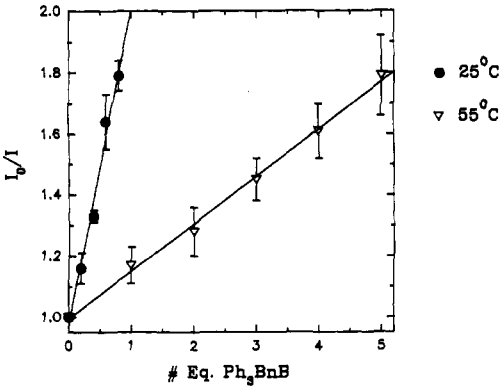

Figure 3. Effect of temperature on the fluorescence quenching of 0.27 μM I by II in DPPC liposomes (0.1 mg/mL).

II alone should render the liposome anionic, removing the need for an anionic phospholipid. In fact, addition of I and II to pure DOPC liposomes followed by irradiation yields results very similar to those obtained with anionic liposomes.

The effect of the physical state of the lipid bilayer on PET between I and II was studied using dipalmitoylphosphatidylcholine (DPPC) liposomes. The time required to bleach 50% of the dye was measured over a 30 °C temperature range (Figure 2). The reaction is 3.5 times faster at 25 °C than at 55 °C, with the rate changing significantly at the phase-transition temperature (T_m) of the membrane.[14] Figure 3 shows that the fluorescence quenching of the dye by the borate in DPPC liposomes is also more efficient (by a factor of 6.5) below T_m. Whereas the photobleaching experiment probes the net PET reaction, fluorescence quenching allows study of the forward reaction separate from the back transfer. The greater sensitivity to temperature observed in the emission data compared to the photobleaching reaction suggests that BET is also favored below the T_m.

A reasonable explanation for these results is suggested by the greater disorder of the acyl chains in the L_α phase above the T_m, which could allow a deeper penetration of II into the bilayer, with two important ramifications. First, the donor-acceptor distance of separation increases, and second, the borate moves deeper into a low dielectric constant region. Both factors would retard the rate of electron transfer,[15] consistent with the data shown in Figures 2 and 3.[16]

In conclusion, the net photoinduced electron transfer from triphenylbenzylborate (II) to water-soluble cyanine I is greatly enhanced by binding of both reactants to PC bilayers. The photoredox reaction involves transfer of an electron from the hydrophobic interior of the bilayer to its surface. The efficiency of the process is dependent on the physical state of the bilayer. A significant amount of the excitation energy is stored in the form of the reduced dye and benzyl radical liberated by decomposition of the oxidized borate. Current efforts are directed toward utilizing this energy to drive subsequent chemical reactions.

Acknowledgment. We thank the National Science Foundation for partial support of this research.

(13) Estimates for the location of tetraphenylborate in phospholipid bilayers place it slightly (<5 Å) below the ester carbonyls in the lipid tails. See ref 12c and the following: Flewelling, R. F.; Hubbell, W. L. *Biophys. J.* **1986**, *49*, 541-552.

(14) The T_m for DPPC is shifted from 41.4 °C to 39.7 °C upon addition of I and II in the relative amounts used in the photobleaching and quenching experiments. Differential scanning calorimetry was performed with a Microcal MC-2 calorimeter at a scan rate between 10 and 15 deg/h.

(15) Marcus, R. A. *J. Chem. Phys.* **1956**, *24*, 966-978. Marcus, R. A. *J. Chem. Phys.* **1957**, *26*, 867-871. Marcus, R. A.; Sutin, N. *Biochim. Biophys. Acta* **1985**, *811*, 265-322.

(16) Calculations based on the fluorescence quenching data and considering only the effect of distance on electron-transfer rates indicate that the borate moves no further than 2 Å deeper into the bilayer above T_m. The actual distance is likely to be smaller when the dielectric constant of the medium is taken into account.

