Combinatorial Chemistry

Screening mixtures using affinity NMR

The synthesis of combinatorial libraries in mixtures offers considerable savings in time and resources. However, the biological testing of mixtures is problematic as it is often impossible to distinguish readily between mixtures containing a single potent compound and those containing several weaker compounds. New spectroscopic techniques offer a solution to this problem and a recent addition is the technique of affinity NMR [Lin, M. *et al. J. Org. Chem.* (1997) 62, 8930–8931].

The method depends on the observation that the diffusion coefficient of a receptor-bound compound will be significantly different from the unbound compound. Using pulsed field gradient techniques, the principle has been demonstrated using a mixture of four carboxylic acids in the presence of the artificial 'receptor' hydroquinone 9-phenanthryl ether. One advantage of this technique is that binding to the receptor can be detected by the NMR spectrum of the ligand itself, leading to direct analysis of the active compound.

'Tuning' the system

By changing the concentration of the receptor, the system may be 'tuned' for the most potent compounds. In this example, by using only 0.35 equivalents of the hydroquinone 9-phenanthryl ether, the most strongly binding compound, dichloroacetic acid, was observed in the NMR spectrum. By increasing the ratio of the 'receptor' compound, the other carboxylic acids appear in a sequence that reflects their receptor affinity. Affinity NMR is currently being applied to the analysis of mixtures tested against real biological targets.

Microdroplet combinatorial libraries

The mix and split synthetic approach to combinatorial libraries allows the generation of huge numbers of compounds. But to use the library effectively requires methods for the screening of the individual library components. In the past this has been achieved either by distributing individual beads into reaction wells for screening or by distributing beads onto an agar plate for whole-cell assays. A new approach for the screening of such libraries uses the generation of nanodroplets that contain both beads and cellular targets [Borchardt, A. *et al. Chem. Biol.* (1997) 4, 961–968]. Distributing single beads into droplets of very small volume allows the production of relatively high test compound concentrations.

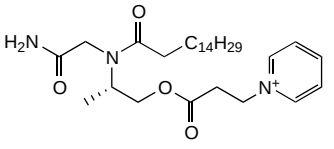
Engineering droplet size

A spray gun has been employed to generate a very fine mist of droplets of 50–200 nl, each containing a stochastic distribution of beads. The droplet size and bead concentration can be engineered such that the majority of droplets contain just one bead and thus constitute a separate screening experiment. The effectiveness of this approach was demonstrated by an experiment that used rapamycin to inhibit the growth of fungal cells. Rapamycin was attached to red-dyed beads through a light-sensitive linker, and these were mixed with colourless control beads. Droplets containing beads plus *Saccharomyces cerevisiae* yeast were generated and then irradiated to release rapamycin.

It was observed that yeast growth was inhibited in the droplets containing red beads, although growth was normal in droplets containing no beads or only colourless control beads. A separate experiment used rapamycin-resistant yeast cells deficient in an essential gene but transformed with a rapamycin-inducible form of that missing gene. In this case, the presence of rapamycin generated by photolysis from the beads resulted in the ability of the cells to undergo cell division. Growth of the yeast cells was observed in the majority of droplets containing red-dyed beads.

Further applications

The same group have used the microdroplet approach in a miniaturized array [You, A.J. *et al. Chem. Biol.* (1997) 4, 969–975]. A combination of photolithography and polymer moulding allowed the generation of 6,500 wells in one plate, each with a volume of 50–150 nl. As with the sprayed nanodroplet technique, beads and yeast cells could be readily distributed to each of the wells. A fungal growth inhibitor (**1**) previously identified through the deconvolution of a 125,000 member library was released photolytically from beads and was shown to prevent the growth of yeast colonies.



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Alternatively, bead libraries could be distributed into wells already containing mammalian cell cultures; this technique was used to demonstrate the podophyllotoxin-induced apoptosis of mink lung cells. The approach also holds promise for the testing of compound libraries against whole organisms such as nematode embryos.

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