Highly Efficient Propane-1,3-dithiol Mediated Thiol–disulphide Interchange: a Facile and Clean Methodology for S–S Reduction in Peptides

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Propane-1,3-dithiol, at room temperature and in the absence of any promoter, neatly brings about the S–S → 2SH change, and the latter can be isolated as acrylonitrile adducts; the methodology has been tested with several substrates including c-lysozyme, insulin and oxytocin, and spontaneous imidazole promoted *S*-deprotection was observed in the case of Z-Cys(S-CH_2CH_2CN)His-OMe.

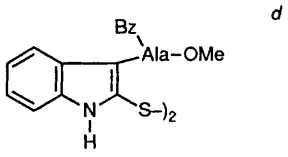
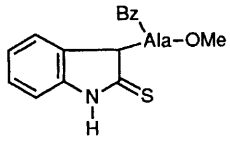
The reagent chosen for the reduction of the disulphide bond by interchange in proteins is dithiothreitol (DTT). The favourable equilibrium constant (*ca.* 10^4) for reductions with DTT has been ascribed to the ready formation of a six-membered ring containing a disulphide bridge. The reactions are done at pH 7–8 in aqueous medium with an excess of DTT.[1] In the course of our studies related to the thiol Zn^{II} tetrahedral templates,[2] we needed an exchange reagent that could be effective in near equivalent amounts and in unbuffered MeOH. From the observation that 1,2-dithiolane, which is the product of thiol–disulphide interchange from propane-1,3-dithiol (PDT), is rapidly transformed into a methanol insoluble polymer,[3]† we therefore felt that the readily available PDT would be most efficient in thiol–disulphide interchange; this turned out to be the case.

In typical procedures, synthetic disulphide bond containing substrates (Table 1*a*) were left stirring under nitrogen at room temperature with PDT (1.5 equiv.) in dry MeOH. The

† Interestingly, when a 10^{-2} mol dm^{-3} solution of PDT in MeOH was left aside at room temperature protected from light for *ca.* 12 days in a volumetric flask (10 ml), the dithiolane polymer (m.p. 69–70 °C) was found deposited on the walls.

Table 1 Propane-1,3-dithiol (PDT) promoted thiol–disulphide interchange

a Synthetic substrates

Entry	Substrate (m.p.)	Method	Product (m.p.)	Yield (%)[a]
1	Z-NHCH[CH_2SSCH_2CH(NHZ)CO_2Me]CO_2Me (52–53 °C)	i PDT–MeOH (dry) ii H_2C=CHCN (A)	Z-Cys[SCH_2CH_2CN]OMe (oil)	71
2	BzNHCH[CH_2SSCH_2CH(NHBz)CO_2Me]CO_2Me (168–70 °C)	A	Bz Cys[SCH_2CH_2CN]OMe (54–55 °C)	80
3	Z-NHCH[CH_2SSCH_2CH(NHZ)COHisOMe]COHisOMe (155–157 °C)[b]	A	Z-Cys[SCH_2CH_2CN] HisOMe (99–101 °C)	90
4	(See entry 3)	PDT–MeOH (dry) (B)	Z-CysHisOMe (112–114 °C)[c]	95
5	$PhCH_2SSCH_2Ph$ (60–62 °C)	B	$PhCH_2SH$ (oil)	42
6	 [d]	B		85

b Natural substrates

Entry	Substrate	No. of disulphide bridges	Method	No. of disulphide bridges reduced
7	Cystine	1	i PDT–MeOH: pH 8 buffer (1 : 10) ii H_2C=CHCN, MeOH (C)	1[e]
8	c-Lysozyme	4	i PDT (neat) ii H_2C=CHCN (D)	2
9	Bovine insulin	3	D	2
10	Oxytocin acetate salt	1	D	1

[a] Parallel runs with dithiothreitol (DTT) according to the procedure described by G. Jung, C. Carrera, H. Bruckner and W. G. Bessler, *Ann.*, 1983, 1608, using 4 equiv. of reagent and acrylonitrile addition, with entries 1 and 3, afforded respectively 41 and 8% of the products. Removal of excess reagent and oxidized product is much cleaner with PDT than with DTT. This would account for the better yields with PDT (we are grateful to a referee for suggestion to make a comparative study).[b] Prepared from Z-Cys-bis-ONp and HisOMe (yield 70%). [c] Reaction with acrylonitrile afforded the adduct (entry 3) in 79% yield. [d] Prepared in 55% yields by reaction of BzTrpOMe with S_2Cl_2. [e] Yield based on 33% of recovered starting material.

exchange, usually complete in *ca.* 5 hours, can be monitored by TLC. At this stage, the thiol could be isolated by evaporation of MeOH *in vacuo* followed by trituration of the residue with hexane and benzene to remove unused PDT and dithiolane polymer. Alternatively, the thiol product could be *S*-protected by addition of acrylonitrile (2 equiv.) to the methanolic reaction mixture, followed by additional stirring for 5 hours, evaporation of MeOH and trituration with hexane and benzene. The products thus obtained were quite pure, as demonstrated by TLC and ^1H NMR comparison with pure samples obtained by preparative TLC‡ (PTLC) (Table 1*a*).§ The ^1H NMR of the crude products revealed the complete absence of the dehydroalanine unit.¶ Our experience tends to suggest that this is likely, when the reactions are carried out in basic media.‖