A Chemical Model for Phosphomutases: A Dissociative Thiophosphoryl Transfer Reaction Proceeding with Retention of Configuration

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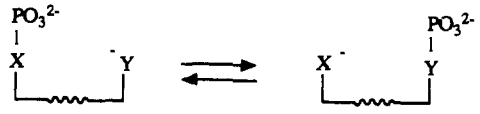
Phosphomutases catalyze an apparent intramolecular phosphoryl transfer reaction, eq 1. We report here a simple chemical model for a dissociative mutase-like reaction and demonstrate that this proceeds with overall excess retention of configuration despite the fact that only a single thiophosphoryl transfer is involved.



(1)

Phosphomutases have been shown to catalyze reactions with overall retention of configuration.[1] This observation is explicable in terms of a double displacement involving a bis-phosphorylated cofactor for one class of such enzymes and for the other class a phosphoenzyme intermediate. The question of whether retention of configuration is a *necessary and sufficient* observation from which to conclude a double displacement (two phosphoryl transfers) is an intriguing one.

The majority of enzyme-catalyzed phosphoryl transfer reactions, in particular kinases, have been shown to proceed with inversion of configuration.[2] In contrast, mutases and some phosphatases have been shown to catalyze phosphoryl transfer with overall retention of configuration. In many of these cases there is good independent evidence for a double-displacement mechanism. The conclusion therefore must be that single phosphoryl transfer steps occur with in-line geometry. The debate over whether individual phosphoryl transfer steps are formally associative or dissociative has rumbled on, in part because model studies demonstrate that although monomeric metaphosphate is not an intermediate in such reactions in dilute aqueous solutions,[3] it does participate in reactions in a variety of organic media including *tert*-butyl alcohol,[4] and it is not clear how the active sites of proteins should be viewed in terms of the environment that they provide for the reaction. Indeed it is tantalizing to imagine the functional significance of the movement of "hinged" regions of proteins (common in phosphotransferases) that effectively sequesters the substrate (and intermediates) away from water. In this present study we have probed the stereochemical constraints on the dissociative pathway and have developed a simple chemical model for a phosphomutase-type reaction.

(R_p)-2-(Hydroxymethyl)-4-nitrophenyl [^{16}O, ^{18}O]thiophosphate (**1**) can be readily synthesized by our published route.[5] We have deliberately chosen to study a thiophosphoryl transfer reaction since we have confidence that in alcohols this type of reaction is fully dissociative.[6] Furthermore, our stereochemical analysis is

(1) Blätter, W. A.; Knowles, J. R. *Biochemistry* **1980**, *19*, 738.

(2) Knowles, J. R. *Ann. Rev. Biochem.* **1980**, *49*, 877; Frey, P. A. *Tetrahedron* **1982**, *38*, 1541; Eckstein, F. *Angew. Chem., Int. Ed. Engl.* **1983**, *22*, 423; Lowe, G. *Acc. Chem. Res.* **1983**, *16*, 244; Gerlt, J. A.; Coderre, J. A.; Mehdi, S. *Adv. Enzymol.* **1983**, *55*, 29; Cullis, P. M. in *Enzyme Mechanisms*, eds. Page, M. I.; Williams, A.; Royal Society of Chemistry, **1987**, p. 178.

(3) Buchwald, S. L.; Friedman, J. M.; Knowles, J. R. *J. Am. Chem. Soc.* **1984**, *106*, 4911; Ramirez, F.; Maracek, J.; Minore, J.; Srivastava, S.; le Noble, W. J. *J. Am. Chem. Soc.* **1986**, *108*, 348; Bourne, N.; Williams, A., *J. Am. Chem. Soc.* **1984**, *106*, 7591; Skoog, M. T.; Jencks, W. P. *J. Am. Chem. Soc.* **1984**, *106*, 7597.

