COLOR MONITORED SOLID PHASE PEPTIDE SYNTHESIS

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Completeness of the coupling is the pre-requisite of successful peptide synthesis performed on the solid support. Up to now usually the testing of coupling is performed on a sample of the synthetic material. However the sample cannot be used for the further synthesis, since free amino groups are destroyed. The only monitoring method which is non-destructive and continuous was described by Atherton et al.(1). This test is based on the color change of 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine from yellow to colorless depending on the presence of free amino groups.

We have shown (2) that for the coupling progress monitoring various acid-base indicators can be used, the best properties having been found for bromophenol blue. In the presence of free amino groups the carrier is colored deeply blue and after all amino groups were acylated the carrier turns to yellow. The intermediate stages are greenish-yellow. We compared the sensitivity of this monitoring method, with the classical ninhydrin test and we have found the new method superior. Bromophenol blue (BB) method can be used either qualitatively for the continuous monitoring of acylation progress or it can be used also for the quantitative determination of free amino groups (either on total volume or on sample analogously to the picric test). However, the greatest advantage of this monitoring is its applicability in the cases when no other monitoring method is feasible

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Table 1
Examples of Peptides Synthesized with the Use "BB" Monitoring

Peptide	Carrier[a]	Mode
Somatostatin	CMPS	Shaker
/Nle^5/EK	CMPS	Shaker
/Mpa^1/OXT	pMBHA	Shaker
dC^{1}OXT	pMBHA	Shaker
/D-Tyr^2/dC^{1}OXT	pMBHA	Shaker
/D-Phe^2/OXT	pMBHA	Shaker
/Mpa^1, Lys^8, Ala^9/VP	pMBHA	Tea-bag[b]
/Lys^8, Ala^9/VP	pMBHA	Tea-bag[b]
/Lys^8, ß-Ala^9/VP	pMBHA	Tea-bag[b]
/Arg^8, ß-Ala^9/VP	pMBHA	Tea-bag[b]
/Lys^8, Sar^9/VP	pMBHA	Tea-bag[b]
HIV-1 p24 Val-His-Ala-Gly-Pro-Ile-Ala-Pro-Gly--Gln-Met-Arg-Glu-Pro-Arg-Gly-Ser-Asp-Ile-Ala	pMBHA	Presep[c]
HIV-2 p24 Pro-Ile-Pro-Gly-Pro-Leu-Pro-Ala-Gly--Gln-Leu-Arg-Glu-Pro-Arg-Gly-Ser-Asp-Ile-Ala	pMBHA	Presep[c]
HIV-1 p31 Ile-Gln-Asn-Phe-Arg-Val-Tyr-Tyr-Arg--Asp-Ser-Arg-Asn-Pro-Leu-Trp-Lys-Gly-Pro-Ala	pMBHA	Presep[c]
HIV-2 p31 Leu-Lys-Asp-Phe-Arg-Val-Tyr-Phe-Arg--Glu-Gly-Arg-Asp-Gln-Leu-Trp-Lys-Gly-Pro-Gly	pMBHA	Presep[c]
HIV-1 gp41 Arg-Pro-Glu-Gly-Ile-Glu-Glu-Glu-Gly--Gly-Glu-Arg-Asp-Arg-Asp-Arg-Ser-Ile-Arg-Leu	pMBHA	Presep[c]
HIV-2 gp41 Ala-Asn-Glu-Glu-Thr-Glu-Glu-Asp-Gly--Gly-Ser-Asn-Gly-Gly-Asp-Arg-Tyr-Trp-Pro-Trp	pMBHA	Presep[c]
Tyr-Val-Pro-Lys-Acp-Ala	Paper	d
Tyr-Glu-Gly-Thr-Acp-Ala	Paper	d
Tyr-Lys-Gln-Ile-Acp-Ala	Paper	d
Tyr-Thr-Pro-Val	Paper	d
Tyr-Lys-Pro-Val	Paper	d
Tyr-Thr-Leu-Val	Paper	d
Ser-Leu-Lys-Val	Paper	d
Ile-Ala-Lys-Val	Paper	d
Tyr-Pro-Thr-Lys-Phe-Leu-Gly-Lys-Ala-Phe-Val	Paper	d
Tyr-Pro-Ala-Gly-Val-Leu-Ala-Thr-Pro-Phe-Val	Paper	d
Tyr-Leu-Ala-Lys-Val-Pro-Gly-Thr-Ala-Phe-Leu	Paper	d
Tyr-Leu-Thr-Gly-Phe-Pro-Ala-Lys-Pro-Phe-Leu	Paper	d
Lys-Pro-Lys-Pro-Gly-Gly-Phe-Phe-Gly-Leu-Leu	Paper	d

[a] pMBHA, p-methylbenzhydrylamine resin; CMPS, chloromethylated polystyrene; [b] Synthesis performed as described in (4). Generous gift of tea-bags of dr. Houghten is acknowledged. [c] Multiple continuous flow arrangement - see (3,5). [d] See contribution of Eichler et al. in this proceedings; Acp, ε-aminocaproic acid.

- for example in the cases of synthesis performed on non-traditional carriers (polyethylene rods, paper), or on classical carriers in the arrangement designed for multiple synthesis (cartridges (3), tea bags (4), flow reactors with moving piston (5)).

In the several syntheses performed in the classical batchwise manner (somatostatin, enkefalin, oxytocin analogs) we have proven that the continuous monitoring does not compromise the purity of the synthesized peptide. However, for the successful application of BB monitoring method several points have to be fulfilled: (i) Triethylamine as a base must not be used, because it forms quaternary ammonium salt with the residual chloromethyl groups on the Merrifield resin - diisopropylethylamine should be used instead. (ii) As little as possible of the monitoring dye should be applied.

We have applied this monitoring method also in the special cases of synthesis performed in the "tea bags", in the Presep cartridges in multiple continuous flow arrangement or on the paper. Examples of the peptides synthesized by various methods are given in Table 1. The syntheses were performed in the usual way with the only one difference that the monitoring color - bromophenol blue - was applied. In the case of "tea bags" syntheses the bags were shaken in the 0.01% solution of bromophenol blue in dichloromethane prior to the coupling. In the case of synthesis on the Presep cartridges the color was added into the reaction mixture.

Monitoring of peptide coupling by bromophenol blue is extremely simple to perform and it can be advantageously applied also in the special cases of multiple peptide synthesis performed on various carriers.

References

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