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APPLICATION OF DIMETHYLSULPHOXIDE(DMSO) / TRIFLUOROACETIC ACID(TFA) OXIDATION TO THE SYNTHESIS OF CYSTINE-CONTAINING PEPTIDE

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Summary: S-protected cysteine derivatives [Cys(Trt), Cys(MBzl), Cys(Dbs), Cys(Bzh)] as well as cysteine were converted to cystine by the action of DMSO / TFA; as examples, two model peptides, oxytocin and an α-human calcitonin gene-related peptide (α-hCGRP), were prepared by this reaction.

In terms of the efficient and/or unambiguous synthesis of complex peptides containing several cystine residues, the existing methods are still less than satisfactory[1]. One of our research interests has therefore been focused on methodological improvements for the chemical synthesis of cystine containing peptides. Recently, we developed two new disulphide bond-forming reactions; *via* thallium(III) oxidation[2] and *via* acid catalized reaction involving S-protected cysteine sulphoxides[3]. These two methods have been successfully applied to the synthesis of several cystine-containing peptides. Both methods can be performed in trifluoroacetic acid(TFA), which eliminates the solubility problems usually encountered during the usual disulphide bond-forming reactions, *e.g.* air-oxidation or glutathione oxidation *etc.*

In this paper, we wish to report a third method which can also be performed in TFA by using dimethylsulphoxide(DMSO) as an oxidant. This oxidation reaction of thiol to disulphide employing DMSO was first introduced by Wallace *et. al.* [4]. The reaction was said to be catalyzed by tri(n-butyl)amine or acetic acid.

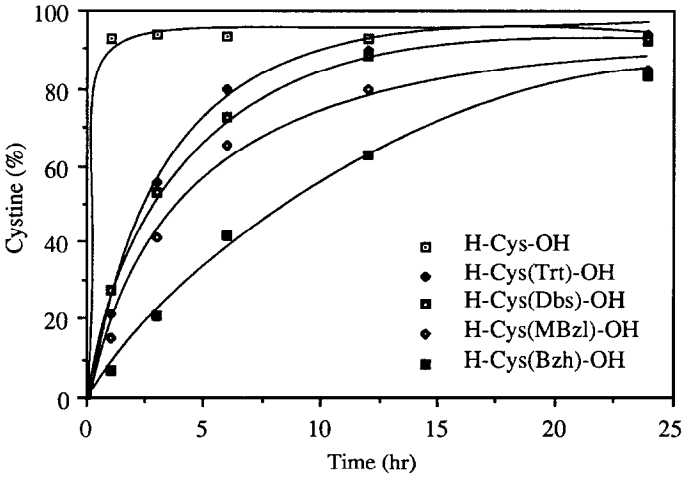

Fig.1.Cystine Formation from Cysteine or S-protected Cysteine Derivatives

We noted this DMSO oxidation and investigated the usefulness for the synthesis of cystine containing peptides. We adopted 10% DMSO / TFA-anisole as a reaction condition. Firstly, we confirmed that cysteine was easily oxidized to cystine by employing 10 % DMSO / TFA. Secondly, each Cys-derivative was treated with 10 % DMSO / TFA at room temperature and the progress of the oxidation was monitered with an amino acid analyzer (Fig.1). Among the S-protecting groups so far examined here, Trt[5], MBzl[6], Dbs[7] and Bzh[8] were cleaved to form cystine as the sole product. These groups were partially deprotected by TFA to form cysteine which by the action of DMSO gave cystine (Fig.2).

