$$Hb + H_2O_2 + H^+ \rightarrow metHbH_2O + HO\cdot \quad (4)$$

HbO_2–10% Hb than for air-saturated solutions. The HO·, which may form via eq 4,[21] can also promote oxidation and protein damage.[22]

In summary, GSH serves effectively as an electron donor to free O_2 but not to O_2 as a ligand to heme iron. Furthermore, in the absence of GSH peroxidase GSH does not prevent oxidative damage to protein by H_2O_2 or H_2O_2 reaction products. GSH must be added to the list of Hb,[13,14] ascorbate, and flavins as likely electron donors for $O_2^{-}\cdot$ generation in red cells.[23,24] This reaction of GSH is undoubtedly disadvantageous to the red cell. However, the hemoglobin remains only partially saturated with O_2 during normal O_2 transport and can maintain the free O_2 concentration at low levels. It is clearly advantageous to red cell function for GSH to be a much better electron donor to metHb than to HbO_2.

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Registry No. GSH, 70-18-8; O_2, 7782-44-7; HbA, 9034-51-9; superoxide, 11062-77-4; hydroxyl radical, 3352-57-6.

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(24) Preliminary results indicate that ascorbate also promotes heme oxidation and precipitation similar to GSH since catalase stops both the reactions.

Time-Dependent Inhibition of Isopentenyl Pyrophosphate Isomerase by 2-(Dimethylamino)ethyl Pyrophosphate

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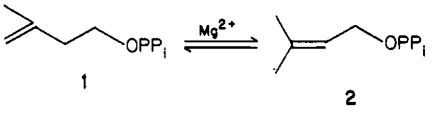
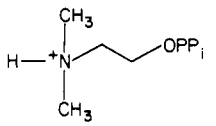
Isopentenyl pyrophosphate isomerase[1] (IPPI) (EC 5.3.3.2) catalyzes the isomerization shown in eq 1.



1 2 (1)

The available evidence indicates that a two-base mechanism is involved but is consistent with either a carbonium or carbanion mechanism.[2] To distinguish between the two mechanisms, we synthesized 2-(dimethylamino)ethyl pyrophosphate[3] (**3**) and examined its effect on the reaction catalyzed by IPPI. If the reaction proceeds by a carbonium ion mechanism, then **3** could be a transition-state analogue.



