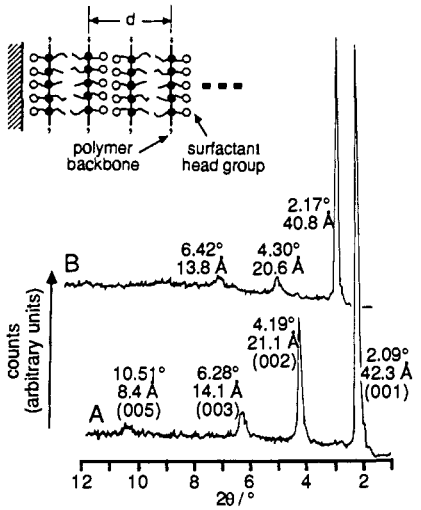

Figure 2. X-ray diffraction (Cu Kα irradiation, λ = 1.5418 Å) patterns of poly(**2**) before and after ion exchange: (A) as-grown poly(**2**) (values of 2θ (deg) and *d* (Å) and Miller indices are shown by each reflection peak); (B) after treatment with 1 M aqueous $HClO_4$, pattern offset by 0.5°. Top: Schematic representation of the head-to-head lamellar structure of poly(**2**) inferred from X-ray diffraction pattern.

from dilute solutions of **2** is probably a consequence of molecular self-assembly such that, although the bulk concentration of surfactant pyrrole is only 5 mM, the *effective* local concentration around a reactive pyrrole radical cation is much higher. Polymerization then proceeds, in preference to diffusion of the radical cation away from the electrode.

Thicker layers were deposited by potentiostatic polymerization at +650 mV in a gently stirred solution. The electrode was disconnected at a positive potential, and the resulting films were briefly rinsed in water, dried under reduced pressure, and peeled from the electrode to afford free-standing films. Transmission Fourier transform infrared spectroscopy and X-ray photoelectron spectroscopy were in accord with the proposed polymeric structure and indicated the absence of nitrate (NO_3^-). The infrared spectrum of the polymer has only weak adsorptions in the region for pyrrole C-H out-of-plane bending, which were observed as strong adsorptions in the spectrum of the monomer **2**. The films were completely soluble in chloroform, swollen and partially soluble in water, and insoluble in dimethyl sulfoxide and acetonitrile.

X-ray diffraction of as-grown films on gold or ITO glass gave four reflections, shown in Figure 2A. The spacings indicate a lamellar structure with an interlayer spacing of 42.2 ± 0.2 Å. The diffraction pattern closely resembles that of Langmuir-Blodgett multilayers[13] and is consistent with a head-to-head structure, shown schematically in Figure 2 (inset). These as-grown films are highly resistive. Immersion of the films in 1 M $HClO_4$ brings about cation exchange of potassium for proton (XPS: no K signal, no ClO_4^- incorporation), with a concomitant contraction of the lamellar spacing to 41.1 ± 0.3 Å (Figure 2B). Preliminary measurements on the ion-exchanged films indicate an anisotropic electrical conductance with a conductivity parallel to the surface ($\sigma_\parallel$) of 10^{-3} $\Omega^{-1}\cdot cm^{-1}$ and a perpendicular conductivity ($\sigma_\perp$) of 10^{-6} $\Omega^{-1}\cdot cm^{-1}$. The conduction mechanism (electronic versus ionic) has not been determined.

The formation of this head-to-head bilayer type assembly by polymerization of pyrrole-containing surfactants from micellar solutions implies that assembly of the monomers, or growing polymer chain, at the electrode (i.e., as a hemimicelle) predominates over the head-first delivery of the surfactant to the electrode by the micelle. The homopolymers assemble into a layered morphology, producing a film which is highly ordered relative to other poly(heterocycles). This method can be used without the need for an ordered supporting medium, and with the distinct advantage of operational simplicity over the use of the Langmuir-Blodgett technique for the deposition of such materials.

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Supplementary Material Available: Characterization (NMR, IR, XPS) of **2**, poly(**2**), and ion-exchanged poly(**2**) (1 page). Ordering information is given on any current masthead page.

