Selective Reduction of Disulfides by Tris(2-carboxyethyl)phosphine

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Received October 15, 1990

Tris(2-carboxyethyl)phosphine (TCEP) reduces disulfides rapidly and completely in water at pH 4.5. It preferentially reduces more strained disulfides, in accordance with the usual mechanism postulated for reduction of disulfides by phosphines in water. The reagent can be synthesized conveniently in large quantities by acidic hydrolysis of the commercially available tris(2-cyanoethyl)phosphine.

Introduction

Trialkylphosphines reduce organic disulfides to thiols smoothly and quantitatively in water (eq 1).[1] The strength

$$R'_3P + RSSR + H_2O \rightarrow R'_3P{=}O + 2RSH \quad (1)$$

of the phosphorus–oxygen bond renders reduction irreversible. Because trialkylphosphines are kinetically stable in aqueous solution, selective for the reduction of the disulfide linkage, and unreactive toward many other functional groups, they are attractive as reducing agents in biochemical systems. Their utility has been limited by the low solubility of most simple trialkylphosphines in water, and their acceptability as reagents (especially in biochemistry) by the odor and ease of autoxidation of many species with low molecular weights.

Water-soluble phosphines have been used as reducing agents, as chelating agents, and as ligands. Sulfonylated triphenylphosphines have been used extensively as ligands for water-soluble metal catalysts.[2] They are the subject of several patents.[3] A commercially available water-soluble triarylphosphine, tris(4-carboxyphenyl)phosphine, is relatively expensive.[4] Other water-soluble phosphines have been made.[5,6] Tris(hydroxymethyl)phosphine, a liquid with an unpleasant odor, can generate formaldehyde and other byproducts derived from it in reductions of proteins.[7] These characteristics have prevented the general use of phosphines as reducing agents for water-soluble disulfides.

Here we describe a convenient preparation of tris(2-carboxyethyl)phosphine hydrochloride (TCEP·HCl, 1·HCl) by acidic hydrolysis of the commercially available tris(2-cyanoethyl)phosphine (2) (eq 2) and demonstrate the

$$\underset{2}{(NCCH_2CH_2)_3P} \xrightarrow[\text{reflux}]{\text{aq HCl}} \underset{1\cdot HCl\ (88\%)}{(HO_2CCH_2CH_2)_3PH^+Cl^-} \quad (2)$$

utility of TCEP as a selective reducing agent for representative dialkyl disulfides in aqueous solutions. TCEP·HCl is a nonvolatile, water-soluble solid that is easily manipulated in air. It is a strong reducing agent that has previously been reported to cleave disulfides:[8] it rapidly reduces even very stable alkyl disulfides such as *trans*-4,5-dihydroxy-1,2-dithiane (DTT^{ox}) at room temperature and pH 5. Qualitative studies of the relative reactivities of TCEP with several disulfides also establish the potentially useful fact that these reductions are kinetically, not thermodynamically, controlled. For example, 1 reduces the disulfide group of lipoic acid (Lip^{ox}) to the corresponding dithiol (Lip^{red}) more rapidly than it reduces that of 2-hydroxyethyl disulfide (ME^{ox}), although Lip^{red} itself reduces ME^{ox} almost quantitatively at thermodynamic equilibrium.

Results

Synthesis of TCEP. TCEP·HCl was made conveniently in 50-g quantities by hydrolysis of tris(2-cyanoethyl)phosphine in refluxing aqueous HCl (eq 2). TCEP oxidizes rapidly in aqueous base (see below), making basic hydrolysis more cumbersome and less clean than acidic hydrolysis: separation of TCEP and TCEP oxide is difficult.[6,9,10] TCEP has also been made by reaction of aluminum phosphide with acrylic acid.[11] Previous preparations are much less convenient than the one reported here.

Properties of TCEP. TCEP·HCl is a water-soluble (310 g/L), odorless, white crystalline solid that is stable in air for several months. The pK_a of the phosphonium center has been estimated by titrimetry to be 7.66.[9] Dilute solutions of TCEP oxidize only slowly in air when the pH is less than that pK_a (Figure 1).