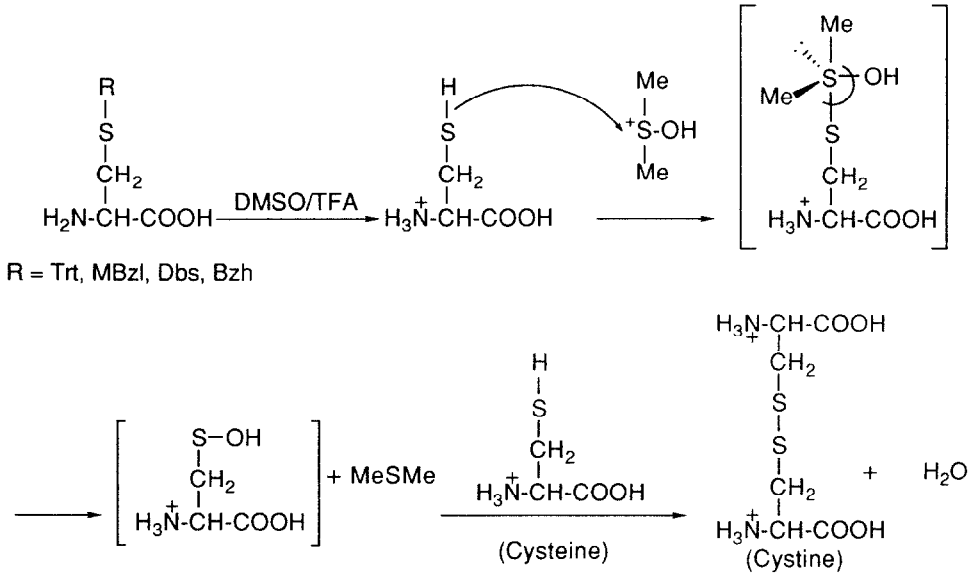

Fig.2. Possible Reaction Mechanism of DMSO / TFA Oxidation

On the other hand, Cys(Ad)[9] and Cys(4-MeBzl)[10], which are stable to TFA, were not converted to cystine upon treatment with 10% DMSO / TFA. Unmasked Trp suffered modification (recovery of Trp, 32.9%). Met was partially oxidized to the corresponding sulphoxide(recovery of Met, 78%, 12hr). Amino acids, including Tyr were recovered unchanged after the above 10% DMSO / TFA treatement.

In order to examine the usefulness of DMSO / TFA for the intra-molecular disulphide bond formation, two model cystine-containing peptides, oxytocin[11] and α-human calcitonin gene-related peptide (α-hCGRP)[12], were prepared and compared with authentic samples by HPLC. By using this procedure, the oxidation step for disulphide bond formation was greatly simplified.

Protected oxytocin, Z(OMe)-Cys(MBzl)-Tyr-Ile-Gln-Asn-Cys(MBzl)-Pro-Leu-Gly-NH_2, prepared by the conventional solution method, was treated with 10% DMSO / TFA in the presence of anisole (25°C, 12hr). After gel-filtration on Sephadex G-15, a highly pure product with an HPLC retention time identical with an authentic sample of oxytocin was obtained in 73.5% yield (Fig. 3)[13].

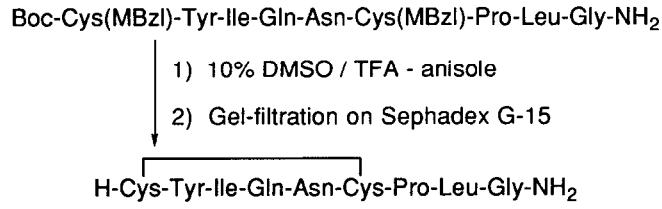

Fig. 3. Deprotection for The Synthesis of Oxytocin

Using the present method, α-hCGRP was re-synthesized[14]. The protected 37-residue peptide was first treated with 1 M TMSOTf-thioanisole / TFA[15] to remove all protecting groups (Ad from Cys, Mts from Arg, Bzl from Ser, and Z from Lys), then the ether-precipitated SH-peptide was treated with 10% DMSO / TFA in the presence of anisole (25°C, 1hr) to give crude α-hCGRP. After purification by HPLC, the product, identical with an authentic sample, was obtained in 17% yield (Fig.4)[16].

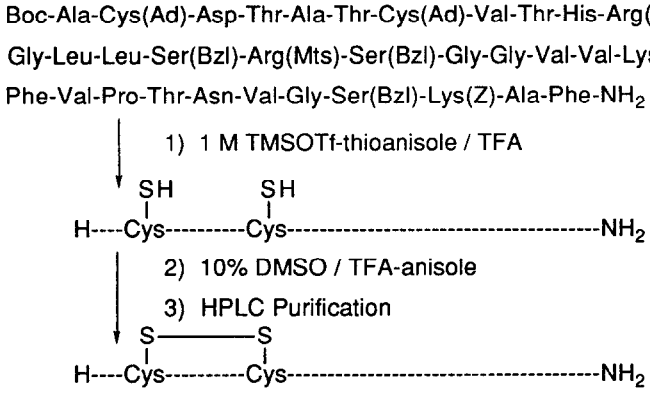

Fig. 4. Deprotections for The Synthesis of α-hCGRP

As demonstrated in these experiments, S-protected cysteine peptides can be directly converted to cystine peptides, when Trt, MBzl, Dbs, or Bzh protection is employed, and the time-consuming air-oxidation step for disulphide bond formation in highly dilute aqueous media is eliminated. This technique may prove to be useful for the synthesis of peptide containing several disulphide bonds, since one such bond can be established between two cysteine residues protected by Trt, MBzl, Dbs, or Bzh groups, while leaving other S-protected residues, Cys(R) (R = Ad, 4-MeBzl) intact.

