Formate Activation in Neutral Aqueous Solution Mediated by a Polyammonium Macrocycle

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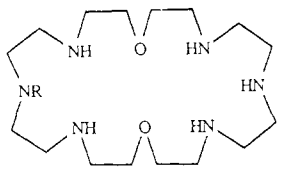
Abstract: The polyammonium macrocycle 1,13-dioxa-4,7,10,16,19,22-hexaazacyclotetracosane, [24]N_6O_2, **1**, activates formate in the presence of ATP and calcium(II) or magnesium(II) ion, the final product being the formylated macrocycle **3**. The reaction sequence proceeds via a phosphorylated macrocycle (**2**), the result of phosphoryl transfer from ATP. The analogous reaction using acetyl phosphate to generate **2** was successful, proving that **2** is the key species in the reaction. Reaction of formate with the phosphoramidate **2** gives formyl phosphate as a transient intermediate, which is readily dephosphorylated by attack of a macrocyclic amino group on the formyl carbon. The final product is the formylated macrocycle **3**. The reaction may be patterned after the stepwise sequence of carboxylate activation and amide formation by certain synthetases.

The activation of carboxylate anions in neutral aqueous media in the biosynthesis of amides presents a major feat in biological systems. The problem appears to be solved through the formation of enzyme-bound carboxyl phosphate anhydrides by a variety of enzymes, e.g., glutamine synthetase,[1] glutathione synthetase,[2] and N^{10}-formyltetrahydrofolate synthetase.[3] These enzymes are dependent on a divalent metal ion, usually Mg^{2+}, and function by catalyzing the transfer of the γ-phosphoryl group of adenosine triphosphate (ATP) to the carboxylate, forming the mixed carboxyl phosphate anhydride and adenosine diphosphate (ADP).

Recent studies using polyammonium macrocycles as chemical models of biological reactions, i.e. biomimics, have shown these molecules to be efficient catalysts in the dephosphorylation of ATP[4] and acetyl phosphate.[5] The influence of the polyammonium macrocycle on ATP chemistry prompted an investigation into other ATP-mediated biological events. As a result of a study of formyl phosphate, a new and more extensive role for the macrocycle has been discovered. Formate can be activated by the polyammonium macrocycle **1**, in conjunction with ATP as phosphate source, in a reaction which proceeds through a phosphorylated macrocycle, **2**, to a formylated macrocyclic product, **3**.



1, R = H
2, R = PO_3^{2-}
3, R = N-formylated **1**

Experimental Section

Materials and Methods. The [^{13}C]formate (99%) and [^{18}O]water (97%) were obtained from Cambridge Isotope Laboratories, Woburn, MA. The macrocycle [24]N_6O_2 (**1**) was prepared by literature procedures.[6] Formyl phosphate was prepared in 50–55% yield from formyl fluoride and K_2HPO_4 by using the procedure described by Jaenicke and Koch.[7] The intermediate, formyl fluoride, was synthesized from anhydrous formic acid and KHF_2 in the presence of benzoyl chloride, by the method of Olah and Kuhn.[8] The formyl phosphate used in these studies was approximately 50–60% pure. The concentrations of the impurities, formate, phosphate, and pyrophosphate, were calculated by ^{1}H and ^{31}P NMR.

NMR spectra were recorded on a Varian XL-300 spectrometer at 300 MHz for ^{1}H, 75.43 MHz for ^{13}C, and 121.4 MHz for ^{31}P. Peak assignments were referenced to (trimethylsilyl)propanesulfonic acid sodium salt (DSS) and phosphoric acid (85%). The probe temperature was regulated by a variable-temperature accessory. Mass spectra were obtained with Ribermag R-10-10 and VG-ZAB spectrometers. Adjustments to the desired pH of samples containing the ligand and substrate were made with 0.2 M NaOD or HCl in D_2O. Yields of the formylated macrocycle **3** were estimated by integration of the ^{31}P and ^{1}H NMR spectra of the ^{18}O-labeled compounds with the use of internal standards.

