Reaction of Thiols and Disulfides with Phosphite Radicals. A Chain Mechanism and RS•/PO₃²⁻• Equilibrium

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Thiyl and phosphite radicals exist in equilibrium, $PO_3^{2-} + RSH \rightleftharpoons RS + HPO_3^{2-}$, with equilibrium constants of 800 where RSH is ethyl mercaptan and 1500 where RSH is penicillamine. Rate constants for the respective forward reactions are 3.0×10^8 M⁻¹ s⁻¹ for both compounds, and for the back reactions, 2.0×10^5 and 3.8×10^5 $10^5 \text{ M}^{-1} \text{ s}^{-1}$. In solutions containing both phosphite and disulfide an S_H^2 reaction, $PO_3^{2-} + RSSR \rightarrow RSPO_3^{2-}$ + RS, yields phosphate thioester and thiyl radicals. At higher phosphite concentrations, a chain reaction mechanism is established, based on re-formation of PO32- radicals in the reverse reaction of the above equilibrium. G values of up to about 30 are observed for thiol formation and disulfide destruction in these systems. One of the factors controlling the extent of the chain mechanism seems to be the thiyl/phosphite radical equilibrium.

Introduction

Phosphite radicals formed via hydrogen abstraction from phosphite by OH radicals and H atoms^{1,2} are known to exist in an acid-base equilibrium

$$HPO_{3} \rightarrow \Rightarrow PO_{3}^{2} \rightarrow + H_{ag}^{+}$$
(1)

with a $pK_a = 5.75^{3,4}$ Both $HPO_3 \rightarrow and PO_3^{2} \rightarrow have been$ found to undergo a variety of reactions (reduction, oxidation, addition, and substitution reactions) and several rate constants have been measured.⁴ The electron-transfer reaction

$$RS^{-} + PO_{3}^{2-} \rightarrow RS + PO_{3}^{3-} (HPO_{3}^{2-})$$
(2)

for example, occurs with 1.0×10^8 and 2.0×10^8 M⁻¹ s⁻¹ for the thiolate from ethyl mercaptan and penicillamine, respectively. The corresponding reactions of the undissociated thiols

$$RSH + PO_3^{2-} \rightarrow RS + HPO_3^{2-}$$
(3)

which constitute a hydrogen abstraction, were found to have a rate constant of 3.0×10^8 M⁻¹ s⁻¹ for both the above thiols.

A particularly interesting and rather complex mechanism, warrenting a more detailed study, was inferred in the course of our earlier studies of irradiated solutions containing thiols and/or disulfides together with phosphite.⁴ Further results on these systems obtained from pulse-radiolysis experiments are now reported in this communication.

Experimental Section

Solutions were prepared from commerical compounds of the highest available purity. The purity of the sulfurcontaining compounds was checked gas chromatographically. Where necessary, fractional distillation was used to achieve at least 98% purity. Phosphite was generally added as H₃PO₃ and the pH was then adjusted by addition of NaOH.

The solvent was deionized, Millipore-filtered water, the quality of which corresponds to triply quartz distilled water.

Solutions were generally saturated with N₂O to convert hydrated electrons, formed with the same yield as OH. radicals as primary reactive species in the radiolysis of aqueous solutions, into OH· radicals also (N₂O + $e_{aq} \rightarrow$ $N_2 + OH^- + OH_2$). The total yield of OH radicals in such solutions is G = 5.6 species per 100 eV absorbed energy. In terms of concentration this is equivalent to about $5 \times$ 10^{-6} M OH· radicals for a 1- μ s pulse of 1.55-MeV electrons or about 0.6 μ mol per 1 J of absorbed energy. In addition to OH radicals hydrogen atoms are also formed with G(H)= 0.6. Since both OH and H undergo the same (hydrogen abstraction) reaction with phosphite the total yield of primary phosphite radicals in solutions containing phosphite (phosphite concentration \gg thiol/disulfide concentration) amounts to G = 6.2.

The pulse radiolysis experiments were carried out by applying short (0.5–5 μ s) pulses of high energy (1.55 MeV) electrons from a Van de Graaff accelerator to the solutions. Dosimetry was based on the reduction of tetranitromethane in solutions containing 2-propanol. Details of the experimental arrangement, dosimetry, and the evaluation of data have been described elsewhere.⁵

 γ -Radiolysis experiments referred to in this paper were carried out by exposing solutions to the field of a 15000-Ci ⁶⁰Co source with an absorbed dose rate of 3×10^3 Gy/h $(3 \times 10^5 \text{ rd/h or } 3 \times 10^3 \text{ J kg}^{-1}/\text{h}).$ All experiments were carried out at room temperature.

