

Suppression of Side Reactions Associated With Use of the N^{α} -benzyloxymethylhistidine by Performing the HF reaction in the Presence of Hydroxylamine Derivatives

Misako Taichi*, Yuji Nishiuchi, and Terutoshi Kimura.

SAITO Reserch Center, Peptide Institute, Inc., Ibaraki, 567-0085, Japan.
E-mail: taichi@peptide.co.jp

Introduction

His derivatives are known to be extremely prone to epimerization in activating and coupling steps involving the π -nitrogen of the imidazole moiety. Therefore, regioselective protection of the π -nitrogen is believed to reliably suppress this undesired side reaction [1]. In Boc chemistry, the N^{α} -benzyloxymethyl (Bom) group is widely accepted as a protecting group for the His residue since it can effectively suppress the risk of epimerization and can be readily removed by HF [2]. However, the use of Boc-His(Bom) in peptide synthesis occasionally entails serious obstacles: 1) failure of N^{α} -Boc deprotection from His(Bom) occurs during TFA treatment for the standard SPPS, resulting in amino acid deletion products generated at the N -terminus of His(Bom) [3], and 2) formaldehyde generated from the Bom group during the HF treatment leads to modification with other functional groups in the same peptide. In particular, formaldehyde can almost completely react with a Cys-peptide to produce a thiazolidyl (Thz)-peptide during isolation from a HF cleavage mixture (Fig. 1) [4, 5]. Also, this leads to conversion of the N -terminal Trp and the N -methylanthranlyl (Nma) group on the amino function to tetrahydro- β -carboline and dihydroquinazoline derivatives, respectively [6]. These modifications could be efficiently suppressed by performing the HF reaction in the presence of Cys·HCl as a scavenger. In practical peptide synthesis, however, the use of Cys·HCl led to intractable and sticky solids after removal of HF. In the present study, we examined the effect of scavengers being added to the HF treatment to help to minimize these side reactions by scavenging formaldehyde.

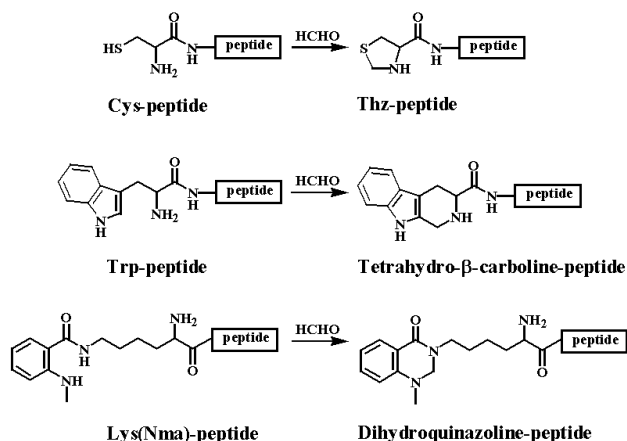


Fig. 1. Side reactions associated with the use of His(Bom).

Results and Discussion

The model peptides, Cys-Ang II (**1**) (Ang II: Asp-Arg-Val-Tyr-Ile-His-Pro-Phe), Trp-Ang II (**2**) and Lys(Nma)-Ang II (**3**), were synthesized by the standard SPPS using Boc strategy on PAM resin to examine the modification caused by formaldehyde. These protected peptides were treated with HF/*p*-cresol (8/2, v/v) at -2° to -5°C for 1 h in the presence or absence of a formaldehyde scavenging agent. The resulting peptides were extracted with 0.1% TFA and analyzed by RP-HPLC to assess the extent of their modification with N -terminal amino acid (Table 1). These modification could be essentially suppressed by the performing the HF reaction in the presence of HONH₂·HCl, MeONH₂·HCl or BnONH₂·HCl. They could be also prevented by the addition of MeONH₂·HCl to the residuals immediately after removal of HF. This indicated that the side reaction did not occur during HF reaction but after removal of HF, as had been reported previously [4]. A reactive formaldehyde, remaining in the HF reaction mixture probably in a N -hydroxymethylated form on the side chain of the His residue, could be liberated and then similarly initiate these modifications when peptides are dissolved in aqueous media for the isolation and purification procedures.