Reduction of Structurally Simple Disulfides in Water: Competition Experiments. The reaction of TCEP with disulfides is so fast at pH 4.5 that it is difficult to measure accurately. We have, instead, measured *relative* reactivities of representative disulfides toward TCEP by competition experiments. The competitive reductions were carried out by allowing 0.8 equiv of TCEP to react with 1.0 equiv of each of two disulfides in D_2O at pH 4.5. At the concentrations we used (0.2–1.0 mM), the reaction was complete within 5 min for all disulfides.

The NMR spectra place lower boundaries on the selectivity between each pair of disulfides. The ratio of the rates of reduction of Lip^{ox} and 1,2-dimethyl-3,8-dioxo-

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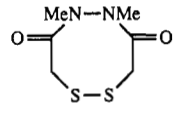
1,2,5,6-diazadithiocane (3)[12] is >10:1. The corresponding



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ratio for 3/DTT^{ox} is >10:1 and for DTT^{ox}/ME^{ox}, 2.5:1. The ratios of rates are calculated from the concentrations of both disulfides and both thiols by the method of Sih (eq 3, 4; $[A_0] = [A^{red}] + [A^{ox}]$).[13] The method assumes that

$$X + A^{ox} \rightarrow Y + A^{red} \qquad \text{rate} = k_A[A^{ox}]f([X],[Y]) \qquad (3)$$

$$\ln([A^{red}]/[A_0])/\ln([B^{red}]/[B_0]) = k_A/k_B \qquad (4)$$

the reaction is first-order in disulfide, is irreversible, and has the same rate expression for each substrate; it does not depend on the participation of other species (X, Y in eq 3). The signal-to-noise ratio in our spectrometer prevents us from measuring selectivities higher than ~10:1.

Discussion

TCEP reduces disulfides rapidly and cleanly in water at pH 5 at room temperature. Because it is easy to make and convenient to use, it should find wide applicability as a reducing agent for water-soluble disulfides, particularly in biochemical applications. Our determinations of the selectivity of the reaction proceeded with minimal difficulty.

The observed order of rates of reduction of disulfides by TCEP (Lip^{ox} > 3 > DTT^{ox} > Me^{ox}) is kinetically determined: the order of thermodynamic reactivity, and the order expected if rates of reduction correlated with the free energy of reduction of the disulfides under acidic conditions, is ME^{ox} > 3 > Lip^{ox} > DTT^{ox}.[14] The order of rates observed for TCEP reduction of disulfides does, however, correlate qualitatively with the order of rates of thiolate-disulfide self-exchange (eq 5);[15] the rate constant for ex-

$$\overline{\text{SRS}} + {}^{-}\text{SRS}^{-} \rightleftarrows {}^{-}\text{SRS}^{-} + \overline{\text{SRS}} \qquad (5)$$

change between 1,2-dithiolane and 1,3-propanedithiolate is 650 times larger than that between 1,2-dithiane and 1,4-butanedithiolate. We have suggested that rate constants for thiolate–disulfide interchange are determined in large part by the ground-state strain in the CSSC group;[15] we infer that the susceptibility of these disulfides to reduction by TCEP is largely determined by the same factor.

This kinetic selectivity offers the opportunity to manipulate the distribution of species in a mixture of disulfides and thiols in a way that would be difficult to achieve by other means. The reduction of disulfides by TCEP proceeds readily at low pH. At these values of pH, thiolate–disulfide interchange is effectively prevented because only low levels of thiolate are present. It is therefore possible to generate thiol from disulfide with kinetic selectivity under conditions in which thiolate–disulfide interchange does not occur. For example, we were able to reduce Lip^{ox} with good selectivity in the presence of ME^{ox} using TCEP at pH 4.5.

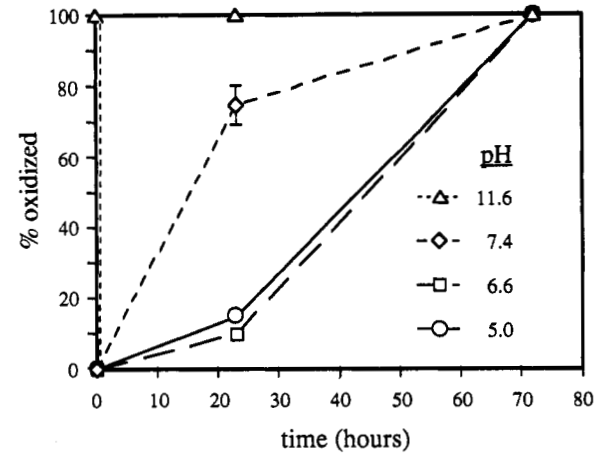
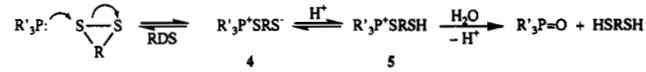