Experimental limits of error are estimated at $\pm 10\%$ unless otherwise stated.

Results and Discussion

(1) Reaction of PO_3^{2-} Radicals with Thiols. Figure 1 (full circles) shows the yield of PenS radicals formed in the reaction

$$PO_3^{2-} + PenSH \rightarrow PenS + HPO_3^{2-}$$
 (4)

as a function of PenSH (penicillamine) concentration, measured from the well-characterized PenS- radical absorption at 330 nm,⁶ in neutral, pulse-irradiated, N₂O-saturated solutions of 10^{-1} M phosphite with PenSH concentrations ranging from 3×10^{-5} to 1.5×10^{-3} M. The curve shows an increase from G = 1.35 at the lowest

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Figure 1. Yields of PenS- radicals from pulse-irradiated, neutral, N₂O-saturated solutions containing various concentrations of PenSH, and 10^{-1} M phosphite (full circles), and 5×10^{-2} M phosphite and 10^{-3} M PenSSPen (open circles), respectively.



Figure 2. Plot of eq 8 for determination of K_6 . Full and open circles refer to corresponding points in Figure 1.

PenSH concentration to a constant value around G = 6.2at concentrations above 10^{-3} M PenSH, i.e., to a yield corresponding to that of phosphite radicals initially formed in the reactions

$$HPO_{3}^{2-} + OH \cdot / H \cdot \rightarrow PO_{3}^{2-} \cdot + H_{2}O/H_{2}$$
 (5)

The low PenS radical yields, particularly in the $10^{-4}-10^{-3}$ M PenSH concentration range, cannot be explained by failure of reaction 4 to go to completion as a result of competition from $PO_3^{2-} + PO_3^{2-}$ deactivation since the latter reaction only occurs with $k = 3 \times 10^7$ M⁻¹ s⁻¹.⁷ Another reaction, $PO_3^{2-} + HPO_3^{2-}$, which has been suggested as a possibility⁴ (and which would lead to regeneration of phosphite radicals) is also not expected to compete with reaction 4. The results shown in Figure 1 can, however, be explained quantitatively by assuming reaction 4 to be reversible, i.e., with the general equilibrium

$$PO_3^{2-} + RSH \rightleftharpoons RS + HPO_3^{2-}$$
 (6)

A number of observations and experimental findings for solutions containing thiol and disulfide which support this equilibrium will be presented in the following.

For the penicillamine system, the equilibrium constant is given by

$$K_{6} = \frac{k_{6}}{k_{-6}} = \frac{[\text{PenS}\cdot][\text{HPO}_{3}^{2-}]}{[\text{PO}_{3}^{2-}\cdot][\text{PenSH}]}$$
(7)

In the pulse radiolysis experiments the actual radical concentrations are proportional to the respective G values and since $G(PO_3^{2-}) = G(OH \cdot) - G(PenS \cdot) = 6.2 - G(PenS \cdot)$, eq 7 rearranges to

$$\frac{G(\text{OH}\cdot) - G(\text{PenS}\cdot)}{G(\text{PenS}\cdot)} = \frac{k_{-6}}{k_{6}} \frac{[\text{HPO}_{3}^{2^-}]}{[\text{PenSH}]}$$
(8)

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TABLE I: Rates and Equilibrium Constantsfor Equilibrium 6

thiol	$k_{6}, a M^{-1} s^{-1}$	$k_{-6}, M^{-1} s^{-1}$	K ₆	_
C ₂ H ₅ SH	$3.0 imes10^{8}$	2×10^{5}	1500	-
C ₂ H ₅ S ⁻	$1.0 imes10^{8}$	$2 imes 10^{\mathfrak{s}}$	500	
PenŠH	$3.0 imes10^{8}$	$3.8 imes 10^{s}$	800	
PenS ⁻	$2.0 imes10^{s}$	$3.8 imes10$ s	525	

^a Reference 4.



Figure 3. (a) Optical absorption/time curve at 250 nm in pulse-Irradiated, N₂O-saturated solution of 2×10^{-2} M phosphite and 2.5 $\times 10^{-4}$ M diethyl disulfide at pH 11.8; 2- μ s pulse, dose ~ 10 Gy (1000 rd). (b) pH dependence of the yield of the stable absorption from Figure 3a.

Accordingly, a plot of the ratio on the left-hand side of eq 8 vs. the phosphite/penicillamine concentration ratio should give a straight line going through origin with a slope of $k_{-6}/k_6 = 1/K_6$. As can be seen from Figure 2 (full circles) this is indeed the case. From the slope, $K_6 = 800$ is calculated for the penicillamine system.