Formyl-1,13-dioxa-4,7,10,16,19,22-hexaazacyclotetracosane (3) by Reaction with Formyl Phosphate. A 0.1 M solution of [24]N_6O_2·6H-Br·4H_2O in 0.3 mL of D_2O was adjusted to pH 7.0 with 0.2 M NaOD. The solution was cooled in an ice bath and added to a weighed sample of formyl phosphate to give a final concentration of 0.054 M formyl phosphate. This and similar reactions were monitored by time-dependent changes in the ^{1}H NMR signals for formyl phosphate (8.55, 8.53 ppm), formate (8.44 ppm), and formylated macrocycle **3** (8.21 ppm), ^{13}C signals for formate (171.02 ppm) and formylated macrocycle **3** (166.96 ppm), and ^{31}P NMR signals for phosphoramide (9.75, 9.63 ppm), phosphate (2.76 ppm), and formyl phosphate (0.37 ppm). After 2 h, the signals due to formyl phosphate had disappeared and were replaced by new major resonances assigned to inorganic phosphate in the ^{31}P NMR and formylated macrocycle **3** in the ^{13}C NMR. The ^{1}H NMR showed no evidence of formate formation. The product **3**, obtained after 6 h, of the reactions at several pH values showed similar NMR spectra: ^{1}H NMR (D_2O) 8.21, 8.20, 8.13 (NC*H*O), 3.76–3.41 (CH_2O), 3.10–2.80 ppm (CH_2N); ^{13}C NMR (D_2O) 166.96 plus three minor peaks (N*C*HO), 65.22, 65.11, 64.07, 63.53 (CH_2O), 45.89, 45.70, 45.64, 45.34, 45.16, 44.40, 44.19, 44.09, 43.79, 43.55, 42.80, 39.68 ppm (CH_2N). A sample for mass spectral analysis was obtained by adjusting the solution to pH 10 and extracting it with methylene chloride: MS (CI/NH_3), *m/e* (relative intensity) 375 (10, **1**·CHO$^+$ + 1), 374 (8, **1**·CHO$^+$), 373 (45, **1**·CHO$^+$ − 1), 359 (20), 357 (25, **1**·CHO − OH$^-$), 347 (100, **1**$^+$ + 1).

Activation of Formate Using ATP To Generate Phosphoramidate 2. A D_2O solution (1.0 mL) of ATP, **1**, and $MgBr_2$ (0.03, 0.03, and 0.045 M, respectively) and sodium formate (1.0 M) was adjusted to pH 7.0 and heated at 60 °C for 10 h. Yield of **3**: 39%. In a similar reaction a D_2O solution (0.5 mL) of ATP, **1**, $CaBr_2$, and sodium formate (0.07 M in each) was adjusted to pH 7.0 and allowed to stand at 25 °C for 7 days. Yield of **3**: 5%. Compound **3** was not found in a control reaction performed under analogous conditions in the absence of ATP.

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Activation of Formate Using Acetyl Phosphate To Generate Phosphoramidate 2. Formylated macrocycle **3** was also obtained by reacting a 0.7 mL solution containing acetyl phosphate and **1** (0.1 M in each) and [^{13}C]formate (0.2 M) adjusted to pH 7.0. The mixture was allowed to react at room temperature for 48 h, at which time DSS was added (0.60 mM). Yield of **3**; 8%: ^{13}C NMR (D_2O, pH 7.0) 181.40 (CH_3COO^-), 171.15 ($HCOO^-$), 166.96 ppm (NCHO); ^{1}H NMR (D_2O, pH 7.0) 8.22, 8.15, 8.11 (NC*H*O). A reaction in which acetyl phosphate and **1** (0.06 M in each) were reacted for 3 h at room temperature at pH 7.0 followed by addition of 1 M [^{13}C]formate was also performed.

