Stereochemical Evidence for Monomeric Thiometaphosphate as an Intermediate in the Hydrolysis of (R_P) and (S_P)-Deoxyadenosine 5'-[β-^{17}O]-β-thiodiphosphate

Sara P. Harnett and Gordon Lowe*

The Dyson Perrins Laboratory, Oxford University, South Parks Road, Oxford OX1 3QY, U.K.

(R_P)-Deoxyadenosine 5'-[β-^{17}O]-β-thiodiphosphate is hydrolysed in (^{18}O)water at pH 7.2 (where it is present as its trianion) to AMP and inorganic [^{16}O,^{17}O,^{18}O]thiophosphate with complete inversion of configuration at phosphorus, whereas at pH 4.5 (where it is present as its dianion) hydrolysis occurs with 50% racemisation and 50% inversion, indicating that 'liberated' metathiophosphate can exist and has a finite lifetime in aqueous solution under these conditions.

Although monomeric metaphosphate was first postulated as an intermediate in the hydrolysis of phosphate monoesters more than 30 years ago,[1] compelling evidence that it has a finite lifetime in aqueous solution has proved elusive.[2] Thus the recent stereochemical experiments showing that phosphoryl transfer proceeds with inversion at phosphorus in protic solvents,[3] and the lack of positional isotope exchange in recovered adenosine 5'-[β-^{18}O$_4$]diphosphate after partial hydrolysis in aqueous solution,[4] provide powerful evidence against the involvement of free monomeric metaphosphate as an intermediate in phosphate monoester hydrolysis. These observations, combined with kinetic[5] and thermodynamic evidence,[6] can best be accommodated by a preassociative concerted mechanism with an 'exploded' transition state, or a preassociative stepwise mechanism.[7]

Thiophosphate *O*-monoesters show little sensitivity to the nature of the nucleophile in substitution reactions,[8] and are hydrolysed faster than the corresponding phosphate monoesters either as mono- or as di-anions under comparable conditions.[9] Moreover, monomeric metaphosphate has been detected only in the gas phase,[10] whereas trithiometaphosphate has been isolated as its tetraphenylarsonium salt,[11] suggesting that thiometaphosphate may possess greater kinetic stability than metaphosphate. In order to explore this possibility we have undertaken a stereochemical study of the hydrolysis of deoxyadenosine 5'-[β-^{17}O]-β-thiodiphosphate. The stereoisomers were prepared as outlined in Scheme 1 and their configurations established by phosphorylation with pyruvate kinase and phosphoenolpyruvate which is known to give predominantly the S_P-stereoisomer of ATPβS.†[12] The position of the ^{17}O (the ^{17}O site was actually 46% ^{16}O, 39% ^{17}O, and 15% ^{18}O) was readily established from the ^{31}P n.m.r. spectrum.[13]

The highest pK_a value of the thiodiphosphate side chain of ADPβS is 5.2.[14] Preliminary experiments with dADPβS showed that hydrolysis occurs faster at pH 4.5, where the monoprotonated species should predominate, than at pH 7.2 where the side chain should be fully ionised. This is in accord with the bell-shaped pH–rate profiles typical of phosphate and thiophosphate monoesters,[8,15] and has been ascribed to a pre-equilibrium proton transfer to the leaving group in the monoprotonated species.

Deoxyadenosine 5'-[(*R*)-β-^{17}O]-β-thiodiphosphate was partially hydrolysed in (^{18}O) water (99 atom % ^{18}O) at pH 7.2 and 4.5 and 50 °C for 5.6 and 1.5 h, respectively. The residual nucleotide was isolated and phosphorylated with pyruvate kinase and phosphoenolpyruvate and the product analysed by ^{31}P n.m.r. spectroscopy. There was no observed loss of isotope and no change in the chirality at phosphorus of the deoxyadenosine 5'-[(*R*)-β-^{17}O]-β-thiodiphosphate. The inorganic [^{16}O,^{17}O,^{18}O]thiophosphate was isolated and its chirality determined by the analytical method recently developed in this laboratory.[16] Comparison of the peak intensities derived from the ^{31}P n.m.r. spectrum with those calculated from the known isotopic composition of the ^{17}O and ^{18}O sites, taking into consideration possible loss in isotope and racemisation, shows that at pH 7.2 hydrolysis proceeds (within experimental error) stereospecifically with inversion of configuration at phosphorus (see Table 1). Analysis of the chirality of the inorganic [^{16}O,^{17}O,^{18}O]thiophosphate derived from the experiment run at pH 4.5, shows that although the S_P-enantiomer predominates, a substantial amount of racemisation has occurred. Comparison of the relative peak intensities with the computed best fit shows that 73% has the S_P and 27% has the R_P configuration (data not shown). In view of the importance of this result the experiment was repeated with deoxyadenosine 5'-[(*S*)-β-^{17}O]-β-thiodiphosphate at pH 4.1. Again the observed relative peak intensities from the ^{31}P n.m.r. spectrum when compared with the computed best fit showed that the inorganic [^{16}O,^{17}O,^{18}O]thiophosphate was about 25% S_P and 75% R_P (Table 2).