Table 1. Effects of scavengers on the synthesis of model peptides by HF method

Substrate	Additive	Ratio desired : modified ^a
1	None	30 : 70 ^b
	Cys·NH ₂ (30 eq)	98 : 2 ^b
	MeONH ₂ ·HCl (5 eq)	98 : 2
	MeONH ₂ ·HCl (5 eq) ^c	94 : 6
	BnONH ₂ ·HCl (5 eq)	98 : 2
2	HONH ₂ ·HCl (5 eq)	97 : 3
	None	83 : 17 ^b
	Cys·HCl (10 eq)	99 : 1 ^b
3	MeONH ₂ ·HCl (5 eq)	99 : 1
	None	54 : 46 ^b
	Cys·HCl (30 eq)	99 : 1 ^b
	MeONH ₂ ·HCl (5 eq)	99 : 1

^a The modified peptides describes thiazolidine-, tetrahydro- β -carboline- or dihydroquinazolin- containing peptides for Cys-, Trp- or Lys(Nma)-AngII, respectively.

^bData from literature [6].

^c The additive was added after removal of HF.

The suppressive ability of these hydroxylamine derivatives (5 equiv.) proved to be comparable to that of Cys·HCl (30 equiv.), although the latter is accompanied by sticky solids after removal of HF and also a mixture of Cys(X) scavenging carbocations, which frequently overlap with the retention time of the desired product on RP-HPLC. When treating the model peptide **4** with HF in the presence of anisole, however, a side reaction with Met residue, *i.e.* a Met(MBzl) sulfonium salt **6**, was found to have occurred during HF reaction (Fig. 2). This *p*-methoxybenzyl group was considered to be formed from the reaction between anisole and formaldehyde in HF. Although MeONH₂·HCl could not circumvent the sulfonium formation, no corresponding sulfonium salt was observed when *m*- or *p*-cresol was substituted for anisole in the HF reaction (Table 2).

In conclusion, the addition of hydroxylamine derivatives in place of Cys·HCl proved to be essential for suppressing the modification arising from the generation of formaldehyde during the HF reaction. We demonstrated that the use of MeONH₂·HCl in the final deprotection procedure using HF in the presence of *p*-cresol should be essential for the facile synthesis of His-containing peptides.

Ac-Cys(MeBzl)-Met-Trp(CHO)-Glu(OcHex)-His(Bom)-Tyr(BrZ)-NH₂ (**4**)

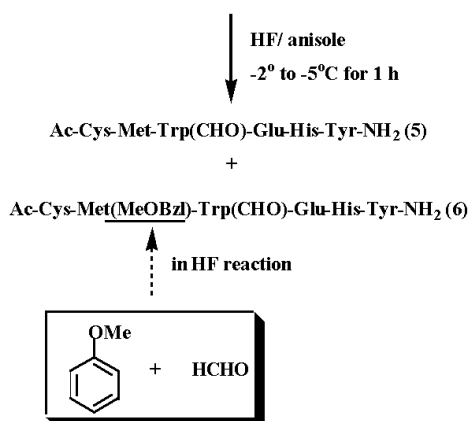


Fig. 2. Met sulfonium formation induced from the reaction between formaldehyde and anisole.

Table. 2. Effects of additives on the synthesis of model peptide **4** by HF method^a.

Additives	modified peptide 6 (%)
anisole	9.5
anisole/MeONH ₂ ·HCl	8.5
<i>p</i> - or <i>m</i> -cresol	0

^a The protected peptide **4** was treated with HF/anisole, *p*- or *m*-cresol (8/2, v/v) in the presence or absence of MeONH₂·HCl (10 eq) at -2° to -5°C for 1 h.

References

1. Jones, J. H., Ramage, W. I., and Witty, M. J. (1980) *Int. J. Pept. Protein Res.*, **15**, 301-303.
2. Brown, T., Jones, J. H., and Richards, J. D. (1982) *J. Chem. Soc. Perkin Trans. I*, 1553-1561.
3. Yoshizawa-Kumagaye, K., Nishiuchi, Y., Nishio, H., and Kimura, T. (2005) *J. Pept. Sci.*, **11**, 512-515.
4. Kumagaye, K. Y., Inui, T., Nakajima, K., Kimura, T., and Sakakibara, S. (1991) *Pept. Res.* **4**, 84-87.
5. Mitchell, M. A., Runge, T. A., Mathews, W. R., Ichhpurani, A. K., Harn, N. K., Dobrowolski, P. J., and Eckenrode, F. M. (1990) *Int. J. Pept. Protein Res.*, **36**, 350-355.
6. Yoshizawa-Kumagaye, K., Ishizu, T., Isaka, S., Tamura, M., Okihara, R., Nishiuchi, Y., Kimura, T. (2005) *Protein Pept. Lett.* **12**, 579-582.