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Evidence for Monomeric Metaphosphate as an Intermediate in the Hydrolysis of μ-Monothiopyrophosphate

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NMR analysis of the cleavage of μ-monothiopyrophosphate trianion (MTP) by tris(hydroxymethyl)aminomethane (Tris), in competition with water under hydrolytic conditions, shows that Tris competes with water for accepting the phosphoryl group from MTP; however, the kinetics fails to implicate Tris as a nucleophile in the transition state for P-S bond cleavage. The simplest mechanism that is consistent with both the kinetic and trapping results is a preassociation stepwise mechanism, in which metaphosphate monoanion (PO_3^-) is produced from MTP in the rate-limiting step, and PO_3^- is instantly trapped by a nucleophilic species participating in the solvation of MTP. Tris at high concentrations occupies the solvent cage and traps PO_3^- in competition with solvating water.

Monomeric metaphosphate has long been proposed to be an intermediate in phosphoryl group transfer reactions such as the solvolysis of alkyl phosphate monoanions and of aryl and acyl phosphate dianions with very good leaving groups ($pK_a < 7$).[1] Phosphoryl transfer in alcoholic solutions was found to proceed with inversion of configuration at chiral P and failed to give evidence for the existence of diffusible PO_3^-.[2] In poorly solvating organic reaction media, racemization of chiral P in the course of solvolysis has been observed and offered as evidence for the participation of PO_3^- as a discrete, intermediate species.[3] The existence of PO_3^- in organic solvents such as acetonitrile and *tert*-butyl alcohol has also been proposed based on studies of kinetics and product composition.[4] However, kinetic studies in aqueous solutions showed that attacking nucleophiles are partially bonded to P in the transition state for phosphoryl transfer in reactions of acyl phosphates and phosphoramidates.[5]

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Table I. Effects of Added Nucleophiles on Rate Constants for Cleavage of MTP[a]

nucleophile	concn,[b] M	pH	k_{av},[c] min^{-1}	k_{hydrol},[d] min^{-1}
$(HOCH_2)_3CNH_2$	0.10-0.30	9.87	0.055 ± 0.014	0.059 ± 0.021
	0.10-0.30	9.15	0.187 ± 0.008	0.25 ± 0.05
	0.74-1.5[e]	9.71	0.069 ± 0.009	0.082 ± 0.007
	2.0-3.0	9.90	0.055 ± 0.01	0.055 ± 0.006
H_2NNH_2	0.1-0.3	9.40	0.131 ± 0.005	0.155 ± 0.013
$HONH_2$	0.1-0.3	9.33	0.176 ± 0.007	0.178 ± 0.013

[a] The reaction mixtures included, at 25 °C, 10-30 mM K-CHES buffer and 63-76 μM Li_4MTP; the ionic strength was 0.5 maintained with KCl unless otherwise stated. The LiMTP was synthesized by a modification of the procedure described by Loewus and Eckstein.[6a,7] Cleavage of MTP to thiophosphate was monitored spectrophotometrically at 227 nm.[6b] [b] At each pH value, single rates were measured at each of three concentrations of nucleophile within the ranges indicated. [c] The values of k_{av} are the average of rate constants from three runs at different concentrations of added nucleophile within the ranges indicated in column 1, and the uncertainties listed are the mean deviations of individual rate constants from k_{av}. [d] The first-order rate constants for hydrolysis of MTP at the indicated pH were obtained by calculation from the pH-rate profile for hydrolysis.[6b] These are the rates at 25 °C and ionic strength 0.1 (KCl). [e] 0.1 M KCl.

Table II. Phosphoryl Group Capture by Tris and Water in the Cleavage of MTP[a]

[Tris], M	pH	% $(HOCH_2)_3$-$NHPO_3^{2-}$	% $H_2N(CH_2OH)$-$CH_2OPO_3^{2-}$	% PO_4^{3-}
1.7	9.8	16.5	14.5	69
3.0	9.9	28.8	23.3	48

[a] The partitioning of phosphoryl groups from MTP into P_i, *N*-phosphoryl-Tris, and *O*-phosphoryl-Tris was determined by ^{31}P NMR. The following values for chemical shifts were observed: PSO_3^{3-}, δ 35.2 ppm; PO_4^{3-}, δ 3.4 ppm; *N*-phosphoryl-Tris, δ 7.0 ppm; *O*-phosphoryl-Tris, δ 5.1 ppm (triplet, J = 5.8 Hz). The assignments of organic phosphates were based on the phosphorus-hydrogen coupling pattern for *O*-phosphoryl-Tris; this was confirmed by the observation that the species exhibiting the signal at δ = 7.0 ppm is labile in dilute acid, a property of phosphoramidates but not of phosphomonoesters.

