Peptides for the New Millennium

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New technique for high-throughput synthesis of peptides, peptidomimetics and nonpeptide small organic molecule arrays

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Introduction

The high demands for quick supply of new peptides can be satisfied only by massive parallel synthesis. Parallel synthesis can also bring down the prices of custom peptides. We have developed a technique which can produce up to 768 peptides in one batch.

Results and Discussion

The synthesis employs classical solid supports, which are placed in the wells of shallow-well polypropylene microtiterplates. Eight plates containing 5 mg of solid support in each well are placed on the perimeter of a centrifugal rotor and fixed at a tilt of 9 degrees. The rotor can be spun at a speed which creates a relative centrifugal force (RCF) of 50 G at the perimeter, removing all contents of the microtiterplate well, with the exception of the "pocket" created by the tilt and centrifugation force (Fig. 1). We call this principle the ilted centrifugation [1].

The synthesis is performed in a very simple way: (i) solid support is distributed into individual vessels (3 to 5 mg/well); (ii) plates are placed on the perimeter of the rotor; (iii)

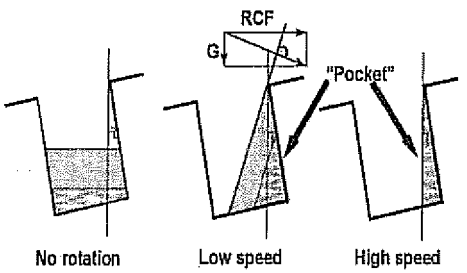

Fig. 1. Formation of the pocket in the well of a tilted plate during centrifugation.

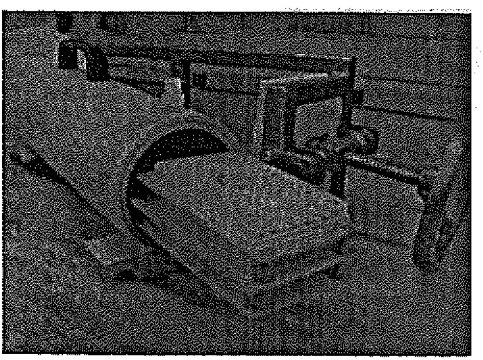

Fig. 2. Chamber for cleavage by gaseous hydrogen fluoride.

appropriate solvent is added by actuating a (motorized) syringe pump and delivering the solvent selected by a (motorized) selector valve through a 96 channel manifold; (iv) plates are shaken either by intermittently moving the rotor forward and backward, or by alternating periods of low speed rotation and stopping; (v) solvent is removed by centrifugation (volume in excess of "pocket" volume spills over the edges of the wells); (vi) steps iii to v are repeated as many times as needed; (vii) solutions of protected amino acids and coupling reagents are added by either manual pipetting or