3

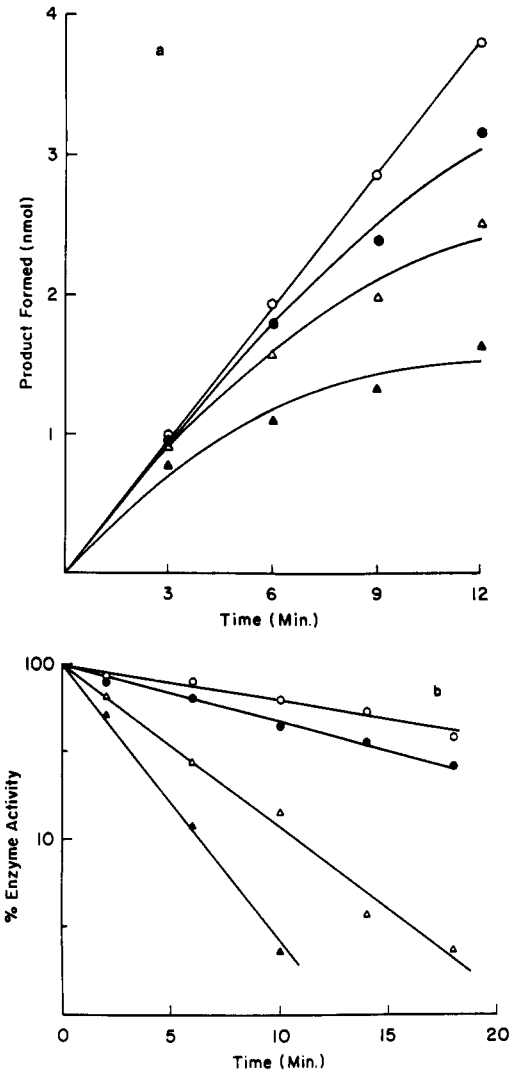


Figure 1. Time-dependent inactivation of isopentenyl pyrophosphate isomerase by 2-(dimethylamino)ethyl pyrophosphate. (a) Assay mixtures contained 0.1 M sodium maleate, pH 6.3, 10 mM $MgCl_2$, 100 μM [4-^{14}C]isopentenyl pyrophosphate, and **3** [(○) 0.0; (●) 0.67; (△) 1.33; (▲) 5.67 μM] in a final volume of 1.5 mL at 37 °C. The assays were started by addition of isomerase (1.7 units). Aliquots, 250 μL, were removed at time intervals and quenched with 15 μL 5 N HCl, and the amount of product formed was determined.[8] (b) Incubation mixtures contained 0.1 M sodium maleate, pH 6.3, 10 mM $MgCl_2$, isomerase (23 units), and **3** [(○) 1.33 μM + 500 mM isopentenyl pyrophosphate; (●) 0.67 μM; (△) 1.33 μM; (▲) 2.0 μM]. At time intervals 4-μL aliquots were diluted into an assay mixture (250 μL) containing 0.1 M sodium maleate, pH 6.3, 10 mM $MgCl_2$, and 350 μM [4-^{14}C]isopentenyl pyrophosphate. After 6 min at 37 °C, 15 μL of 5 N HCl was added and the amount of product formed was determined.[8]

The effect of **3** on the isomerization of **1** is shown in Figure 1a. The data show that the onset of inhibition is slow, suggesting that either combination of enzyme and inhibitor is slow or that

(1) Isopentenyl pyrophosphate isomerase was isolated from Bakers yeast (specific activity 6.5 nmol/min/mg).[7] Enzyme purified in this manner was essentially free of prenyl transferase activity and was assayed as described.[8] A unit of enzyme activity is defined here as 1 nmol/min.
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(3) **3** was synthesized from *N*-CBZ-ethanolamine pyrophosphate.[11,12] The CBZ group was removed by catalytic hydrogenation over 10% Pd–carbon. Reductive methylation with formaldehyde/$NaBH_4$ (Means, G. E.; Feeney, R. E. *Biochemistry* **1968**, *7*, 2192) gave the desired product. **3** was purified by chromatography on QAE Sephadex A25 using a linear triethylammonium bicarbonate gradient from 0.0 to 0.45 M. Fractions containing product were pooled and the buffer was removed by rotary evaporation in vacuo. A samaple for NMR analysis was prepared by passage through Dowex 50·Na^+ form. ^{1}H NMR (D_2O) δ 4.24 multiplet (2 H), 3.43 triplet (2 H), 2.92 singlet (6 H). A sample was further purified by paper chromatography in methanol/water/28% (w/w NH_3) ammonium hydroxide (60:30:10) for use in enzyme assays.

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Table I.[a] Inhibition of Isopentenyl Pyrophosphate Isomerase by Substrate Analogues

compound[b]	K_i, M
isopentenyl pyrophosphate	3.5×10^{-5} (K_m)
3-bromo-3-butenyl pyrophosphate	4.5×10^{-5}
methyl pyrophosphate	7×10^{-5}
isoamyl pyrophosphate	4×10^{-4}
2-(dimethylamino)ethyl phosphate	2.0×10^{-3}

[a] Assay mixtures contained 0.1 M sodium maleate pH 6.3, 10 mM $MgCl_2$, [4-^{14}C]isopentenyl pyrophosphate (10–100 μM), inhibitor, and isomerase (0.5 units) in 0.25-mL total volume. Assays were initiated by addition of enzyme. After 6 min at 37 °C, 15 μL of 5 N HCl was added and the amount of product formed was determined.[8] In all cases, K_i values were determined from the slope replots of $1/v$ vs. $1/[IPP]$ plots. Three inhibitor concentrations and four substrate concentrations were used. [b] 2-(Dimethylamino)ethyl phosphate was synthesized by a published procedure[9] and purified by paper electrophoresis at pH 8.9. 3-Bromo-3-butenol was synthesized according to ref 10. Isopentenyl phosphate was synthesized according to ref 11. Isopentenyl pyrophosphate was synthesized by using the diphenyl phosphorochloridate coupling method.[12] All other pyrophosphate monoesters were synthesized by the above procedures and purified by chromatography on QAE Sephadex using a linear triethylammonium bicarbonate gradient from 0.0 to 0.6 M. The purity of all phosphate and pyrophosphate monoesters was determined by ^{1}H NMR and paper electrophoresis at pH 3.5 and/or 8.9. Samples from paper electrophoresis and paper chromatography were visualized with ammonium molybdate spray.[13] Concentrations of substrate and inhibitor samples for use in enzyme assays were determined by phosphate analysis.[14]