[^{18}O]Water Incorporation Studies. The incorporation of [^{18}O]water was examined in a 300-μL solution of acetyl phosphate and **1** (0.06 M in each) and formate (0.12 M) in 97% [^{18}O]water adjusted to pH 7.0. The solution was incubated at room temperature for 48 h and then diluted with an equal volume of D_2O. Ethylenediaminetetraacetic acid (EDTA) (2 mM) was added to the mixture, and the ^{31}P and ^{13}C NMR spectra were obtained: ^{31}P NMR 1.99 ($HP^{16}O_4^{2-}$, 25%), 1.97 ppm ($HP^{16}O_3{}^{18}O^{2-}$, 75%); ^{13}C NMR 166.91, 166.79, 166.20 (NCH^{16}O) 166.88, 166.76, 166.17 (NCH^{18}O), 171.02 ppm ($HC^{16}O_2^-$). A reaction was run under analogous conditions without formate: ^{31}P NMR (D_2O, pH 7) 1.85 ppm ($HP^{16}O_3{}^{18}O^{2-}$). Another reaction was run in which acetyl phosphate (0.06 M) and formate (0.12 M) were reacted for 8 h at pH 7 and 40 °C in 50% [^{18}O]water: ^{13}C NMR 181.26 (CH_3COO^-), 171.03 ppm ($HCOO^-$).

A reference sample of the five labeled isotopomers of phosphate equilibrated in 50% [^{18}O]water was examined: ^{31}P NMR (D_2O, pH 7.0) 2.05 ($HP^{16}O_4^{2-}$), 2.02 ($HP^{16}O_3{}^{18}O^{2-}$), 2.00 ($HP^{16}O_2{}^{18}O_2^{2-}$), 1.98 ($HP^{16}O^{18}O_3^{2-}$), 1.95 ppm ($HP^{18}O_4^{2-}$). Equilibration of formate for 20 min at room temperature in 50% [^{18}O]water at pH 1 gave a mixture of the three isomers: ^{13}C NMR (D_2O, pH 7) 171.14 ($HC^{16}O_2^-$, 25%), 171.12 ($HC^{16}O^{18}O^-$, 50%), 171.09 ppm ($HC^{18}O_2^-$, 25%). The peak assignments for the samples were confirmed by analysis of the ^{31}P and ^{13}C NMR spectra of a solution of the individual samples and each of the reference solutions.

Results

Formylation of Macrocycle by Formyl Phosphate. The reaction of formyl phosphate and **1** was found to result in formylated macrocycle **3** as the principal product. The release of phosphate from formyl phosphate and the disappearance of formyl phosphate in the presence and absence of **1** were monitored by ^{1}H, ^{13}C, and ^{31}P NMR in D_2O and mixtures of D_2O/H_2O at 5 °C and pH 7.0. Both formate and phosphate were observed as hydrolysis products in the absence of **1**. Analysis of the ^{13}C NMR spectrum of the products showed one major carbonyl signal along with several minor signals, assigned to NCHO isomers, four CH_2O signals, and 12 CH_2N peaks, one of which was shifted 5 ppm upfield to 39.68 ppm. The latter is indicative of the restricted rotation of the amide in **3** and corresponds to findings in other amides.[9] The ^{1}H NMR spectrum showed one major resonance at 8.21 ppm and additional minor resonances in that region corresponding to the NC*H*O signal, along with the anticipated resonances of CH_2O and CH_2N. The ^{31}P NMR spectrum showed the formation of only a small amount of phosphorylated macrocycle **2** (ca. 2%). Mass spectral analysis of the product of the reaction of the macrocycle **1** with formyl phosphate at pH 7 also indicated the formation of one major product, which is proposed to be the monoformylated macrocycle.

Formylation of the Macrocycle Using ATP, Metal Ions, and Formate. The reaction of ATP, metal ions (calcium or magnesium), and sodium formate in the presence of **1** resulted in a formylated macrocycle. The reactions were performed at pH 7 in the presence of either $CaBr_2$ or $MgBr_2$ at varying ratios and temperatures, and all showed the presence of a formylated macrocycle with the characteristic ^{13}C and ^{1}H NMR resonances for the formamide carbon and proton. In control experiments all conditions and reagents were kept the same with the exception that ATP was absent. No evidence of a formylated macrocycle was observed in the ^{13}C NMR of the controls, even in the presence of a 33-fold excess of formate. The approximate yield of **3** for reactions using equimolar portions of reagents was 5%, but it increased to 39% when carried out with the 33-fold excess formate at elevated temperatures. The increased yield was possibly due to a more favorable equilibrium phenomenon resulting in higher concentrations of the complex **2**·formate at higher formate concentrations.