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Abbreviation

Trt = triphenylmethyl, MBzl = *p* - methoxybenzyl, Dbs = dibenzosuberyl, Bzh = benzehydryl, Ad = 1-adamantyl, Acm = acetamidomethyl, 4-MeBzl = 4-methylbenzyl, Mts = mesityrene-2-sulfonyl, Bzl = benzyl, Z = benzyloxycarbonyl, TMSOTf = trimethylsilyl trifluoromethanesulfonate.

References

1) S. Kumagaya, H. Kuroda, K. Nakajima, T. Watanabe, T. Kimura, T. Masaki, and S. Sakaibara, *Int. J. Peptide Proteine Res.*, **32**, 519(1988).
2) N. Fujii, A. Otaka, S. Funakoshi, K. Bessho, and H. Yajima, *J. Chem. Soc., Chem.*, **1987**, 163; N. Fujii, A. Otaka, S. Funakoshi, K. Bessho, T. Watanabe, and H. Yajima, *Chem. Pharm. Bull.*, **35**, 2339(1987).
3) N. Fujii, A. Otaka, T. Watanabe, H. Arai, K. Bessho, and H. Yajima, *J. Chem. Soc., Chem, Commun.*,**1987**, 1676; N. Fujii, A. Otaka, S. Funakoshi, T. Watanabe, H. Arai, K. Bessho, and H. Yajima, *J. Protein Chem.*, **7**, 151(1988).
4) T.J. Wallace and J.J. Mahon, *J. Org. Chem.*, **30**, 1502(1965).
5) G. Amiard, R. Heynes, and L. Velluz, *Bull. Soc. Chim. Fr.*, **1956**, 689.
6) S. Akabori, S. Sakakibara, Y. Shimonishi, and Y. Nobuhara, *Bull. Chem. Soc., Jpn.*, **37**, 433(1964).
7) J. Pless, *Helv. Chim. Acta*, **59**, 499(1976).
8) L. Zervas, I. Photaki, A. Cosmatos, and D. Borovas, *J. Am. Chem. Soc.*, **87**, 4922(1965).
9) O. Nishimura, C. Kitada, and M. Fujino, *Chem. Pharm. Bull.*, **26**, 1576(1978).
10) B.W. Erickson, and R.B. Merrifield, *J. Am. Chem. Soc.*, **95**, 3570(1973).
11) V. du Vigneaud, C. Ressler, J.M. Swan, C.W. Roberts, P.G. Katsoyannis, and S. Gordon, *J. Am. Chem. Soc.*, **75**, 4879(1953).
12) H.R. Morris, M. Panico, T. Etienne, J. Tippins, S.I. Girgis, and I. MacIntyre, *Nature(London)*, **308**, 746(1984).
13) Protected oxytocin(46.8 mg, 37.5μmol) was treated with 10% DMSO / TFA (5.5 ml)-anisole(50μl) to establish a disulphide bond at 25°C. After 12hr, dry ether was added to the reaction mixture to afford a powder. The product was isolated by gel-filtration on Sephadex G-15 using 1 N AcOH as an eluant.
14) N. Fujii, A. Otaka, S. Funakoshi, M. Nomizu, K. Akaji, H. Yajima, I. Yamamoto, K. Torizuka, K. Kitagawa, T. Akita, K. Ando, T. Kawamoto, Y. Shimonishi, and T. Takao, *Chem. Pharm. Bull.*, **34**, 620(1986).
15) N. Fujii, A. Otaka, O. Ikemura, K. Akaji, S. Funakoshi, Y. Hayashi, Y. Kuroda, and H. Yajima, *J. Chem. Soc., Chem. Comuun.*, **1987**, 274.
16) Ether precipitated SH-peptide(4.1μmol) was treated with 10% DMSO / TFA (5.0 ml)-anisole(50μl) to establishi a disulphide bond at 25°C. After 1hr, dry ether was added to the reaction mixture to afford a powder. The product was isolated by HPLC on a μ-Bondasphere 5μ C18-100Å(19mm x150mm) column with a linear gradient of MeCN (25 to 45%, for 40 min) in 0.1% TFAaq. at a flow rate 17.0 ml / min.

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