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General method for rapid synthesis of multicomponent peptide mixtures

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A method is suggested for the synthesis of multicomponent peptide mixtures. The method is a solid phase synthesis modified in order to give a closely equimolar mixture of peptides with predetermined sequences. The main point of modification is that before every coupling cycle the resin is divided into equal parts and each portion is coupled with a different amino acid. Then the portions are mixed and before the next coupling cycle the resin is again distributed into equal portions. The method is illustrated by the synthesis of a mixture of 27 tetrapeptides and that of 180 pentapeptides.

Key words: peptide mixtures, synthetic; peptide synthesis, new method; peptide synthesis, solid phase; peptides, electrophoretic identification; peptides, HPLC separation

Due to the outstanding importance of peptides in biological processes there is an increasing need for synthetic peptides in a variety of applications. Although the introduction of the solid phase method (1) and its automation have considerably speeded up the synthetic procedure itself, the one by one synthesis of peptides still seems to be slow to comply with the need. A possible strategy to improve the productivity of the synthetic methods is the simultaneous synthesis of two or more-even several hundreds of-peptides. Different methods have been developed to achieve this goal. van Rietschoten et al. (2) succeeded in synthesizing two peptides on two easily separable resins. Gorman (3) constructed a multi-vessel apparatus and successfully applied it for the simultaneous synthesis of four peptides. The multiple continuous-flow solid phase method devised by Krchnak et al. (4) made possible the synthesis of a decapeptide and its nine omission analogs in a single run. Geysen et al. (5) synthesized 208 hexapeptides on polyethylene rods and tested them without removal from the solid support. In the remarkable method of Houghten (6) 40-80 peptides were simultaneously synthesized on 40-80 portions of resin placed in solvent-permeable

bags. Frank & Döring (7) applied paper discs as solid support in their synthesis and the coupling operations were carried out on 100 discs at a time. Much labor can be saved by using simultaneous synthetic methods, which is well demonstrated by Houghten's experiments in which 260 different 13-residue peptides were synthesized in less than 4 weeks.

Further improvement can be expected in the efficiency of the synthesis if one compromises by using peptide mixtures instead of individual peptides. Tjoeng *et al.* (8) succeeded in synthesizing mixtures of four to seven oligopeptides in a single run. The mixture was synthesized on solid support by using, in one of the coupling cycles, a mixture of four to seven acylating amino acid derivatives.

By exploiting the additional possibilities inherent in the Merrifield method, a new synthetic strategy can be introduced assuring, besides a dramatic reduction in the number of the coupling cycles, the closely equimolar formation of the components of the peptide mixture (9).

The principle of the method. A mixture of a large number of peptides, each of them containing the same number of residues but different sequences (which can be deduced from that of a parent peptide by varying amino acids in all or several positions), can be synthesized in a single run. A normal solid phase synthesis is carried out, but before every coupling step the resin is

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Abbreviations follow the recommendations of the IUPAC-IUB Joint Commission on Biochemical Nomenclature (*European J. Biochem.* **138**, 1984, 9–37).

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divided into equal parts (the number depending on the number of amino acids intended to vary in that particular position). Each portion is coupled with the desired amino acid, and the samples are then mixed. The number of the components in the mixture synthesized this way is given by the product of the numbers of the amino acids varied in the different positions.

MATERIALS AND METHODS

Reagents and solvents were products of Fluka AG (Buchs, Switzerland). Boc-amino acids were purchased from Reanal (Budapest, Hungary). Side chain protecting groups were: OBzl for Glu and Z for Lys. Boc-Ala-resin (0.74 mmol Ala/g) was prepared from Bio-Beads S-X1 chloromethyl resin (Bio-Rad Laboratories, Richmond, CA) by Gisin's method (10).

