ac-Tyr-Ile-Arg in the mixture, and its activity should be comparable to the activity of the positive control mixture represented in column 4. As can be seen, activities comparable to the activities of control mixtures were found in groups 7, 10, and 11. Activities similar to those represented by groups 8 and 9 were not considered significant. Our original intention was to follow the iterative protocol of leads from groups 10 and 11 where the known ligands were expected, however, the activities of two subgroups from groups 18 and 46 prompted us to follow the alternative strategy, and deconvolute these, obviously more active, unexpected leads.

Two step deconvolution of the lead from group 46 gave the structure Aaa-Bbb-Ccc-Ddd (the full structure has not yet been released for publication) with Ki=0.65 μM. This activity is actually significantly lower than the activity of an unrelated lead that we have previously discovered, Ac-2Nal-Chg-Arg (Ki=0.27 μM), which was synthesized as one of components of the positive library mixtures (column 8 in group 11). This result clearly illustrates the danger inherent in the use of iterative techniques - the most active compound is not identified in the screening due to the fact that its most important

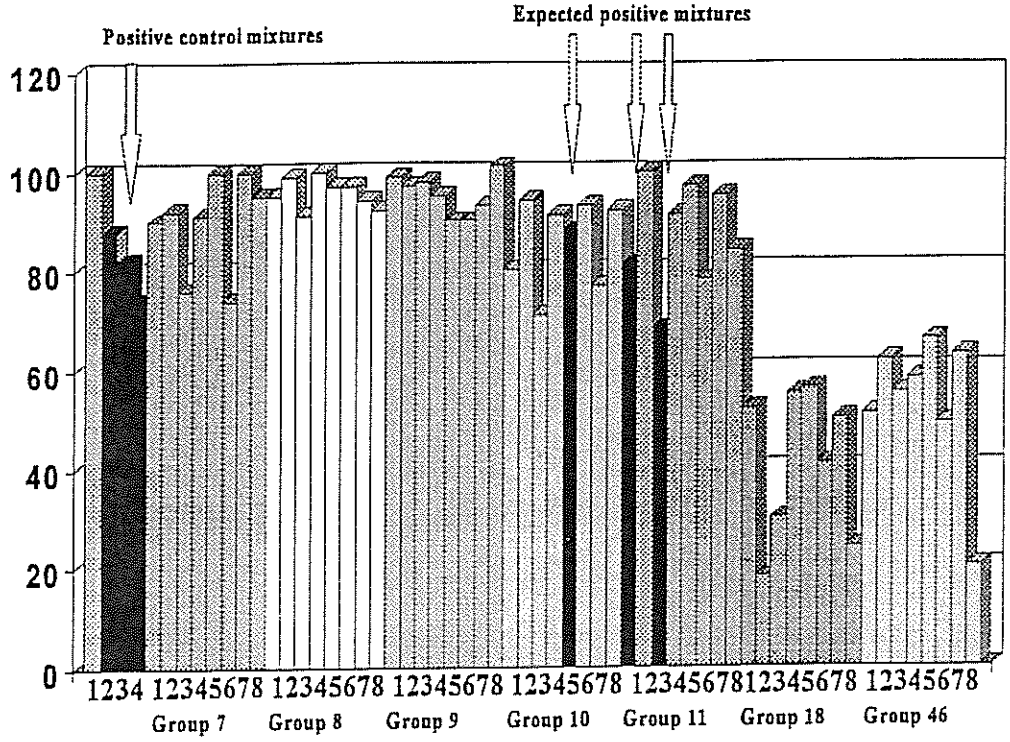

Figure 1. Results of screening of seven representative groups from iterative library. X axis -- controls and individual mixtures, for definition see text; Y axis -- % of response (hydrolysis of chromogenic substrate by factor Xa)

features are not defined in the early steps of iteration. The iterative pathway then follows the structural type which may not be optimal. We tested this hypothesis using model libraries having very different starting points for iteration. The results of the testing of model mixtures are given in Table I. Both the new structure, Aaa-Bbb-Ccc-Ddd, and the known ligand, Ac-2Nal-Chg-Arg, were used as the templates for synthesis of "left" and "right iteration" models. In these models the leftmost (or rightmost) building block of the structure was defined, the second bulding block from the left (or right) was semi-defined (mixture of five building blocks was used in this position), and the last two building blocks were completely randomized. As can be seen from the activities in the table, left-iteration model mixture of Aaa-Bbb-Ccc-Ddd is more active than left-iteration model mixture of Ac-2Nal-Chg-Arg. For the right-iteration model mixtures just the opposite is true. The left-iteration model mixture of Aaa-Bbb-Ccc-Ddd is three times more active than right-iteration model mixture of the same motif, whereas right-iteration model mixture of Ac-2Nal-Chg-Arg is three times more active than its left-iteration model mixture. Obviously, the critical residues in both motifs are on opposite sides of the molecule, and it is not surprising that iteration starting from the left side leads to the Aaa-Bbb-Ccc-Ddd motif, which would not be found with iteration starting from the right side (note that iteration from the right side is technically substantially more difficult than iteration from left side). The one-bead-one-compound method found the best motif due to the fact that individual structures rather than partially defined mixtures were used in screening. We can only speculate at this moment what would be the result of screening of the "library of

Table I. Activities of leads and model mixtures

Compound/Mixture	Structure	Activity[a]
OBOC lead	Ac-2Nal-Chg-Arg	Ki = 0.27 μM
Left iteration model	Ac-(2Nal)-Xxx-Xxx	22 %
Right iteration model	Xxx-Xxx-(Chg)-Arg	45 %
Iteration lead	Aaa-Bbb-Ccc-Ddd	Ki = 0.65 μM
Left iteration model	Aaa-(Bbb)-Xxx-Xxx	27 %
Right iteration model	Xxx-Xxx-(Ccc)-Ddd	11 %

[a] Ki value, or percent of inhibition at 100 nM concentration

libraries", which may represent the iterative library with all possible combinations of two or three building blocks (12). It would also be interesting to compare the results for positional scanning libraries (13), and for the newly described technique of non-iterative deconvolution, using multidimensional orthogonal mixtures of compounds prepared during robotic synthesis (Felder and Kris, IBC Symposium on Combinatorial Chemistry, Jan 24, 1996, San Diego, CA).

In conclusion, the iterative technique produced an alternative, structurally dissimilar inhibitor of factor Xa, which may prove valuable in the development of the new anticoagulant. The one-bead-one-compound method found the compound with the highest activity, yet may overlook alternative structures. Importantly, the results from iterative techniques depend on the position from which the iteration starts and may miss the most active compounds. It may be advisable to apply both screening techniques for those cases where the biological test is amenable to assay both by direct on-bead binding and by inhibition in solution.

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