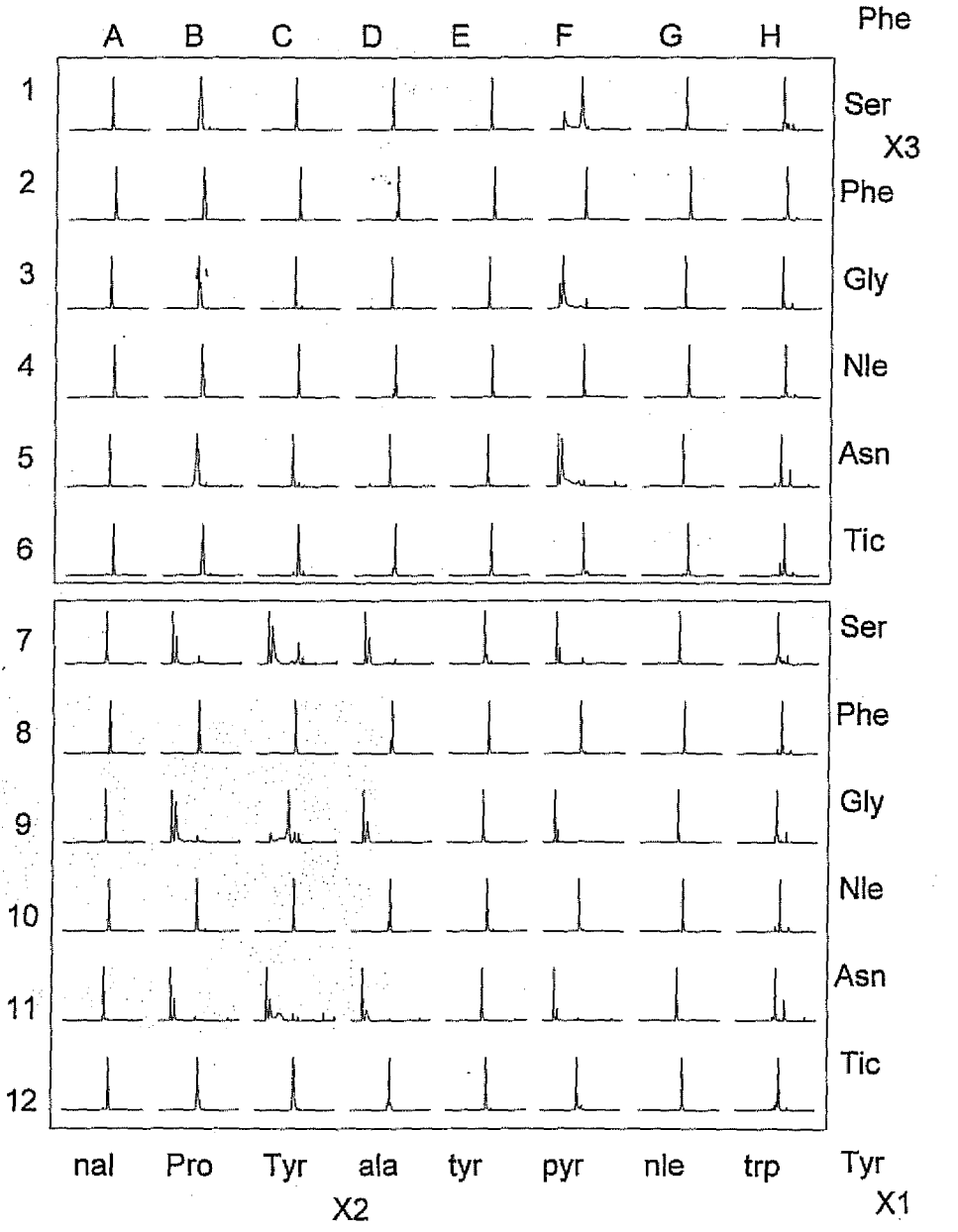

Fig. 3. HPLC traces of X1-X2-X3-Arg-NH_2. The major peak was proven to contain predicted molecular weigh product. Additional peaks in F1,F3,F5,B7,C7,D7,F7,B9,D9,F9,B11,C11,D11, F11 (column break through - too large injection), D2,B3,D8, and D10 (conformational equilibrium) contained a product with identical molecular weight to the major peak.

utilizing integrated x-y-z pipetting systems; (viii) plates are incubated with occasional shaking (as described in iv) until the coupling is completed (a convenient way of monitoring the progress is the use of bromophenol blue [2], coupling completion is determined by the loss of blue color in the well); (ix) liquid is removed from the wells by centrifugation, and washing is performed as described in (iii) to (vi); (x) deprotection reagent (piperidine/DMF) is added by 96 channel manifold; (xi) plates are incubated for 20 min; and (xii) after washing [as in (iii) to (vi)] the plates are ready for the next step of the synthesis. In all steps of the synthesis the resin must sediment in the particular solvent system – if the resin would float, it would be lost by centrifugation. The floating problem can be solved by dilution of the solvent with a cosolvent of lower density, or by evaporation of the solvent before the next step. At the end of the synthesis, the plates are dried and cleavage from the support is achieved in the HF chamber (Fig. 2) by the action of gaseous hydrogen fluoride [3,4]. We have found the two step deprotection and cleavage advantageous. In the first step the side-chain protecting groups are removed using a deprotection reagent (TFA with appropriate scavengers – the chemical link to the solid support must be stable at this stage; we have used benzhydrylamine resin – alternatively safety catch linkers can be applied [5]). In the second step the link to the resin is cleaved by gaseous HF.

We have applied tilted centrifugation for the synthesis of several thousands of peptidic and nonpeptidic molecules. As an example, Fig. 3 shows HPLC (MS total ion current detection) of products prepared in one plate of a synthesis which consisted of 768 analogs of opiate receptor ligands. All analogs shown contained arginine, which usually creates problems in sequences also containing tryptophan [6]. As illustrated in the figure, the two step deprotection led to acceptable results. Similar results were reported in parallel synthesis and two step deprotection of 576 betides, peptides containing a high proportion of beta amino acids [7]. We have demonstrated successful synthesis of both short and long peptides (up to 21 mers), containing an array of unnatural residues, and we believe that tilted centrifugation synthesis can revolutionize the parallel synthesis of peptides, making them as available and inexpensive as oligonucleotides are today.

Acknowledgment

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