included within the cavity of the host molecule **2b**·4HCl. The whole 1:1 complex sits on a center of symmetry, which means that the guest molecule is located exactly at the middle of the cavity.[18] The conformation of the host molecule is as follows. The four benzene rings are perpendicular to the mean plane of the macroring ("face" conformation[19,20]), and the bridging chain moieties take the trans-planar conformation except for the gauche conformation about the N(1)-C(2) and N(20)-C(21) bonds. As a result a cavity is formed which has rectangularly shaped open ends (~3.5 × 7.9 Å)[21] and a depth of 6.5 Å. The mode of inclusion of the guest molecule is as follows. As expected the benzene ring fits well with the cavity, being nearly parallel to the inner wall, and the methyl groups which are oriented to the outside protrude partly from the cavity. The closest contacts between the host and guest molecules (<3.80 Å) are shown in Figure 1 with dotted lines. Since durene is a nonpolar substrate and the complex was obtained from aqueous solution, it is indicated that hydrophobic interaction plays an important role and that polar interactions (i.e., electrostatic interaction and hydrogen bonding) do not participate in the complex formation between **2b**·4HCl and durene.

On the basis of the direct evidence of 1:1 inclusion described above, water-soluble paracyclophanes will be generally useful to trap and fix nonpolar substrates of definite shape and size in aqueous solution. Modification of the nature of the cavity and introduction of functional groups are now in progress.

Supplementary Material Available: Perspective view of host-guest complex with atomic numbering, positional parameters, thermal parameters, F(obsd)-F(calcd), bond distances, and bond angles of **2b**·4HCl·durene·4H_2O (25 pages). Ordering information is given on any current masthead page.

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(6) (a) Satisfactory elemental analyses and ^{1}H NMR spectra were obtained. (b) Satisfactory ^{13}C NMR spectrum was obtained. (c) Molecular ions were observed in their mass spectra.
(7) Measured in a KCl–HCl buffer of pH 1.95 at 25.0 ± 0.1 °C. Concentrations of 1,8-ANS and **2b** were 2.00 × 10^{-6} and 1.39–13.9 × 10^{-5} M, respectively. Excitation wavelength was 375 nm.
(8) (a) Brand, L.; Gohlke, J. R. *Annu. Rev. Biochem.* **1972,** *41,* 843. (b) Penzer, G. R. *Eur. J. Biochem.* **1972,** *25,* 218. (c) Kumbar, M.; Maddaiah, V. T. *Biochim. Biophys. Acta* **1977,** *497,* 707.
(9) Benesi, H. A.; Hildebrand, J. H. *J. Am. Chem. Soc.* **1949,** *71,* 2703. Fluorescence intensity was measured at 505 nm.
(10) Dissociation constants of 1.8 × 10^{-3},[2a] 6.3 × 10^{-4},[2b] and 9.1 × 10^{-5} M[3a] have been reported for 1:1 complex formation between 1,8-ANS and a water-soluble paracyclophane in aqueous solution.
(11) Measured in a DCl–D_2O solution of pD 1.2 at ambient temperature of 28 ± 2 °C. Concentrations of 2,7-dihydroxynaphthalene and **2b** were 2.5 × 10^{-2} and 5.0 × 10^{-2} M, respectively. Me_4Si was used as external standard. pD was adjusted according to Glasoe, P. K.; Long, F. A. *J. Phys. Chem.* **1960,** *64,* 188.
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(13) v. Braun, J. *Ber.* **1908,** *41,* 2145.
(14) The largest chemical shift change ($\Delta\delta$) of 0.12 ppm was observed for the proton at C-4 under the same condition as described in note 11.
(15) In the cases of water-insoluble substrates, a HCl–H_2O solution of **2b** and a hexane solution of the substrate were shaken, and the resulting precipitates were crystallized from 0.1 N HCl.
(16) On the basis of the elemental analyses of C, H, N, Cl, and the LC determination using LiChrosorb RP-2 with acetonitrile–methanol–water–28% ammonium hydroxide (55:10:34:1).
(17) Here the term "host–guest complex" was used according to Cram, D. J.; Cram, J. M. *Acc. Chem. Res.* **1978,** *11,* 8.
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(19) Tabushi, I.; Yamada, H.; Kuroda, Y. *J. Org. Chem.* **1975,** *40,* 1946. Predominance of "face" conformation in solution was suggested for polyparacyclophane system on the basis of ^{1}H NMR study.
(20) In **2b** the "face" conformation would be favored by the diphenylmethane skeletons. Cf.: (a) Cram, D. J.; Antar, M. F. *J. Am. Chem. Soc.* **1958,** *80,* 3103. (b) Kawato, T.; Inazu, T.; Yoshino, T. *Bull. Chem. Soc. Jpn.* **1971,** *44,* 200.
(21) The four corners of the rectangle are composed of the two methylene carbons of diphenylmethane skeletons [C(13) and C(32)] and the two N–C bonds having the gauche conformation [N(1)–C(2) and N(20)–C(21)]. The angle between the two benzene rings of diphenylmethane skeleton is 109.8°.