Figure 1. Solutions of TCEP (5 mM) in acetate or phosphate buffers (0.4 M) autoxidized slowly at pH <7. The solutions were stirred vigorously under air at room temperature and analyzed periodically by ^{1}H NMR spectroscopy.

Scheme I. Mechanism of Reduction of Disulfides by Phosphines in Water



The selectivity of this reaction supports the mechanism usually postulated for reduction of disulfides by phosphines: cleavage of the disulfide bond appears to be the rate-determining step (RDS) (Scheme I).[16,17] The order of kinetic reactivities is the same as the order of reactivity of disulfides as measured in thiolate–disulfide exchange between dithiolates and their corresponding disulfides.[15] We infer from that correspondence that the S–S bond is partially broken in the transition state, as it is in the transition state for thiolate–disulfide interchange.[18] If the RDS were to occur after the cleavage of the S–S bond, the kinetic selectivity would be different. For this type of mechanism, assuming little interaction between the thiophosphonium moiety and the thiolate moiety (intermediate 4) or the thiol group (intermediate 5), the transition states derived from those intermediates have no S–S bond and resemble the thiol *products* of the thiolate–disulfide interchange. As a result, the rates of reduction by phosphines should follow the thermodynamic order of disulfide reactivity, not the kinetic order.

Conclusions

Although the reaction of phosphines with disulfides in the presence of water to form thiols (eq 1) has been known since 1935,[1] little use has been made of the reaction because the commonly available phosphines are malodorous or insoluble in water. TCEP is a convenient phosphine for reduction of disulfides in water: TCEP·HCl, an odorless, crystalline, air-stable solid, is soluble in water and reacts rapidly (<5 min) with disulfides at room temperature in dilute (1 mM) solutions.[8] Related phosphines with different charges (e.g., $P(CH_2CH_2CONH_2)_3$) are also available from 2 by similar synthetic routes.[4,5] A test of

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the mechanism of the reaction suggests that it is an S_N2 mechanism, with the RDS being attack of the phosphine nucleophile on the S-S bond.

Experimental Section

General. Tris(2-cyanoethyl)phosphine was obtained from American Cyanamid.[19] Deuterated materials were obtained from Cambridge Isotope Laboratories. Other chemicals were obtained from Aldrich.

Tris(2-carboxyethyl)phosphine Hydrochloride. A slurry of tris(2-cyanoethyl)phosphine (44.6 g, 0.231 mol) in concd aqueous HCl (150 mL) was heated at reflux under an argon atmosphere for 2 h. A clear white precipitate formed when the hot clear solution was cooled to 0 °C. The precipitate was isolated by filtration. Recrystallization from water (200 mL), filtration, and drying in vacuo afforded 28.5 g of white crystals (99.4 mmol, 43%). The combined supernatants were concentrated to 110 mL by boiling. More white crystalline precipitate formed when the mixture was cooled to 0 °C. Filtration, rinsing with 20 mL of water at 0 °C, and drying in vacuo gave 29.5 g of white crystals (88% yield for the combined crops). ^{1}H NMR (D_2O): δ 2.76 (dt, 6 H, $J_{1,P}$ = 18.2 Hz, $J_{1,2}$ = 7.0 Hz), 2.47 (dt, 6 H, $J_{2,P}$ = 13.9 Hz). Mp: 176 °C (lit.[6] mp 175-177 °C). UV: λ_{max} = 218 nm, ϵ = 180 L mol^{-1} cm^{-1}; λ_{max} = 192 nm, ϵ = 150 L mol^{-1} cm^{-1}. Anal. Calcd for $C_9H_{16}O_6PCl$: C, 37.71; H, 5.63; P, 10.81; Cl, 12.37. Found: C, 37.61; H, 5.65; P, 10.99; Cl, 12.38.