(4) Cullis, P. M.; Rous, A. J. *J. Am. Chem. Soc.* **1985**, *107*, 6721; *ibid.* **1986**, *108*, 1298; Friedman, J. M.; Knowles, J. R.; *J. Am. Chem. Soc.* **1985**, *107*, 6126; Cullis, P. M.; Nicholls, D. *J. Chem. Soc., Chem. Commun.* **1987**, 783; Freeman, S.; Friedman, J. M.; Knowles, J. R. *J. Am. Chem. Soc.* **1987**, *109*, 3166.

(5) Cullis, P. M.; Iagrossi, A.; Rous, A. J. *J. Am. Chem. Soc.* **1986**, *108*, 7869.

(6) Cullis, P. M.; Iagrossi, A. *J. Am. Chem. Soc.* **1986**, *108*, 7870; Cullis, P. M.; Misra, R.; Wilkins, D. J., *J. Chem. Soc., Chem. Commun.* **1987**, 1594; Burgess, J.; Blundell, N.; Cullis, P. M.; Hubbard, C. D.; Misra, R. *J. Am. Chem. Soc.* **1988**, *110*, 7900.

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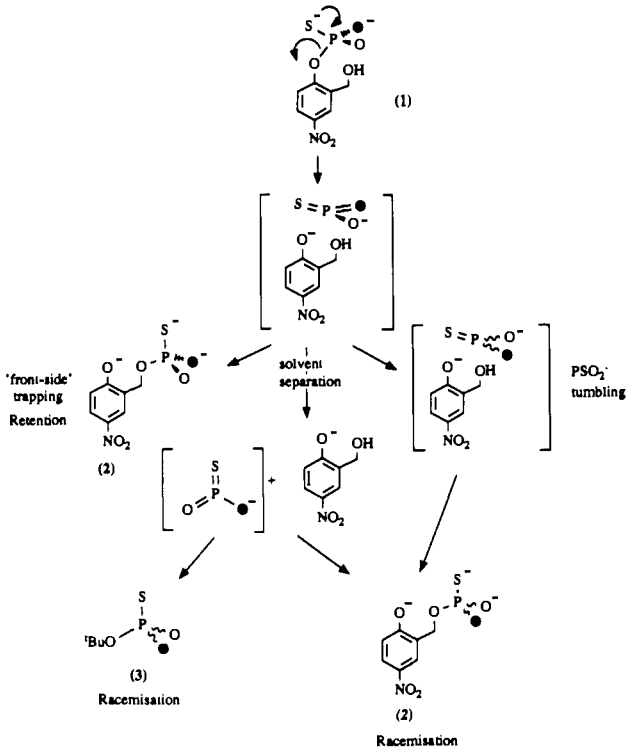
Scheme I



considerably more convenient than that for isotopically chiral phosphate esters.[7] When a concentrated aqueous solution of the dianion of **1** is rapidly diluted into a large volume of *tert*-butyl alcohol, **1** reacts rapidly to give comparable yields of 2-hydroxy-5-nitrobenzyl thiophosphate (**2**) and *tert*-butyl thiophosphate (**3**), with only small amounts of inorganic thiophosphate, Scheme I. The starting material **1** and the two products **2** and **3** were subjected to our published stereochemical analysis.[7] The starting material was shown to be present in ≥90% ee, and the assignment of the R_p configuration follows from the synthesis. As expected, the *tert*-butyl [^{18}O]thiophosphate (**3**) was completely racemic, confirming a fully dissociative mechanism involving a "free" monomeric thiometaphosphate intermediate, in accord with expectations based on our previous studies.[6] Although the product (**2**) of the formally intramolecular thiophosphoryl transfer reaction experienced some racemization (ca. 60%), there was a significant stereospecific pathway. On the basis of the absolute configuration deduced from the stereochemical analysis and confirmed by comparison with independently synthesized material, this pathway proceeds with retention of configuration.