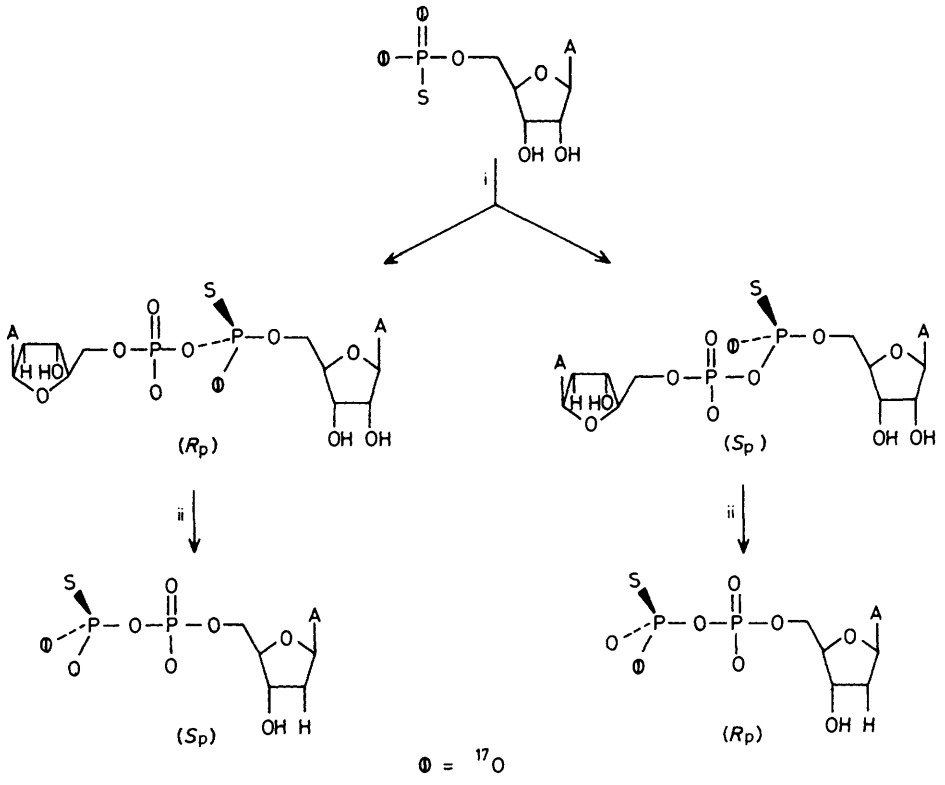
Table 1. Observed relative peak intensities of the ^{31}P resonances of the isotopomeric esters formed in the stereochemical analysis of the inorganic [^{16}O,^{17}O,^{18}O]thiophosphate obtained by hydrolysing deoxyadenosine 5'-[(*R*)-β-^{17}O]-β-thiodiphosphate at pH 7.2 and 50 °C. The stereochemistry is determined from the ratio of the intensities of the two mono-^{18}O esters. The computed best fit to the experimental data for the *cis*-ester indicates that 5% loss of label has occurred and that the reaction has proceeded with complete inversion of configuration within experimental error.

	Observed	Calculated
MeO–P=O	0.82	0.80
Me●–P=O	1.00	1.00
MeO–P=●	0.60	0.64
Me●–P=●	0.17	0.14