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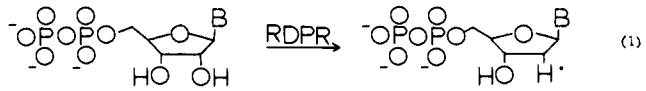
Inorganic Pyrophosphate Is Released from 2′-Chloro-2′-deoxyuridine 5′-Diphosphate by Ribonucleoside Diphosphate Reductase

Sir:

Ribonucleoside diphosphate reductase (RDPR) (E.C. 1.17.4.1) catalyzes the reduction of ribonucleoside 5′-diphosphates to the corresponding 2′-deoxyribonucleotides (eq 1). This enzyme has been purified to homogeneity by Eriksson



(1)

and co-workers[1] and consists of two nonidentical subunits B1 and B2 which form an active (1:1) complex in the presence of magnesium ions.[2] Protein B1 (mol wt, 160 000 daltons), a dimer of the general structure $\alpha\alpha'$,[1] contains active thiols and the binding sites for the nucleoside diphosphate substrates and the nucleoside triphosphate allosteric regulators. Protein B2 (mol wt, 78 000 daltons), a dimer of general structure $\beta\beta$, contains two antiferromagnetically coupled Fe(III)'s and an unusual tyrosine radical essential for activity.[2] Recently, Thelander and co-workers[3] reported that 2′-chloro-2′-deoxynucleoside 5′-diphosphates in the presence of the reductase did not undergo the normal reduction sequence, but instead was degraded to chloride ion, free base (e.g., uracil), and a phosphosugar tentatively identified as 2-deoxyribose 5-diphosphate. In addition, inactivation of B1 was observed accompanied by modification of several thiol groups. We felt that the elucidation of the mechanism of this enzyme-catalyzed degradation required the absolute identification of this phosphosugar. We report that in our hands *inorganic pyrophosphate* is quantitatively liberated from 2′-chloro-2′-deoxyuridine 5′-diphosphate by the action of the reductase. This finding demonstrates a remarkable loss of all substituents from the ribose moiety and has important mechanistic implications.

Incubation of 2.6 μmol of [β-^{32}P]-2′-chloro-2′-deoxyuridine 5′-diphosphate (12 000 cpm/μmol) with RDPR in the presence of the positive effector dTTP afforded >80% uracil formation.[4] Chromatography on DEAE Sephadex resulted in the isolation of 2.2 μmol of an unknown diphosphate.[5] ^{1}H NMR analysis of this material using a Brüker 270-MHz Fourier transform spectrometer revealed small amounts of contaminants which were present in the starting material. The amazing feature of this spectrum was the lack of any new protons in the anomeric sugar region, the 2-deoxy region, or the 5-hydroxymethylphosphate region. These findings suggested the possibility that inorganic pyrophosphate was the product. Analysis by ^{31}P NMR (Figure 1) revealed a singlet at −7.7 ppm which was in agreement with a known sample of tetrasodium pyrophosphate in the same buffer.