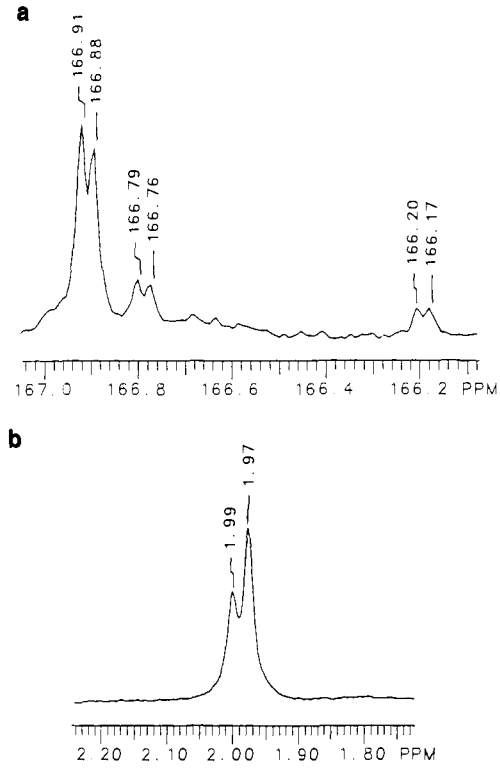

Figure 1. NMR spectra of the carbonyl and inorganic phosphate region of the reaction product of acetyl phosphate and **1** (0.06 M in each) and sodium formate (0.12 M) in 97% [^{18}O]water adjusted to pH 7.0. The upfield peak of the doublets in both spectra corresponds to the ^{18}O-enriched product. (a) ^{13}C spectrum of the NCHO resonances including one major and two minor isomers after dilution to 50% [^{18}O]water; (b) ^{31}P spectrum of the resonance due to inorganic phosphate.

Formylation of the Macrocycle by Acetyl Phosphate and Formate. The reaction of acetyl phosphate with the macrocycle **1** to generate an intermediate phosphorylated macrocycle, **2**, was examined in order to ascertain that **2** is the crucial species in the reaction. This reaction was performed in the presence of a 2-fold excess of ^{13}C-labeled formate at pH 7. After standing at room temperature until all of the phosphoramidate was consumed, approximately 48 h, the ^{13}C NMR was examined, and the presence of formylated macrocycle was evident by the formamide carbon resonance. A modified procedure was also followed in which acetyl phosphate and **1** were reacted for 3 h at room temperature and pH 7, at which time all of the acetyl phosphate had been consumed, followed by the addition of a 16-fold excess of ^{13}C-labeled formate. The ^{13}C NMR indicated the presence of the formylated macrocycle product **3**. Although the ^{31}P NMR spectra were examined carefully during the course of the reaction, no indication of intermediate formyl phosphate was observed. With a 2-fold excess of formate, the yield of **3** was estimated as approximately 8% by comparison of the ^{1}H signals of the product NC*H*O with those of a DSS standard.

Reactions in [^{18}O]water were performed to determine whether a formyl phosphate transient intermediate was present in the reaction. The hydrolysis of acetyl phosphate in the presence of **1** in [^{18}O]water showed only one signal for inorganic phosphate, which corresponded to phosphate containing one ^{18}O atom. The analogous experiment, performed in the presence of formate, resulted in two ^{31}P resonances, in a ratio of 1:3, corresponding to phosphate containing no and one ^{18}O after a period of 48 h. The ^{13}C NMR spectrum of this reaction mixture showed, in addition to the formamide peak, a single peak for formate that corresponded to the ^{16}O compound and three doublets for the formamide resonance, indicating ^{18}O incorporation (Figure 1). Control experiments in which formate and phosphate were incubated with **1** in [^{18}O]water for the same length of time showed