Solid phase synthesis. The coupling protocol of Gutte & Merrifield (11) was adapted with slight modifications: diisopropylcarbodiimide (12) and 1-hydroxybenzotriazole (13) were used; the Boc-amino acids were added in 100% molar excess; the activated Bocamino acid derivatives were dissolved in dichloromethane-dimethyl formamide mixture (3:1, v/v). The progress of the coupling reaction was followed with Kaiser's ninhydrin test (14).

Mixing and portioning of the resin samples were performed on Boc-protected peptidyl resins. The different resin samples (ca. 120 mg of each) were suspended in 10-10 mL of dimethylformamide and poured into a common vessel. The mixture was shaken for 10 min and, to avoid sedimentation, quickly divided into equal volumes.

Cleavage of peptides from the resin (50 mg) was carried out by trifluoromethanesulfonic acid (15). The reaction mixture was filtered and washed with trifluoroacetic acid into 25 mL dry ether. The mixture was allowed to stand overnight at -20° C, then the precipitate was collected by filtration, washed twice with ether and dried over KOH, then over P₂O₅.

HPLC separation of the peptides was performed on a Vydac 218TP54 C18 ($25 \text{ cm} \times 2.1 \text{ mm}$, i.d.) reversedphase column using a Beckman system (Model 421 Controller, Model 340 Organizer). Elution was isocratic at 100% A (0.1% aqueous trifluoroacetic acid) for 5 min; then followed by a linear gradient of 0–20% B (90% acetonitrile containing 0.1% trifluoroacetic acid) for 20 min, and 20–50% for an additional 20 min. The flow rate was 1 mL/min. The peptides were detected at 214 nm (0.5 AUFS).

Sequential degradation was carried out on a gas-phase sequencer built at the City of Hope according to the method of Hawke *et al.* (16) using the continuous-flow

reactor of Shively *et al.* (17); the phenylthiohydantoin amino acids were identified by using an on-line reversed-phase HPLC system.

Two-dimensional paper electrophoresis. The samples of the peptide mixtures were applied to a 30 cm band on Whatman 3 MM paper (0.3-0.4 mg/cm), together with reference markers of taurine and leucine methyl ester (50 nmol/cm) on both sides. Electrophoresis was accomplished on a horizontal cooled plate apparatus (Labor MIM, Hungary) at 32 V/cm. The first run was made at pH 6.5 for 2 h, using pyridine acetate buffer (18). After drying, two side strips were cut out and stained with cadmium-ninhydrin (19). A guide strip of 2.5 cm (parallel to the side strips) was excised and sewn to a fresh sheet. Electrophoresis was performed, again with markers on the two sides, in a perpendicular direction at pH2.0 applying AcOH-formic acid solution as buffer (20). The peptide map was stained and the migration distances then measured.

For the preparation of the individual peptides, the bands were cut out from the first electrophoretogram, stitched to new sheets. Each sheet was subjected to electrophoresis at pH 2.0 for 2 h. The ninhydrin-positive strips were cut out and the peptides were eluted from the paper with dilute AcOH, then freeze dried.

RESULTS

Synthesis of a mixture of 27 tetrapeptides. The components of the mixture to be synthesized were designed to have Ala at the C-terminus and, in the remaining 3 positions, Glu, Phe and Lys were varied.

 TABLE 1

 Peptides formed on polymer (p) support as result of the three coupling steps in synthesis of 27 tetrapeptides

А-р			
Coupling step 1			
EA-p	FA-p	KA-p	
	Coupling step 2		
EEA-p	FEA-p	KEA-p	
EFA-p	FFA-p	KFA-p	
EKA-p	FKA-p	KKA-p	
	Coupling step 3		
EEEA-p	FEEA-p	KEEA-p	
EEFA-p	FEFA-p	KEFA-p	
EEKA-p	FEKA-p	KEKA-p	
EFEA-p	FFEA-p	KFEA-p	
EFFA-p	FFFA-p	KFFA-p	
EFKA-p	FFKA-p	KFKA-p	
EKEA-p	FKEA-p	KKEA-p	
EKFA-p	FKFA-p	KKFA-p	
ЕККА-р	FKKA-p	KKKA-p	

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