Since the assignment of the presumed phosphosugar by Thelander and co-workers[3] was based on chromatography on polyethyleneimine (PEI) and Whatman 3 MM paper, we compared our unknown diphosphate with authentic [^{32}P]-

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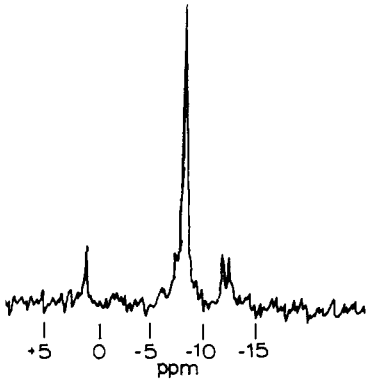
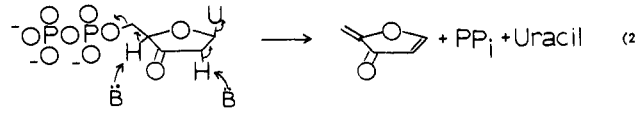


Figure 1. Proton decoupled ^{31}P spectrum of unknown isolated by DEAE-Sephadex A-25 chromatography in 0.05 M sodium succinate (pH 6.8) containing 0.1% EDTA. Shifts are referenced to external 85% H_3PO_4. The chemical shift of the major peak (−7.7 ppm) is in agreement with value obtained for authentic inorganic pyrophosphate. A small amount of inorganic phosphate (+1.5 ppm) is present. The doublet (−11.6 ppm) is the α-phosphate of a contaminating uracil-containing nucleoside 5′-diphosphate present in the starting material. The corresponding doublet for the β-phosphate is located under the pyrophosphate peak.

pyrophosphate and the R_f values reported by these workers (Table I). In both PEI systems (A and B), our unknown, pyrophosphate, and their presumed phosphosugar had identical R_f's. Paper chromatography (system C), however, produced different results. We found that our unknown and pyrophosphate did not migrate from the origin, while Thelander et al. reported an R_f of 0.30 for 2-deoxyribose 5-diphosphate.[6]

Our results clearly establish that pyrophosphate is quantitatively released from 2′-chloro-2′-deoxyuridine 5′-diphosphate by the action of RDPR. Therefore, the enzyme not only catalyzes C-Cl bond cleavage in analogy to C-OH bond cleavage in the normal substrate, but also the anomalous release of uracil and pyrophosphate. A possible intermediate which would account for these processes in the chloro analogue is a 3′-ketonucleoside 5′-diphosphate (eq 2). Generation of a 3′-



(2)

keto moiety would labilize H-2′ and H-4′ and permit elimination of uracil and pyrophosphate, respectively. If such an intermediate is on the normal reaction pathway, it is apparent that the difference in its reactivity when generated from the chloro analogue may be due to the occurrence of Cl^- instead of OH^- (protonate) at the active site. The precise mechanistic sequence for the overall reduction is not known; however, our very recent results suggest the possibility of a radical hydrogen abstraction.

In addition the resulting unsaturated ketone could explain the concomitant loss of sulfhydryl groups and enzyme activity from B1 observed by Thelander and co-workers[3] and verified by us. They postulated, based on an increase of absorbance at 320 nm, that inactivation was due to addition of an "active" sulfhydryl group to a tryptophane residue at the active site. This scheme was proposed because no radioactive label was detected on the inactivated enzyme using labeled substrates.[7] Our findings suggest that, if pyrophosphate, chloride, and base are released, then covalent modification of the enzyme by the substrate could only occur via the remaining ribose fragment. Moreover, we suggest that the carbohydrate produced (A Michael acceptor) could effect inactivation and the observed spectral changes.[8] We are presently preparing substrate labeled in the ribose moiety to determine if radioactivity is bound to the B1 protein and to identify any sugar that is released. These studies should help to elucidate the unique reduction mechanism of RDPR.[9]

Table I. R_f Values

compd	chromatographic system[a] A	B	C
our unknown	0.10	1.0	origin
unknown[3]	0.10	1.0	0.30
PP_i (known)	0.10	1.0	origin
2′-ClUDP	0.48	0.50	

[a] Thin layer chromatography systems: A, 1 M LiCl on polyethyleneimine plate (Brinkman); B, 60 g of ammonium sulfate in 100 mL of 0.1 M KPO_4 buffer (pH 7.0) containing 2 mL of 1-propanol on PEI plate; C, 70 mL of ethanol (95%) and 30 mL of 1 M ammonium acetate (pH 5.0) with 0.1% EDTA on Whatman 3 MM paper (ascending).

