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Letter to the Editor

Noninvasive continuous monitoring of solid-phase peptide synthesis by acid-base indicator

Sir:

The method of Atherton *et al.* (1) for monitoring the progress of coupling in the solid-phase synthesis of peptides, which employs active esters of 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine as indicator, has proved very useful, but the colour change from yellow to colourless limits its applicability rather strictly. We have used this phenol derivative in an indicative amount in couplings effected by active esters of *N*-hydroxybenzotriazole (2). The limiting factor was the colour of the resin, which must be perfectly white.

We therefore attempted to find an alternative indicator that would protonate free amino groups on peptidyl resin and whose ionized form would be deeply coloured. Among the several indicators tested, bromophenol blue (3′,3″,5′,5″-tetrabromophenolsulfophthalein) displayed the best properties: the colour change from yellow (λ_{max} = 429 nm) to dark blue (λ_{max} = 600 nm) is remarkable and the sensitivity high (extinction coefficient ε_{600} = 91800). In the absence of free amino groups the resin exposed to the solution of bromophenol blue becomes yellow-orange and when free amino groups are present the colour turns deep blue. Peptide synthesis was performed in the following ways.

a) Batchwise synthesis. Synthesis was carried out in an ordinary shaker reactor. After the addition of the amino acid, hydroxybenzotriazole, and dicyclohexylcarbodiimide in dimethylformamide or dichloromethane, three drops of a 5% bromophenol blue solution in dimethylacetamide were added. The suspension turned dark blue. After the suspension in the reaction vessel turned greenish yellow, the next step of the synthesis was performed. All experimental details regarding the synthetic protocol, cleavage conditions and purification procedures were the same as described previously (3). By this method the following peptides were synthesized: deamino-oxytocin, deamino-1-carba-oxytocin, [2-D-tyrosine]deamino-1-carba-oxytocin, an antiparallel dimer of deamino-1-carba-oxytocin, [2-phenylalanine]-oxytocin, [[2-D-phenylalanine]oxytocin, [2-alanine]tocinoic acid, [2-alanine,3-alanine]tocinoic acid, [2-alanine,3-alanine,4-alanine]tocinoic acid, [2-alanine,3-alanine,4-alanine,5-alanine]tocinoic acid, and somatostatin.

b) Multiple continuous flow synthesis. The synthesis was carried out in a system of polypropylene flow reactors, adjustable for volume, each being initially charged with 400 mg *p*-methylbenzhydrylamine copoly(styrene–1% divinylbenzene) resin (contents of amino group 0.4 mmol/g). The detailed synthesis protocol is described elsewhere (2, 4). The concatenated flow reactors were disconnected before the condensation reaction. A polypropylene syringe was charged with 5 mL of a 0.2 M solution of the appropriate hydroxybenzotriazole ester in dimethylformamide and 0.1 mL of a 0.01 M solution of bromophenol blue in dimethylformamide. A part of this solution (2 mL) was injected into each reactor separately; 5 min later the rest of the solution was injected. After the disappearance of the blue colour the reactors were washed with dimethylformamide, re-concatenated, and the synthesis continued. A sample of the resin was checked by the ninhydrin test; failure to detect free amino groups by bromophenol blue was never observed. By this method the following peptide-amides were

synthesized: Val-His-Ala-Gly-Pro-Ile-Ala-Pro-Gly-Gln-Met-Arg-Glu-Pro-Arg-Gly-Ser-Asp-Ile-Ala (derived from p24 protein of HIV-1, content of the desired peptide in the crude mixture after HF cleavage — 76%), Pro-Ile-Pro-Gly-Pro-Leu-Pro-Ala-Gly-Gln-Leu-Arg-Glu-Pro-Arg-Gly-Ser-Asp-Ile-Ala (p24 of HIV-2, 83%), Ile-Gln-Asn-Phe-Arg-Val-Tyr-Tyr-Arg-Asp-Ser-Arg-Asn-Pro-Leu-Trp-Lys-Gly-Pro-Ala (p31 of HIV-1, 75%), Leu-Lys-Asp-Phe-Arg-Val-Tyr-Phe-Arg-Glu-Gly-Arg-Asp-Gln-Leu-Trp-Gly-Pro-Gly (p31 of HIV-2, 74%), Arg-Pro-Glu-Gly-Ile-Glu-Glu-Glu-Gly-Gly-Glu-Arg-Asp-Arg-Asp-Arg-Ser-Ile-Arg-Leu (gp41 of HIV-1, 72%), and Ala-Asn-Glu-Glu-Thr-Glu-Glu-Asp-Gly-Gly-Ser-Asn-Gly-Gly-Asp-Arg-Tyr-Trp-Pro-Trp (gp41 of HIV-2, 71%).

Two of the oxytocin analogs (deamino-oxytocin and deamino-1-carba-oxytocin) were synthesized using both this monitoring method and classical ninhydrin monitoring. The comparable results obtained (35 versus 33% yield of purified peptide, respectively, in the case of deamino-oxytocin) proved that bromophenol blue does not compromise the quality or yield of the prepared peptide.

The monitoring method presented is remarkably sensitive and can be used for following the coupling reaction in synthesis performed on an extremely small scale without the possibility of monitoring by classical methods ("tea bags" (5)) or upon alternative carriers (6) (polyethylene rods (7) etc.).

A full paper describing this monitoring procedure will be published elsewhere (8).

Viktor Krchňák, Josef Vágner*, and Michal Lebl+*
**Léčiva-Pharmaceuticals, 143 10 Prague 4,*
+ Institute of Organic Chemistry and Biochemistry,
Czechoslovak Academy of Sciences, 166 10 Prague 6,
Czechoslovakia

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