Knowing K_6 and with $k_6 = 3.0 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$,⁴ one can calculate a rate constant for $k_{-6} = 3.8 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ for the back reaction of equilibrium 6, i.e., for hydrogen atom abstraction from phosphite by PenS- radicals. A comparison of these and other kinetic and equilibrium data is presented in Table I.

A corresponding derivativon of K_6 for other aliphatic thiyl radicals is not possible since these RS- radicals do not generally exhibit a measurable absorption. Equilibrium 6 should, however, affect, for example, the decay of the PO₃²⁻· absorption ($\epsilon = 3200 \text{ M}^{-1} \text{ cm}^{-1} \text{ at } 250 \text{ nm}^{4}$) in the presence of RSH ([HPO_3^{2-}] \gg [RSH]). Thus, a pseudo-first-order decay with $t_{1/2}$ inversely proportional to [RSH] but independent of $[HPO_3^{2-}]$ is found only when the forward reaction of eq 6 occurs almost quantitatively, i.e., in solutions containing relatively low phosphite concentrations. For example, $t_{1/2} = 34 \ \mu s$ for the PO₃²⁻ radical decay in solutions containing 10⁻⁴ M *i*-C₃H₇SH and HPO₃²⁻ concentrations up to 5×10^{-3} M. For higher phosphite concentrations but the same constant thiol concentration, the half-life then increases, and $t_{1/2} = 130 \ \mu s$ is observed for 10^{-1} M phosphite solutions. The fact that phosphite only becomes effective at 10^2-10^3 times higher concentrations than the thiol concentration indicates a similar equilibrium constant K₆ and a similarly low k₋₆ relative to k_6 , as found for the penicillamine system.

(2) Reaction of PO_3^{2-} with Disulfides. Phosphite radicals have been shown to react with disulfides with rate constants of the order of $10^{7}-10^{8}$ M⁻¹ s⁻¹,⁴ but although they exhibit both reducing and oxidizing properties neither of the well-known one-electron reduction or oxidation species, RSSR⁻ and RSSR⁺, are formed from aliphatic and other disulfides (with the apparent exception of certain cyclic disulfides, e.g., an RSSR⁻ radical anion is formed from lipoic acid with about 50% efficiency).⁴ Pulse-radiolysis experiments conducted with solutions containing phosphite and disulfide under basic conditions indicate two processes. Figure 3a shows a typical optical absorption-time curve recorded at 250 nm from an N₂O-saturated solution of 2×10^{-2} M phosphite and 2.5×10^{-4} M C₂H₅SSC₂H₅ at pH 11.8. The initial spike is due to the PO₃²⁻ absorption, and, from its decay, the bimolecular rate constant $k = 1.1 \times 10^8$ M⁻¹ s⁻¹ has been determined for the reaction of PO₃²⁻ radicals with diethyl disulfide.⁴

At longer times the slower formation of a stable absorption is observed. The spectrum of this absorption resembles that of the $C_2H_5S^-$ anion and this assignment is substantiated by the pH dependence of the absorption yield (250 nm), shown in Figure 3b. The point of inflection at pH 10.5 \pm 0.2 is practically identical with the pK of 10.6 for the acid-base equilibrium⁸

$$C_2H_5SH + OH^- \rightleftharpoons C_2H_5S^- + H_2O \tag{9}$$

The $C_2H_5S^-$ anion absorption grows in exponentially, with both the rate and yield of this process increasing with phosphate concentration, which would suggest participation of phosphite in thiolate formation. The following mechanism is compatible with all these observations. The first step is assumed to be a homolytic substitution (S_H2) reaction

$$RSSR + PO_3^{2-} \rightarrow RSPO_3^{2-} + RS$$
(10)

yielding thiyl radicals and phosphate thioester. Such S_{H2} processes are, in fact, known to occur between a variety of radicals and disulfides.^{9,10} In a second reaction, the thiyl radical then reacts with phosphite via

$$RS \cdot + HPO_3^{2-} \rightarrow RSH + PO_3^{2-} \cdot$$
(11)

to generate thiol (or thiolate, depending on pH; eq 9). Reaction 11 is the back reaction of equilibrium 6, i.e., the mechanism of the phosphite radical reaction with a disulfide is coupled to the PO_3^{2-}/RS equilibrium.