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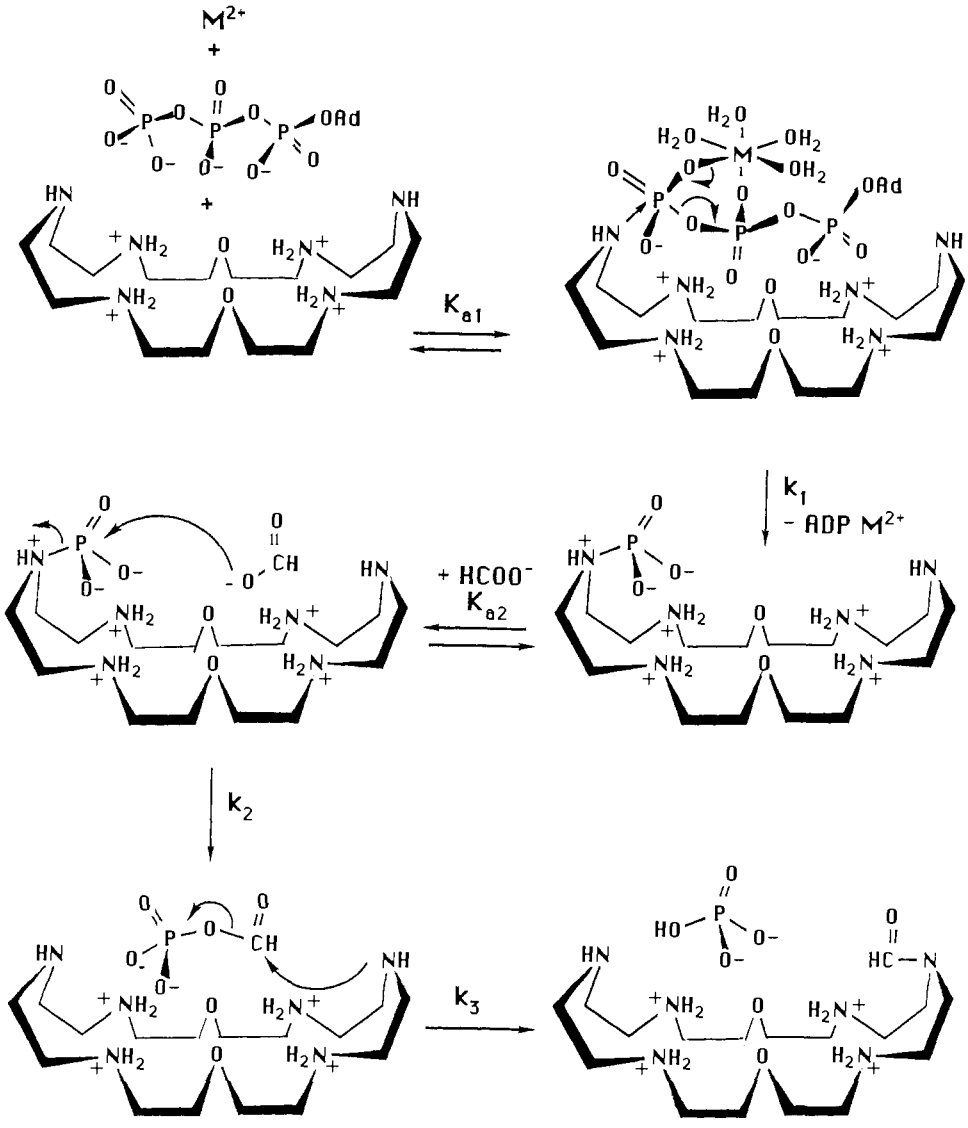
Scheme I. Schematic Representation of the Route for Formate Activation and N-Formylation Utilizing **1**, ATP, M(II), and $HCOO^-$[a]



[a] The geometries of the complexes **1**·ATP·M(II) and **2**·$HCOO^-$ are hypothetical but compatible with potential structures of the species involved. The structure shown for the formylated macrocycle **3** is one of the potential isomers.

no exchange of solvent into formate or phosphate. Also an experiment in which acetyl phosphate and formate were reacted in 50% [^{18}O]water until all of the acetyl phosphate was hydrolyzed showed only one acetate and one formate resonance in the ^{13}C NMR spectrum.