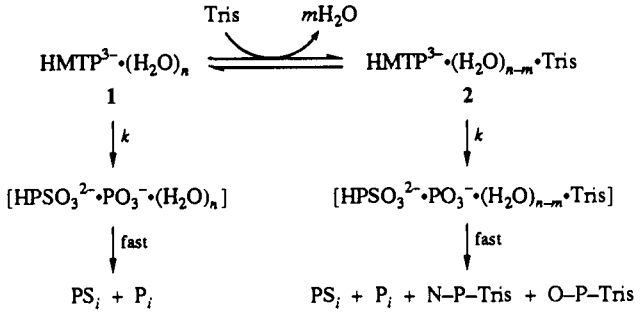
The rates at which phosphomonoesters and *N*-phosphopyridines are cleaved under hydrolytic conditions are enhanced by the presence of added nucleophiles such as carboxylates, bicarbonate, F^-, pyridines, and especially nucleophiles exhibiting the α-effect.[5] Enhanced cleavage rates result from the larger second-order rate constants for the added nucleophiles relative to water.

μ-Monothiopyrophosphate (MTP) is an analogue of pyrophosphate that undergoes hydrolysis millions of times faster than pyrophosphate, owing to the dissociative character of the reaction and the weakness of the P-S bond relative to P-O.[6b] The cleavage rate for MTP is not increased by the addition of any of a variety of nucleophiles, including α-effect nucleophiles. For example, as shown in Table I, Tris at concentrations from 0.01 to 3.2 M does not alter the rate at which MTP is cleaved to thiophosphate at pH values ranging from 9.15 to 9.90.

Although Tris does not affect the rate under hydrolytic conditions, it participates in the cleavage of MTP, as shown by the data in Table II, which shows that Tris at high concentrations captures a phosphoryl group from MTP to form both *N*-phosphoryl-Tris and *O*-phosphoryl-Tris. The phosphoryl groups captured by Tris are partitioned as 54 ± 1% *O*-phosphoryl-Tris and 46 ± 1% *N*-phosphoryl-Tris. Inasmuch as an amino group should be orders of magnitude more reactive than a hydroxyl group when the value of β_{nuc} is in a normal range, the observed capturing ratio is consistent with little or no nucleophilic participation in the transition state (i.e., $\beta_{nuc} = 0$).

Tables I and II show that Tris captures a phosphoryl group from MTP in competition with water, producing both *N*-phosphoryl-Tris and *O*-phosphoryl-Tris in comparable amounts, but it has no significant effect on the rate at which MTP is cleaved to thiophosphate. The simplest interpretation of these facts is that the reaction proceeds by a preassociation, stepwise mechanism such as that in Scheme I for the trianionic form of MTP ($HMTP^{3-}$), the dominant and most reactive form under the conditions of Tables I and II.[6b] Tris reversibly enters the solvation sphere, presumably by displacing water, in a preassociation step. Phosphoryl transfer proceeds *at the same rate* within both solvated complexes **1** and **2**. That phosphoryl transfer to nitrogen and oxygen of Tris is almost random within complex **2** suggests the production of a common, highly reactive intermediate, such as PO_3^-, in the rate-limiting step. Cleavage of MTP in complexes **1** and **2** to PO_3^- (and PS_i) with the same rate constant, k in Scheme I, followed by fast, essentially random capture of PO_3^- by water and Tris in the solvation sphere, accounts for our results.

Scheme I



Earlier studies of phosphomonoesters and phosphoramidates showed that there is nucleophilic participation in phosphoryl group transfer; that is, b_{nuc} is positive. These reactions required cleavage of P-O and P-N bonds, which are much stronger than the P-S bond. The present work shows that phosphoryl group transfer from μ-monothiopyrophosphate in water is insensitive to the nucleophilic reactivity of the acceptor, that is, $\beta_{nuc} = 0$. This is positive evidence that monomeric metaphosphate is a discrete intermediate that is randomly captured by nucleophiles in the solvation sphere.

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Observation of an Imine Intermediate on Dehydroquinase by Electrospray Mass Spectrometry

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We report the direct observation of an imine intermediate (Schiff base) in an enzyme-catalyzed reaction using electrospray mass spectrometry. Dehydroquinase (E.C. 4.2.1.10) catalyzes the third step on the shikimate pathway, the conversion 3-dehydroquinate **1** to 3-dehydroshikimate **6**.[1] The reaction involves loss of the less acidic *pro-R* hydrogen[2] and proceeds via a multistep

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