If we look at Figure 3a, it appears that, similar to the penicillamine system, the rate constant for reaction 11 is rather small, certainly smaller than for reaction 10. An absolute value for k_{11} of about 2×10^5 M⁻¹ s⁻¹ can be determined from the C₂H₅S⁻ built up. A relatively large error limit of at least a factor of 2 should, however, be considered. This results from the influence of equilibrium 6, and the fact that reactions 10 and 11 constitute a chain mechanism which affects the overall kinetics at the higher phosphite concentrations (low phosphite concentrations, which would overcome some of these problems, are not feasible, on the other hand, because OH- radicals could then react directly with the disulfide instead of with phosphite).

With $k_{11} = k_{-6} = 2 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ and $k_6 = 3.0 \times 10^8 \text{ M}^{-1}$ s⁻¹⁴ an equilibrium constant of $K_6 = 1500$ is calculated for the ethyl mercaptan system in solutions where undissociated C₂H₅SH is predominant. At pH values where C₂H₅S⁻ prevails and where $k_6 = 1.0 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$, a value of $K_6 = 500$ is derived. Again, relatively large limits of error should be allowed for these values of K_6 , although they are certainly of the correct order of magnitude.

The equilibrium between thiyl and phosphite radicals is also evident from the following experimental results obtained in irradiated penicillamine disulfide solutions.



Figure 4. Yields of thiol formation as a function of phosphite concentration in pulse-irradiated, N₂O-saturated solutions; pH 11.3 solution of 1.0×10^{-4} M (full circles) and 2.5×10^{-4} M (open circles) diethyl disulfide, respectively.

The measurable yield of PenS- radicals formed in a neutral, N₂O-saturated solution of 10^{-3} M PenSSPen and 5×10^{-2} M phosphite via

$$PO_3^{2-} + PenSSPen \rightarrow PenS + PenSPO_3^{2-}$$
 (12)

which equilibrates according to

$$\operatorname{PenS} + \operatorname{HPO}_{3}^{2-} \rightleftharpoons \operatorname{PenSH} + \operatorname{PO}_{3}^{2-} \cdot \tag{13}$$

is $G(\text{PenS}\cdot) = 1.8$. Addition of even small amounts of PenSH, the corresponding thiol, to the solution at concentrations of 5×10^{-5} and 1.3×10^{-4} M leads to an increase in $G(\text{PenS}\cdot)$ to 3.2 and 3.8, respectively. These values are also shown in Figure 1 (open circles) and, as expected, the PenS· radical yields from these solutions containing 5×10^{-2} M phosphite are higher for a given thiol concentration than in the 10^{-1} M phosphite system (full circles). The above data also fit the straight line in Figure 2 which supports the equilibrium constant K_6 and the associated kinetic data for the penicillamine system.

(3) Chain Reaction in Disulfide/Phosphite Solutions. The chain mechanism set up by reactions 10 and 11 is expected to lead to higher yields for thiol formation and disulfide destruction than the yield of PO_3^{2-} radicals (G = 6.2) initially formed in reaction 5. Termination reactions are considered to be

$$RS \cdot + RS \cdot \rightarrow RSSR \tag{14}$$

$$RS \cdot + PO_3^{2-} \to RSPO_3^{2-}$$
(15)

Mutual deactivation of two phosphite radicals is probably too slow to play any significant role.¹⁻⁴ Under experimental conditions which ensure that reaction 10 is fast compared with reaction 11, G(RSH) and G(-RSSR) should depend only on the phosphite concentration and be independent of the disulfide concentration. This is indeed the case as can be seen from Figure 4, where $G(C_2H_5S^-)$ is plotted vs. HPO₃²⁻ concentration for solutions at two different disulfide concentrations. The yields for thiolate formation were derived from the 250-nm absorption in pulse-irradiated, N₂O-saturated solutions at pH 11.3, containing 1 \times 10⁻⁴ M (full circles) and 2.5 \times 10⁻⁴ M (open circles) diethyl disulfide and various phosphite concentrations. Calculation of the thiolate yield is based upon $\epsilon(C_2H_5S^-)$ = 2500 M⁻¹ cm⁻¹ and ϵ (C₂H₅SSC₂H₅) = 440 M⁻¹ cm⁻¹ at 250 nm, i.e., on $\Delta \epsilon = 2060$ for each equivalent of C₂H₅S⁻ anion formation and $C_2H_5SSC_2H_5$ destruction.¹¹

The curve is nonlinear as a result of decreasing competition from the termination reactions, and seems to level

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Figure 5. Optical absorption/time curve at 250 nm in a pulse-irradiated, N2O-saturated, netural solution of 10⁻¹ M phosphite and 10⁻³ M diethyl disulfide; 2- μ s puise, dose ~10 Gy (1000 rd).

off at about G = 30 at concentrations above 5×10^{-3} M phosphite.