Discussion

Activation of Formate. Formate was found to be activated in the presence of ATP, divalent metal ion, and **1** resulting in N-formylation of **1** to yield **3**, the formylated macrocycle. The analogous process, ATP-mediated activation of formate resulting in N-formylation, is found to occur in the biosynthesis of N^{10}-formyltetrahydrofolate.

As a result of studies of the reaction of the macrocycle **1** with (a) ATP, metal ion, and formate, (b) acetyl phosphate and formate, and (c) formyl phosphate,[10] the sequence in Scheme I is proposed for reaction a.

The pathway of the hydrolysis of ATP and acetyl phosphate in the presence **1** proceeds through a phosphorylated macrocycle, **2**, intermediate.[4,5,11] The formation of **2** is enhanced by metal ions in the case of ATP.[11] The reaction of formyl phosphate with **1** resulted not in phosphorylated macrocycle **2** but rather in the N-formylation of **1** in almost quantitative yield. The different reaction sites may be related to the electronic and steric influences at the carbonyl carbons. While the phosphorylated macrocycle **2** has been found to be unstable, the formylated macrocycle **3** is stable at room temperature and pH 7.0.

Previous findings for ATP hydrolysis in the presence of **1** indicated that the macrocycle can utilize its ditopic capabilities in the formation of pyrophosphate from phosphorylated macrocycle **2** and inorganic phosphate in the presence of certain metal ions.[11] The isolation of a formylated species, **3**, in the current study shows that carboxylate activation, a crucial process in the biosynthesis of amides, is also possible via this route with this macrocyclic system. The three different experimental conditions examined resulted in formate activation: (1) ATP and magnesium ion, (2) ATP and calcium ion, and (3) acetyl phosphate, all in the presence of the macrocycle **1** and formate.

Since prior studies had shown that the magnesium(II) ion promoted the formation of **2**, and magnesium(II) is the metal ion most often used in biological reactions involving ATP, the reaction with formate using magnesium(II) was examined. A 33-fold excess of formate (molar ratio to ATP) resulted in the formylation of the macrocycle in 39% yield when the reaction mixture was heated at 60 °C over 10 h. Calcium(II) ion also was examined

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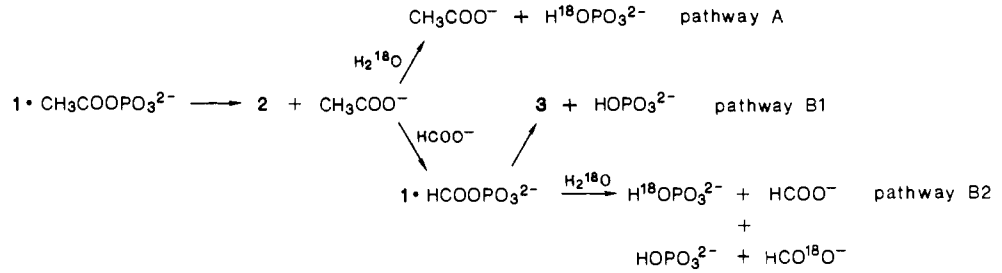
Scheme II. Products of the [^{18}O]Water Labeling Studies



in this reaction because of previous studies[11] in the macrocycle-catalyzed dephosphorylation of ATP, which showed that calcium(II) not only enhanced the rate by a factor of 2 but resulted in the stabilization of the phosphoramidate **2**. The reaction using equimolar concentrations of calcium(II), ATP, **1**, and formate gave a 5% yield of the formylated macrocycle **3**. The control reaction, run without ATP, ruled out the possibility that formate was activated by a means other than the phosphoramidate intermediate, since the ^{13}C NMR spectrum showed none of the formylated macrocycle **3**. A major role of the calcium ion in this case is to help free the macrocyclic cavity by complexing the ADP.[11] This allows for a more facile approach of the formate to the reaction site.

The analogous reaction using acetyl phosphate to generate the intermediate phosphorylated macrocycle **2** was examined in order to verify that **2** was required for the reaction to occur. The isolation of the formylated macrocycle in this reaction indicated that the necessary reactant is **2**.