Tris(2-carboxyethyl)phosphine Oxide. A crystal of iodine was allowed to react with an aliquot (0.5 mL) of a solution of TCEP·HCl (10 mM) in D_2O. ^{1}H NMR (D_2O): δ 2.66 (dt, 6 H, $J_{1,P}$ = 10.9 Hz, $J_{1,2}$ = 8.0 Hz), 2.20 (dt, 6 H, $J_{2,P}$ = 11.5 Hz).

Competitive Reductions. The competitive reductions were carried out by diluting from stock solutions 1 equiv of each of two disulfides and 1 equiv of 2-butyne-1,4-diol (as an internal standard for ^{1}H NMR spectroscopy) in buffer (20 mM acetate-d_3 in D_2O, pD = 4.5). In mild acid, neither autoxidation nor thiol-disulfide interchange occurs at an appreciable rate. An aliquot of TCEP·HCl stock solution in D_2O (0.8 equiv) was added to the reaction mixture. The reaction mixture was transferred to an NMR tube which was flushed with argon, stoppered, and closed with Parafilm. At the concentrations (0.2-1.0 mM) and temperature (22-25 °C) we used, the reaction was complete within 5 min for all disulfides.

Air Oxidation of Dilute Solutions of TCEP. Deuterated buffer solutions were made by neutralizing solutions of acetic acid-d_4 (0.40 M) or phosphoric acid-d_3 (0.35 M) with 40% NaOD in D_2O to pD 5.0 (acetate), 6.6, 7.4, and 11.6 (phosphate). To 4.5 mL of each solution was added 500 μL (25 mmol) of a solution of TCEP·HCl (49 mM in D_2O). The reaction mixtures were vigorously stirred under air. Aliquots were examined by ^{1}H NMR spectroscopy after 30 min, 23 h, and 72 h.

Acknowledgment. We thank our colleague R. H. Holm for the use of a UV spectrometer. This research was supported by the NIH through Grant GM39589 and by the National Science Foundation under the Engineering Research Center Initiative Biotechnology Process Engineering Center (Cooperative Agreement CDR-88-03014). NMR facilities were provided by the National Science Foundation under grant CHE-84-10774. J.A.B. was a National Science Foundation predoctoral fellow, 1986-1989.

(19) American Cyanamid no longer sells tris(2-cyanoethyl)phosphine. It is available from Strem.

Structure Determination of Natural Epoxycyclopentanes by X-ray Crystallography and NMR Spectroscopy[1]

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Received September 18, 1990

Two stereoisomeric epoxycyclopentane cyanohydrin glucosides were isolated, along with several previously described cyclopentene cyanohydrin glucosides, from *Passiflora suberosa* L. (Passifloraceae) and *Kiggelaria africana* L. (Flacourtiaceae); they represent new members of a rare class of natural nonannellated cyclopentane derivatives. The new glucosides were shown, by NMR spectroscopy (including NOE measurements), X-ray crystallography, and enzymatic hydrolysis to the corresponding cyanohydrins, to be (1*R*,2*R*,3*R*,4*R*)- and (1*S*,2*S*,3*S*,4*S*)-1-(β-D-glucopyranosyloxy)-2,3-epoxy-4-hydroxycyclopentane-1-carbonitrile and named suberin A and B, respectively. The crystal structure of suberin A was determined at 110 K and refined to R = 0.036 for 2198 unique reflections; the cyclopentane ring forms an envelope with C5 placed 0.41 Å away from the plane of the remaining four carbon atoms, and to the same side as the three oxygen substituents. In addition to the glucosides, two amides, (1*S*,2*S*,3*R*,4*R*)-2,3-epoxy-1,4-dihydroxycyclopentane-1-carboxamide and (1*S*,4*R*)-1,4-dihydroxy-2-cyclopentene-1-carboxamide, were isolated from *P. suberosa* and characterized; the amides are probably artefacts, and their formation represents a novel enzymatic transformation of plant cyanohydrins.

Introduction

In contrast to the vast abundance of natural products having a cyclopentane ring as a part of a polycyclic system, relatively few naturally occurring nonannellated cyclopentane derivatives are known. Such natural products can be divided into four major groups. These are the prostaglandins,[2] the antibiotics pentenomycins and related mold and bacterial metabolites,[3-5] a few monoterpenes[6] including

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