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(3) Thelander, L.; Larsson, B.; Hobbs, J.; Eckstein, F. *J. Biol. Chem.* **1976**, *251*, 1398-1405.
(4) 2′-Chloro-2′-deoxyuridine (Codington, J. F.; Doerr, I. L.; Fox, J. J. *J. Org. Chem.* **1964**, *29*, 558-564) was converted into the monophosphate by $POCl_3/PO(OEt)_3$ (Yoshikawa, M.; Kato, T.; Takenishi, T. *Tetrahedron Lett.* **1967**, *50*, 5056-5068) followed by purification on DEAE Sephadex using a gradient of triethylammonium bicarbonate (0 → 0.4 M). Conversion into the [β-^{32}P]diphosphate was carried out by activation of the monophosphate with 1,1′-carbonyldiimidazole and condensation with $^{32}P_i$ (Kozarich, J. W.; Chinault, A. C.; Hecht, S. M. *Biochemistry* **1973**, *12*, 4458-4463). Purification on DEAE Sephadex with a triethylammonium bicarbonate gradient (0 → 0.6 M) afforded the desired product. ^1H NMR analysis of this material revealed that it was contaminated with small amounts of arabinofuranosyl uracil 5′-diphosphate and another unidentified diphosphate. These impurities (~15%) were not substrates for the reductase and were isolated intact after the enzymatic reaction. The amount of uracil formation is based on total uracil-containing nucleoside 5′-diphosphates present in the starting material. The >80% uracil formation represents essentially quantitative release of uracil from the chloro compound.
(5) A typical reaction mixture (total volume = 6 mL) contained 0.05 M Hepes (pH 7.6), 15 mM $Mg(OAc)_2$, 1.0 mM dithiothreitol, 1 mM EDTA, 0.46 mM [β-^{32}P]-2′-chloro 2′-deoxyuridine 5′-diphosphate, 0.05 mM dTTP, and RDPR (27 mg; ~0.034 μmol of B1). Immediately before use, the RDPR was passed through a Sephadex G-50 column equilibrated with 0.05 M Hepes (pH 7.6) containing 15 mM $Mg(OAc)_2$ and 1 mM EDTA. A control experiment was run under identical conditions except that dTTP was replaced with 0.1 mM dATP a negative effector for UDP reduction.
Each reaction was incubated for 90 min at room temperature, concentrated in vacuo, and chromatographed on a Sephadex G-50 column (1.2 × 50 cm) equilibrated with Hepes buffer. Sixty 1-mL fractions were assayed for ^{32}P and A_{260} or A_{280}. No radioactivity was detected in the protein region. Fractions 28 through 55 contained the phosphorylated products. These fractions were pooled and chromatographed on DEAE Sephadex A-25 column (2 × 17 cm) by elution with a linear gradient (0 → 0.6 M; 1 L total vol.) of triethylammonium bicarbonate (pH 7.8). One hundred 10-mL fractions were collected and assayed for ^{32}P and A_{260}. Absorbance at 260 nm which eluted at the front was a direct measure of uracil formation which was verified by high pressure liquid chromatography. The reaction afforded 2.2 μmol of uracil; the control, 0.4 μmol. The appropriate diphosphate fractions were pooled and the salt was decomposed by repeated evaporation from H_2O in vacuo. The residue was converted into the sodium salt by exchange on Dowex 50 (Na^+ form), concentrated in vacuo, and dissolved in D_2O for ^1H and ^{31}P NMR. It should be noted that, during the reaction, <1% of the diphosphates were degraded to afford $^{32}P_i$ as detected on DEAE Sephadex.
(6) It is not clear if this is an actual discrepancy between the two results since it appears that the R_f of 0.3 reported by Thelander et al.[3] was for authentic material. In the text of their paper they state "The remaining part of the analog molecules behaved like 2-deoxyribose-5-diphosphate on paper electrophoresis or polyethyleneimine thin layer chromatography". Hence, it is not apparent that the R_f of the unknown was determined in this paper system. This is not surprising since the starting diphosphate had an R_f of 0.26 in this system and it appears that this system was used primarily for the determination of $^{36}Cl^-$ release (R_f = 0.71).
(7) It is interesting to note that the radioactive substrates utilized by Thelander et al. contained β-^{32}P, ^{36}Cl, and ^3H in the base. On the basis of our finding (i.e., PP_i, Cl^-, and base release) no label should be found on the enzyme if