Hence, the sequence of the reaction in Scheme I entails five steps: (1) complexation, (2) phosphorylation of the macrocycle by ATP, (3) complexation of formate in the other receptor site of **2**, (4) reaction of the bound carboxylate in the supramolecular complex with the phosphoramidate group, leading to the complex of formyl phosphate and the macrocycle **1**, and (5) formylation of **1** by the formyl phosphate. While structurally there is no way to say how the formate substrate is oriented in the macrocyclic cavity, the entropic advantage of complexation to give a unimolecular reaction would promote the reaction.

[^{18}O]Water Studies. The presence of a transient formyl phosphate intermediate was indicated by [^{18}O]water studies. The principal routes for [^{18}O]water incorporation into the species involved are outlined in Scheme II. In these studies acetyl phosphate was used to generate the phosphorylated macrocycle **2** in 97% [^{18}O]water to determine ^{18}O incorporation into either acetate or phosphate. Hydrolysis of acetyl phosphate at neutral pH has been reported to proceed by at least 90% P–O cleavage.[12,13] Similarly, in the macrocycle-catalyzed hydrolysis of acetyl phosphate, only one ^{31}P NMR resonance was observed and was confirmed as corresponding to the monolabeled phosphate when [^{18}O]water was used (Scheme II, pathway A).[5]

The hydrolysis of acetyl phosphate in the presence of macrocycle **1** results in essentially 100% monolabeled (^{18}O)phosphate. Formate activation was examined with respect to ^{18}O incorporation into phosphate in that reaction, and it provided indirect evidence for the transient presence of formyl phosphate.

Formate in the presence of acetyl phosphate and **1** in 97% [^{18}O]water yielded two ^{31}P NMR signals, which were identified as non- and monolabeled phosphate in 25% and 75% yield, respectively (Figure 1b). The presence of any nonlabeled phosphate supports the proposal that the reaction proceeds through the intermediate formyl phosphate (pathway B). If the only reactions involved in the sequence were dephosphorylation of acetyl phosphate and hydrolysis of the intermediate phosphoramidate **2**, 100% monolabeled phosphate would be anticipated, as was observed in the absence of formate (Scheme II, pathway A).

A formyl phosphate or formic acetic anhydride intermediate (the latter from attack of the formate on the carbonyl group of acetyl phosphate) was indicated by the presence of nonlabeled phosphate in the product reaction mixture. The occurrence of a formic acetic anhydride was ruled out by the control experiment in which acetyl phosphate was reacted in the presence of formate in 50% [^{18}O]water until completely hydrolyzed. Only a single resonance each was observed in the ^{13}C NMR spectrum for acetate and formate, indicating no ^{18}O incorporation and further indicating that hydrolysis of acetyl phosphate occurred entirely by attack at the phosphorus atom. Further evidence that a mixed acetic formic anhydride is not involved was obtained from the reaction in which acetyl phosphate was completely hydrolyzed to give **2** and acetate before addition of formate. The formylated macrocycle **3** was still an observed product.

In Scheme II, pathway B, formyl phosphate generated by the reaction of formate with the phosphoramidate **2** and subsequent attack by the macrocycle **1** gives nonlabeled phosphate as product (Scheme II, pathway B1). Hydrolysis of the formyl phosphate by [^{18}O]water would result in both labeled and nonlabeled phosphate (Scheme II, pathway B2), since at pH 7, in the absence of **1**, hydrolysis of formyl phosphate occurs with 55% P–O and 45% C–O bond cleavage.[13] (These percentages are revised from an earlier study and include ^{13}C results.[14]) However, labeled formate (C–O bond cleavage) was not observed in the reaction of **1** with formate and acetyl phosphate, which indicates that the dephosphorylation of transient formyl phosphate occurs via aminolysis, not hydrolysis. Thus, the reaction of the intermediate formyl phosphate appears to proceed entirely by C–O bond cleavage, leading to the *N*-formyl species and nonlabeled inorganic phosphate and formate (pathway B1). These results are consistent with the hypothesis that formyl phosphate formed in the reaction reacts rapidly with the macrocycle. That this most probably occurs in the supramolecular complex before dissociation is indicated by the lack of any ^{18}O-labeled formate in the product solution. By calculating the relative percentages of nonlabeled and monolabeled phosphate and correcting for dilutions and impurities, the yield of **3** in the [^{18}O]water experiment based on nonlabeled phosphate was calculated to be 10% from ^{31}P NMR when a 2:1 molar ratio of formate:macrocycle was used. Yields based on integration of the ^{1}H NMR NC*H*O peak of **3** showed 8%.