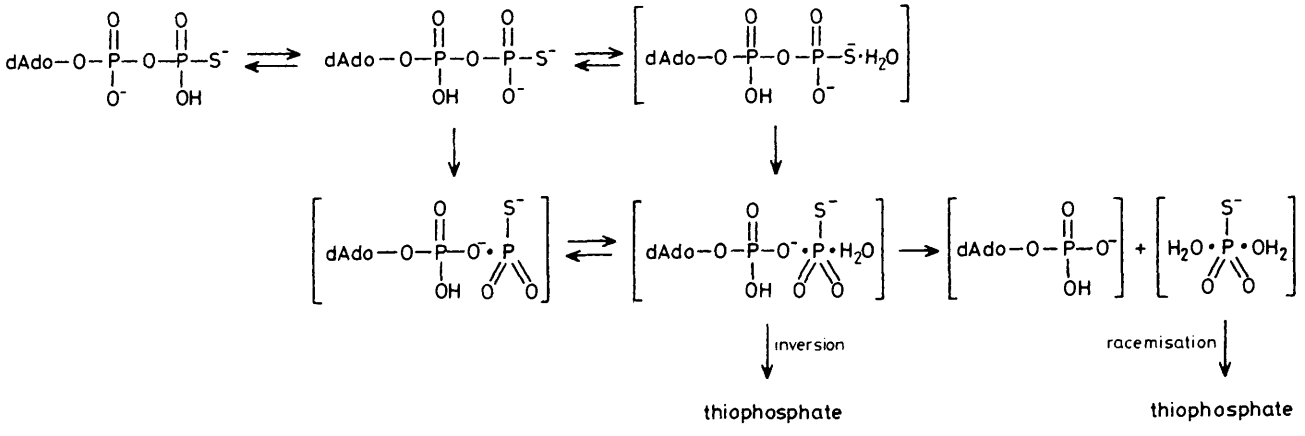
Table 2. Observed relative peak intensities of the ^{31}P resonances of the isotopomeric esters formed in the stereochemical analysis of the inorganic [^{16}O,^{17}O,^{18}O]thiophosphate obtained by hydrolysing deoxyadenosine 5'[(*S*)-β-^{17}O]-β-thiodiphosphate at pH 4.1 and 50 °C. The computed best fit to the experimental data for the *cis*-ester indicates that 50% racemisation has occurred with 5% loss of label. For comparison the computed ratios are shown for complete inversion of configuration.

		Calculated	
	Observed	25% *S* + 75% *R*	100% *R*
MeO–P=O	0.80	0.81	0.78
Me●–P=O	0.80	0.80	0.65
MeO–P=●	1.00	1.00	1.00
Me●–P=●	0.15	0.15	0.13

† *Abbreviations.* ATPβS: adenosine 5'-β-thiotriphosphate; ADPβS: adenosine 5'-β-thiodiphosphate; dADPβS: deoxyadenosine 5'-β-thiodiphosphate.



Scheme 1. Synthesis of the R_P and S_P diastereoisomers of deoxyadenosine 5′-[β-^{17}O]-β-thiodiphosphate. *Reagents:* i, (a) $(PhO)_2POCl$, dioxane; (b) dAMP, pyridine; (c) separate on DEAE-Sephadex; ii, (a) $NaIO_4$; (b) NaOH.



Scheme 2. Proposed reaction mechanism for the hydrolysis of deoxyadenosine 5′-thiodiphosphate as its dianion. Although a distinction between the dissociative and preassociative stepwise mechanisms is uncertain, it seems likely that the preassociative stepwise mechanism (i.e. the pathway *via* [$dADP\beta S^{2-}\cdot H_2O$]) is the lower-energy pathway.[7]

The partial racemisation observed in the hydrolysis of dADPβ[^{17}O]S as its dianion (pH 4.5 and 4.1) provides evidence that a liberated thiometaphosphate ion is generated which can be attacked by water from either face. Since, however, racemisation and inversion occur to about the same extent, the rate of diffusion of thiometaphosphate from the $AMP^{-}\cdot PSO_2^{-}$ ion-pair or the rate of reaction of the liberated thiometaphosphate ion with water must be comparable with the rate of reaction of thiometaphosphate ion in the $AMP^{-}\cdot PSO_2^{-}$ ion-pair with water in the solvent cage. Moreover the stereochemical integrity of the recovered dADPβ[^{17}O]S suggests either that the collapse of the ion-pair back to starting material is slow compared with the forward reaction, or that tumbling of the thiometaphosphate ion within the solvent cage does not occur. In an attempt to distinguish between these possibilities, adenosine 5′[αβ-^{18}O,β-$^{18}O_2$]-β-thiodiphosphate[17] was partially hydrolysed (66%) under the same conditions (pH 4.1). The residual [$^{18}O_3$]ADPβS showed no positional isotope exchange of the $P_\alpha P_\beta$ bridging ^{18}O to the P_α non-bridging position.[18] This suggests that the formation of the $AMP^{-}\cdot PSO_2^{-}$ ion-pair is effectively an irreversible step. A reaction mechanism which accommodates these observations is shown in Scheme 2.

dAMP as its monoanion is apparently a sufficiently good

leaving group to require little or no assistance from the solvent nucleophile. If the hydrolysis of dADPβS at pH 7.2 followed a similar mechanism, solvent separation of the dAMP dianion and the thiometaphosphate anion might, for electrostatic reasons, be expected to occur more rapidly than the solvent separation of the dAMP monoanion from thiometaphosphate anion. The slower reaction and the complete inversion of configuration observed in the hydrolysis of dADPβS at pH 7.2 suggests therefore that the trianion may be hydrolysed by a preassociation concerted mechanism with an 'exploded' transition state, the departure of the dAMP dianion requiring some assistance from the solvent nucleophile.