only the ribose moiety is left.

(8) Thelander et al.[3] observed a rapid loss of titratable sulfhydryl groups followed by a slower increase in absorbance at 320 nm. These observations are nicely explained by a rapid Michael addition of a sulfhydryl group to the ribose fragment followed by a slow step, perhaps a rearrangement or dehydration to yield the chromophore. For instance, a β-mercapto-α,β-unsaturated ketone might be expected to have a λ_{max} at 320 nm.

(9) NOTE ADDED IN PROOF. It has been demonstrated (Pfitzner, K. E.; Moffatt, J. G. *J. Am. Chem. Soc.* **1965**, *87*, 5661-5670) that oxidation of thymidine 5'-monophosphate to afford the presumed 3'-keto analogue results in the generation of inorganic phosphate, thymine, and unidentified sugars. This chemical model provides strong support for our proposal. Moreover, we have recently found that incubation of RDPR with [^{14}C]-2'-chloro-2'-deoxyaridine 5'-diphosphate (uniformly labeled) effects a covalent labeling of the enzyme concomitant with inactivation. This finding is consistent with the modification of the active site by the ribose fragment.

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An ab Initio SCF Study of the Structure and Vibrational Spectrum of Thiirene

Sir:

Although antiaromatic 4n π systems have received considerable attention over the past few years, thiirene is the only heterocyclic example which has yet been prepared.[1,2] Calculations had predicted thiirene to be an extremely antiaromatic system (REPE = -0.114β),[3] and its instability has been confirmed by experiment.[1,2] Both Krantz and Laureni[1] and Strausz and his co-workers[2,4] have recently reported the isolation and IR spectrum of thiirene at low temperature though they are not in complete agreement on the spectrum, in part due to the presence of other substances in the reaction mixture.

We have now calculated the equilibrium structure and IR spectrum of thiirene using an ab initio single-configuration method and the 4-31G basis of Pople and Hehre.[5] Geometry optimization with an assumed C_{2v} structure gave C—S = 1.9782 Å, C=C = 1.2509 Å, C—H = 1.0556 Å, ∠HCC = 154.94°, and an energy of −473.725 975 hartrees.[6] This is nearly 2 hartrees lower than the best previous ab initio calculation on thiirene.[7] Of interest is the unusually long C—S bond and the short C=C bond. The latter is in accord with the reported high stretching force constant.[4] Together these results do reflect the expected antiaromaticity of thiirene; i.e., they show a tendency of the molecule to minimize conjugation between the sulfur atom and the C=C bond. A similar situation arises in cyclobutadiene where in the rectangle the C—C bond is calculated to be unusually long.[8]

Symmetry-adapted 0.01-Bohr displacements of atoms were then taken singly and in pairs to give a force constant matrix for each vibrational symmetry. These were diagonalized to yield the normal modes and frequencies. Dipole moment changes, and hence related infrared intensities, were computed by displacing the atoms along a normal mode by 0.1 au (this corresponds roughly to one half the classical turning point for high-frequency modes and one quarter this distance for those of low frequency). Calculated IR and Raman frequencies and intensities of the IR active bands of thiirene and dideuteriothiirene are listed in Table I. In Figures 1 and 2 the IR bands are plotted for comparison with those found experimentally by Strausz.[4] Although all lie too high, the computed C—H and C—C stretches are of the correct symmetry and relative intensity and are in the correct order for both compounds. The observed shift on deuteration of C—H stretching intensity from the b_2 to the a_1 band is reproduced by the calculations.