The disulfide destruction is exemplified in Figure 5, which shows the optical absorption time curve at 250 nm in a pulse-irradiated, neutral, N_2O -saturated solution of 10^{-3} M $C_2H_5SSC_2H_5$ and 10^{-1} M phosphite. The initial increase in absorption results from the primary yield of PO_3^{2-} formed in reaction 5. The absorption then decreases and a net bleaching remains as a result of disulfide consumption in the chain reaction (reactions 10 and 11), and the fact that the ground-state absorption of the disulfide at 250 nm ($\epsilon = 440 \text{ M}^{-1} \text{ cm}^{-1}$) is now greater than that of (in neutral solution undissociated) \bar{C}_2H_5SH ($\epsilon = 50 \text{ M}^{-1}$ cm⁻¹). The total yield for the bleaching process is given by $G\Delta\epsilon = -7800$. With $\Delta\epsilon = -\epsilon(C_2H_5SSC_2H_5) + \epsilon(C_2H_5SH)$ $= -440 + 50 = -390 \text{ M}^{-1} \text{ cm}^{-1}$, the yield for disulfide destruction is calculated as G(-RSSR) = 20. Generally, the vield of the disulfide destruction calculated on this basis is, however, found to be lower than the thiolate yields in basic solutions. Both yields would become identical, if another product, e.g., phosphate thioester, would contribute to the overall small change in optical density in neutral solution with an extinction coefficient as low as 130 $M^{-1}\,cm^{-1}\,at$ 250 nm. Therefore, a very large limit of error, at least a factor of 2, should be allowed for the yield of disulfide destruction. (PO32- radicals formed during the chain mechanism do not contribute to the absorption at longer times since they will react via reaction 10 immediately upon their formation in reaction 11).

The limiting value of the curve in Figure 4 is probably not only controlled by the termination reactions but also by equilibrium 6. Thus the increasing thiol concentration formed in the chain reaction will, in fact, reduce the efficiency of the chain as the thiol increasingly competes for the PO₃²⁻ radicals. Thus, an overall radical-induced equilibration will result for a disulfide/thiol/phosphite system.

The formation of thiols in the disulfide solutions has been attributed solely to reaction 11, i.e., to the reaction of thiyl radicals with phosphite. Another potential source of thiol would, of course, be the hydrolysis of the phosphate thioester formed in reaction 10:

$$RSPO_3^{2-} + H_2O \rightarrow RSH + HPO_4^{2-}$$
(16)

This reaction has been reported by several sources but is found to be very slow $(k \le 10^{-2} \text{ min}^{-1})$ at pH \ge neutral.¹²⁻¹⁴ Reaction 16 is thus not expected to make any significant contribution on the time scale and within the pH range of our investigations.

The phosphate thioester hydrolysis may, however, be significant for the interpretation of corresponding steady-state γ -radiolysis results. For example, a yield of G(RSH) = 33 was determined for γ -irradiated (Ar-saturated) solutions of 5 \times 10⁻⁴ M disulfide and 3 \times 10⁻² M phosphite.¹⁵ Incidentally, if reaction 16 is considered as having taken place in this solution this would practically leave no significant difference between the high (pulse radiolysis) and low (γ -radiolysis) dose rate experiments. Even if reaction 16 is not taken into consideration, the difference is still small enough to support the suggestion made above that equilibrium 6 is a major chain-lengthcontrolling parameter.

(4) Reactions of HPO_3 . Although only the reactions of PO_3^{2-} radicals have been considered in this discussion, the same mechanism and reactions, in principle, are expected for the acid radical HPO3-. Several rate constants have, in fact, been reported for the reactions of this species with thiols and disulfides in an earlier communication.⁴ Further mechanistic and quantitative studies have not been carried out since absolute data would hardly be accurate in view of the very weak optical absorption of HPO_3^{-} and the fact that the strongly absorbing RS⁻ anion is not formed in the pH range at which HPO₃- exists.

Conclusion

Chemical systems containing phosphite and either thiols or disulfides or both under conditions which allow radical formation are characterized by an equilibrium between thiyl and phosphite radicals and by a chain reaction which leads to thiol formation at the expense of disulfide. This may be of significance not only for purely chemical systems, but also for biochemical and biological systems, especially since one of the radical-induced reaction products of this mechanism (phosphate thioester) has been discussed as a possible agent for phosphate group transfer.^{16,17}

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