A similar degree of racemisation has been observed in the hydrolysis of *p*-nitrophenyl phosphorothioate monoanion.[8] *p*-Nitrophenyl phosphorothioate is also solvolysed *in ethanol* as its dianion with predominant racemisation (80% racemisation and 20% inversion).[19]

It is clear therefore that the thiometaphosphate ion, unlike the metaphosphate ion, does have sufficient kinetic stability in aqueous solution to allow the liberated species to be formed under favourable conditions.

We gratefully acknowledge support for this work through an S.E.R.C. studentship (to S. P. H.).

Received, 11th May 1987; Com. 626

References

1 W. W. Butcher and F. H. Westheimer, *J. Am. Chem. Soc.*, 1955, **77**, 2420; P. W. C. Barnard, C. A. Bunton, D. R. Llewellyn, K. G. Oldham, B. L. Silver, and C. A. Vernon, *Chem. Ind. (London)*, 1955, 760.
2 D. Herschlag and W. P. Jencks, *J. Am. Chem. Soc.*, 1986, **108**, 7938 and references there cited.
3 S. L. Buchwald, J. M. Friedman, and J. R. Knowles, *J. Am. Chem. Soc.*, 1984, **106**, 4911.
4 G. Lowe and S. P. Tuck, *J. Am. Chem. Soc.*, 1986, **108**, 1300.
5 M. T. Skoog and W. P. Jencks, *J. Am. Chem. Soc.*, 1984, **106**, 7597; N. Bourne and A. Williams, *ibid.*, p. 7591.
6 F. Ramirez, J. Maracek, J. Minore, S. Srivastava, and W. le Noble, *J. Am. Chem. Soc.*, 1986, **108**, 348.
7 W. P. Jencks, *Acc. Chem. Res.*, 1980, **13**, 161; *Chem. Soc. Rev.*, 1981, **10**, 345.
8 P. Domanico, V. Mizrahi, and S. J. Benkovic, in 'Mechanisms of Enzymic Reactions: Stereochemistry,' ed. P. A. Frey, Elsevier, Amsterdam, 1986, p. 127, and personal communication.
9 R. Breslow and I. Katz, *J. Am. Chem. Soc.*, 1968, **90**, 7376.
10 S. Meyerson, D. J. Harvan, J. R. Hass, F. Ramirez, and J. F. Maracek, *J. Am. Chem. Soc.*, 1984, **106**, 6877; M. Henchman, A. A. Viggiano, J. F. Paulson, A. Freedman, and J. Wormhoudt, *J. Am. Chem. Soc.*, 1985, **107**, 1453.
11 H. W. Roesky, R. Ahlrichs, and S. Brode, *Angew. Chem., Int. Ed. Engl.*, 1986, **25**, 82.
12 E. K. Jaffe and M. Cohn, *J. Biol. Chem.*, 1978, **253**, 4823.
13 G. Lowe, B. V. L. Potter, B. S. Sproat, and W. E. Hull, *J. Chem. Soc., Chem. Commun.*, 1979, 733; M.-D. Tsai, *Biochemistry*, 1979, **18**, 1468; M.-D. Tsai, S. L. Huang, J. F. Kozolowski, and C. C. Chang, *ibid.*, 1980, **19**, 3531.
14 E. K. Jaffe and M. Cohn, *Biochemistry*, 1978, **17**, 652.
15 C. A. Bunton, D. R. Llewellyn, K. G. Oldham, and C. A. Vernon, *J. Chem. Soc.*, 1958, 3574.
16 J. R. P. Arnold and G. Lowe, *J. Chem. Soc., Chem. Commun.*, 1986, 865; *Bioorg. Chem.*, 1987, in the press.
17 This was prepared by R. C. Bethell using a modification of the method of R. C. Bethell and G. Lowe, *J. Chem. Soc., Chem. Commun.*, 1986, 1341 and *J. Labelled Compd.*, 1987, **24**, 239.
18 R. C. Bethell and G. Lowe, unpublished work.
19 P. M. Cullis and A. Iagrossi, *J. Am. Chem. Soc.*, 1986, **108**, 7870.