Table I. Calculated IR and Raman Frequencies and IR Intensities of Thiirene and of Dideuteriothiirene (in Parentheses)

sym-metry[a]	frequency, cm^{-1}	rel intensity	type
a_1	509 (505)	0.15 (0.30)	ring deformation
	939 (667)	0.01 (0.02)	in-plane C—H bend
	1935 (1779)	0.09 (0.12)	C=C stretch
	3645 (2820)	0.10 (0.21)	C—H stretch
b_1	808 (604)	1.00 (1.00)	C—H out-of-plane bend
b_2	448 (429)	0.00 (0.00)	ring deformation
	996 (767)	0.36 (0.36)	in-plane C—H bend
	3548 (2606)	0.38 (0.39)	C—H stretch
a_2	934 (775)	—	C—H out-of-plane bend

[a] The a_1 frequencies are IR active only; the a_2 is Raman active only; b_1 and b_2 appear in both Raman and IR.

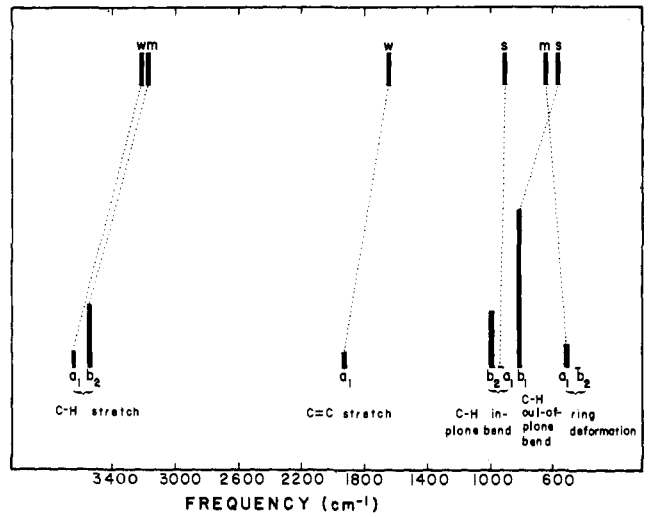

Figure 1. Comparison of observed and computed IR spectra of thiirene. The observed bands (from ref 4) are shown at the top of the figure with relative intensities indicated by strong (s), medium (m), or weak (w). Calculated bands are at the bottom of the figure with relative intensities shown by the height of the lines.

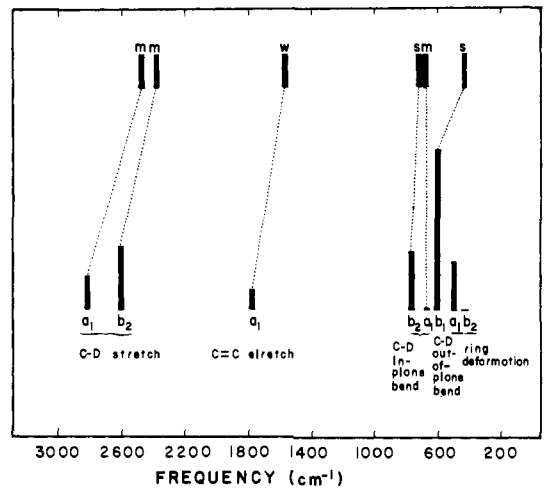

Figure 2. Comparison of observed and computed IR spectra of dideuteriothiirene. See legend of Figure 1.

The strong absorption of C_2H_2S seen at 910 cm^{-1} has been assigned[4] to the a_1 in-plane C—H bend, but, from the calculated intensities, it seems more likely to be the b_2 in-plane bend.[9] (We take the plane perpendicular to the molecular plane to be that distinguishing a from b symmetry.) This would make the bands of third-lowest frequency in C_2H_2S and C_2D_2S

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