The observed racemization during the formation of **2** could be accounted for in a number of ways, including tumbling of the thiometaphosphate within the dissociation complex or return from a solvent-separated species. However, the pathway proceeding with retention must arise from "front-side" trapping of monomeric thiometaphosphate within the initial dissociation complex. The alternative possibility involving formation and pseudorotation of a pentacovalent intermediate is highly unlikely for two reasons: (i) in base, the first-formed pentacoordinate intermediate would involve placement of an oxyanion (or S^-) in the apical position, for which there is no reliable precedence;[8] (ii) increasing amounts of water included in the reaction mixture lead to a decrease in the amounts of the rearranged product (**2**) and an increase in inorganic thiophosphate, presumably because of competition for a common intermediate, namely, caged thiometaphosphate. This is the first demonstration of a dissociative phosphoryl transfer reaction occurring with retention.[9] Interestingly, this study taken with other results demonstrates that a dissociative or preassociative stepwise pathway can occur with *inversion, racemization, or retention*, depending on conditions (solvent, etc.) and the constraints placed on the nucleophile and the reactive intermediate. It also suggests that, if enzymes do accelerate a formally dissociative reaction, there exists the possibility for a front-side nucleophilic displacement on an enzyme-shielded metaphosphate intermediate. This possibility is reminiscent of the difficulties in distinquishing an enzyme-shielded oxycarbonium ion and a genuine glycosyl-enzyme intermediate in glycosyl transferases that proceed with overall retention.[10]

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Registry No. 1, 136537-94-5; **2**, 136537-95-6; **3**, 136537-96-7.

(7) Cullis, P. M.; Misra, R.; Wilkins, D. J. *Tetrahedron Lett.* **1987**, *28*, 4211.

(8) If (1) were to undergo an associative reaction the nucleophile and the leaving group would initially have to span apical-equatorial positions with the nucleophile forced to approach from an adjacent position since a six membered ring cannot span the two apical sites (six membered rings may offer some stabilization of the pentacoordinate intermediate but considerably less than a five membered ring). The pentacoordinate intermediate that this would have to involve would therefore place one of the remaining ligands (-O^- or -S^-) in the other apical position. The low apicophilicity of such groups would make this a relatively high energy pathway.

(9) A previous chemical 'mutase-like' reaction studied by Knowles and coworkers (Buchwald, S. L.; Pliura, D. H.; Knowles, J. R. *J. Am. Chem. Soc.* **1984**, *106*, 4916) reported the stereochemical course of an intramolecular phosphoryl transfer of monophosphates derived from propane diol under acid conditions to be retention of configuration but this reaction is an associative reaction involving an adjacent attack and pseudorotation of the resulting phosphorane, a reaction type known to proceed with retention.

(10) Sinnott, M. L. in *Enzyme Mechanisms*, eds. Page, M. I.; Williams, A.; Royal Society of Chemistry, **1987**, p. 259.

Isolation and Crystal Structure of a Technetium(V) Nitrido Complex Containing a Coordinated Transient State of *N*-(2-Aminoethyl)carbamic Acid

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We reported recently the synthesis and characterization of the technetium(V) nitrido complexes $[TcN(L^1)_2Cl]^+$ and $[TcN(L^2)Cl]^+$ [$L^1 = H_2N(CH_2)_nNH_2$, $n = 2, 3$; $L^2 = H_2N(CH_2)_3NH(CH_2)_2NH(CH_2)_3NH_2$].[1] In developing this chemistry, we carried out the reaction of the complex $TcNCl_2(PPh_3)_2$ with diethylenetriamine ($H_2NCH_2CH_2NHCH_2CH_2NH_2$ = dien), and surprisingly, we isolated the dicationic complex $[TcN(en)_2(L)]^{2+}$ (**1**) [en = ethylenediamine; L = *N*-(2-aminoethyl)carbamic acid ($HOOCNHCH_2CH_2NH_2$)]. The X-ray crystal structure of **1** revealed that the coordinated ligand L lies in a peculiar "transient state" originated by an uncommon weakening of a carbon–nitrogen bond. We report here the synthesis of complex **1** and the description of its crystal structure.

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