a slow event occurs subsequent to formation of enzyme–inhibitor complex. Time dependence of the inhibition was demonstrated more directly by incubating **3** with IPPI. At intervals, aliquots were withdrawn and assayed for enzyme activity. Results are shown in Figure 1b. Loss of catalytic activity is a first-order-order process at all inhibitor concentrations tested. Over the range of concentrations tested, k_{obsd} is proportional to enzyme concentration. No evidence for saturation was seen. Figure 1b also shows that substrate reduces the rate of inactivation, indicating that **3** probably interacts with the active site. When IPPI was incubated with **3** without Mg^{2+} in the presence of 5 mM EDTA, no inhibition occurred. The inhibition is, therefore, Mg^{2+} dependent, as is the catalytic process. Several analogues of **3** were also investigated. All compounds tested were competitive inhibitors, but no time-dependent inhibition was observed. The K_i values are listed in Table I. To determine whether the reaction of IPPI with **3** is reversible, 6.67 μM **3** was added to enzyme (11 units) in the assay buffer in a total volume of 150 μL. After 10 min at 37 °C, it was determined, by assaying an aliquot, that the enzyme was >95% inactivated. The reaction mixture was then subjected to rapid gel filtration[4] to remove excess inhibitor. No enzyme activity was detected after filtration nor was any activity detected 90 min after gel filtration. A parallel experiment was carried out in which enzyme and inhibitor were mixed and subjected to gel filtration within 10 s after mixing. Immediately after filtration the enzyme had 67% of its original activity and 50% after 90 min.

The result presented here shows that inhibition of isopentenyl pyrophosphate isomerase by **3** is time dependent. From the data available so far, it cannot be determined whether dissociation of the enzyme inhibitor complex is extremely slow ($t_{1/2}$ for dissociation >10 h) or whether irreversible modification of the enzyme occurs. Possibly irreversible inactivation could occur by methyl transfer from **3** to a nucleophile at the active site.

We believe that inhibition of isopentenyl pyrophosphate isomerase by **3** provides strong evidence for a carbonium ion mechanism. There are now several examples in which substitution of a carbon atom, which acquires carbonium ion character in the transition state, by a positively charged nitrogen leads to compounds that are very good inhibitors, presumbly transition-state analogues.[5] It is surprising that an ammonium ion can take the place of a carbonium ion, since the geometry of the two structures is quite different. The fact that these compounds are inhibitors indicates that electrostatic interactions and not geometry are of overwhelming importance. It is likely that this interaction occurs with a negatively charged group on the enzyme, which in the catalytic process stabilizes the carbonium ion. Some evidence exists that in glycosidases, where carbonium ion mechanisms are involved, a carboxylate group stabilizes the carbonium ion.[6] The nature of the negatively charged group at the active site of isopentenyl pyrophosphate isomerase is not known. Possibly, it is a sulfhydryl group, since it has been established that the enzyme is sensitive to sulfhydryl reagents.[7]

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Molecular Mechanics Calculations on *cis*-[Pt(NH$_3$)$_2$\{d(GpG)\}] Adducts in Two Oligonucleotide Duplexes

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The anticancer drug *cis*-diamminedichloroplatinum(II) (*cis*-DDP) binds predominantly to d(GpG) sequences in DNA.[1] Since *cis*-DDP binding to DNA destroys substrate recognition for nucleases[2] and polymerases,[3] shortens the duplex,[4,5] and unwinds the double helix,[5] substantial changes in DNA structure, including base pair disruption, have been suggested. On the other hand, NMR studies of platinated octa- and decanucleotide duplexes, while confirming d(GpG)–Pt binding, were interpreted in terms of base-paired structures up to 28 °C.[6]

Molecular mechanics calculations on oligonucleotide chains have been used to explore possible structures and have been shown to predict correctly relative energies.[7] We have applied this method

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