The methodology has been successfully tested with natural cystine containing substrates ranging from the parent to oxytocin, insulin and c-lysozyme (Table 1*b*). A number of solvents were examined. Thus, the interchange can be performed in MeOH : pH 8 buffer (1 : 10) (for cystine), in MeOH : H_2O (1 : 15) (for insulin), in MeOH (for oxytocin) and neat. However, in the range of *ca.* 1 μmol of substrates the reaction is most conveniently carried out in neat PDT. The extent of disulphide reduction was determined, except in the case of cystine, by amino acid analysis on samples that were, after interchange, either directly treated with acrylonitrile (oxytocin, insulin) or as with c-lysozyme, by treatment with urea (6 mol dm^{-3}), followed by acrylonitrile treatment, dialysis and lyophylization. Electrophoresis on sodium dodecyl sulphate–polyacrylamide gel against starting material showed no fragmentation of the enzyme. The reduction of the disulphide bridges in c-lysozyme is sluggish and under mild conditions, only 2 of the 4 are interchanged. The finding that with PDT only 2 of the 4 disulphide bonds are cleaved is in excellent agreement with similar results from DTT, performed in pH 8 phosphate buffer : urea (8 mol dm^{-3}) : EDTA.[4] The partially modified c-lysozyme retains biological activity.[5] We feel that the present procedure for c-lysozyme** would be applicable to other enzymes.

‡ In spite of all care, PTLC [developer; entries 1 and 2, PhH: EtOAc (80 : 20); entry 3, $CHCl_3$: MeOH (80 : 20) (see Table 1*a*)] resulted in loss of material. All sulphur containing substrates used in this study were found to be air-sensitive; even brief exposure resulted in the formation of highly polar compounds (TLC).

§ The structural assignments for all compounds listed in Table 1*a* are supported by excellent IR and ^1H NMR spectroscopy data. Satisfactory elemental analyses have been obtained for the tripeptide (entry 3) and all acrylonitrile adducts.

¶ Even small amounts of dehydroalanine units present can be detected by the very characteristic doublet at δ 6.1 (*J* 1.25 Hz).

‖ In early experiments, when the exchange with PDT was done with added triethylamine, considerable diversion took place *via* dehydroalanine.

** Under a dry nitrogen blanket, c-lysozyme (1 μmol) was mixed with PDT (93 μl). After 0.5 h stirring, additional PDT (50 μl) was added and stirring continued for 8 h. The mixture was triturated with dry hexane (1 × 3 ml), mixed with 0.5 ml of urea (6 mol dm^{-3}) followed by acrylonitrile (60 μl), left stirring for 5 h, evaporated, dried, taken up in distilled water, dialysed and the clear solution lyophilysed to give the product as a white powder.

We feel that the PDT mediated interchange reactions are easier to process than with DTT and this is reflected in enhanced yields (see Table 1*a*; footnote[a]).

The possibility for the facile–$SCH_2CH_2CN \rightarrow SH$ deprotection[6] in conjunction with the methodology shown here, should make the naturally occurring cystine chiron further attractive in the design of peptide synthesis.

A very surprising observation was that the imidazole unit present in histidine side chains can effect the deprotection. Thus, when PTLC pure samples of Z-Cys(SCH_2CH_2CN)-HisOMe were kept in closed vials at room temperature for about one week, it was neatly transformed to the starting compound, namely, Z-NHCH[CH_2SCH_2CH(NHZ)CO-HisOMe] COHisOMe, arising from deprotection and oxidation. In sharp contrast, adducts lacking the histidine unit were found to be stable.

The present methodology, adaptable to a variety of substrates, merits wide testing, particularly with respect to the S–S cleavage in proteins.

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References

1 A. Dabre, *Practical Protein Chemistry—A Handbook*, Wiley, New York, 1986, p. 71–72.
2 S. Ranganathan, D. Ranganathan, S. Bamezai, W. P. Singh, D. Bhattacharyya, G. P. Singh, R. Rathi, S. Saini, N. Jayaraman and B. K. Patel, *Pure Appl. Chem.*, 1990, **62**, 1437.
3 M. Caserio and J. J. Kim, *Phosphorus and Sulfur*, 1985, 304.
4 K. S. Iyer and W. A. Klee, *J. Biol. Chem.*, 1973, **248**, 707.
5 Yu. M. Torchinsky, *Sulfur in Proteins*, Pergamon, Oxford, 1981, p. 202.
6 P. K. Misra, S. A. N. Hashmi, W. Haq and S. B. Katti, *Tetrahedron Lett.*, 1989, 3569.