When the intermediate phosphoramidate **2** was generated by acetyl phosphate in the presence of formate in 97% [^{18}O]water, doublets were observed for the NCHO resonances in the ^{13}C NMR spectrum of the formylated macrocyclic product, indicating ^{18}O incorporation. No ^{18}O incorporation was noted in the formate carbon resonance. This finding suggests that formyl transfer via a cyclic imidazoline intermediate to give other regioisomers is occurring.[13]

Conclusions

In this kinase-like reaction several substrates (ATP, phosphorylated macrocycle **2**, $HCOO^-$, and formyl phosphate) are involved in both bond cleavage and formation. An initial high-affinity (**1**·ATP) complex is formed between the macrocycle and ATP (log K_s = 4.8 for $[\mathbf{1}\cdot H_4]^{4+}$ and ATP[15]), followed by γ-phosphoryl transfer in the complex to produce the phosphoramidate **2** (Scheme I). Given the affinity of polyammonium macrocycles for car-

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boxylate anions,[16] the formation of the binary complex of $\mathbf{2}\cdot HCO_2^-$ is proposed in the next step. Nucleophilic attack by the carboxylate on the reactive phosphoramidate within the complex leads to the formation of a mixed phosphoric anhydride, formyl phosphate. The successful nucleophilic behavior of the formate may well result from the phosphoramidate being in the appropriate mode for attack, i.e., with the nitrogen protonated. The intermediate formyl phosphate, while not spectroscopically visible, was implied by the ^{18}O labeled H_2O studies. The final step of the carboxylate activation occurs within the macrocycle complexed with formyl phosphate, resulting in transfer of the formyl group to a nitrogen and release of phosphate.

The special chemistry of this particular macrocycle, $[24]N_6O_2$ (**1**), has been observed in other catalytic studies.[4,5,17] Part of this uniqueness derives from the ability of the macrocycle to participate in phosphoryl transfer or kinase activity. Of over 30 macrocycles and linear amines which have been investigated as ATPase mimics, only **1** shows the presence of a significant amount of the phosphorylated macrocyclic intermediate **2**, which is crucial to the pathway of formate activation.

Biomimetic chemistry has sought to unravel the elusive pathways involving biosynthetic reactions by using simplified versions of highly evolved catalytic species, i.e. enzymes. This particular reaction sequence may be patterned after the way in which formate is brought into the one-carbon pool of metabolic reactions by N^{10}-formyltetrahydrofolate synthetase. Recently, evidence for a mixed anhydride of formate and phosphate, i.e. formyl phosphate, has been obtained for the enzymatic reaction. These findings include enzyme catalysis of the phosphorylation of ADP by carbamoyl phosphate (a formyl phosphate analogue),[3] the phosphorylation of ADP and formylation of tetrahydrofolate in the presence of enzyme and synthetic formyl phosphate,[14] and the slow formation of formyl phosphate from ATP and formate catalyzed by the enzyme.[18] The results of this study also parallel the enzymatic reaction wherein formyl phosphate is a transient intermediate in the reaction. Evidence for the presence of this species in both cases is indirect. Formyl phosphate in the complex with either enzyme or macrocycle is likely to react more rapidly than it dissociates from the complex, at least on the basis of the [^{18}O]water studies, in which no ^{18}O-labeled formate is observed.

The complexity of the activation sequence as described in this model study, when considered with the apparent aptitude of polyammonium macrocycles such as **1** for a variety of phosphoryl transfer reactions, attests to the value of polyammonium macrocycles in enzyme mimicry and their rapidly growing role in biomimetic chemistry.[19]

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