(453 nm) $Ru(bpy)_3^{2+}$-$Co(C_2O_4)_3^{3-}$ solution as in the previous cell but connected by a salt bridge to a solution containing Fe^{2+} and Fe^{3+} (0.01 M) in 1 N H_2SO_4 and a platinum gauze electrode. The photocurrent arises because of oxidative quenching of $Ru(bpy)_3^{2+*}$ by $Co(C_2O_4)_3^{3-}$ followed by oxidation of Fe^{2+} by $Ru(bpy)_3^{3+}$ but through the electrode circuit (eq 4). The photocurrent responds as expected for a diffusion limited electrochemical cell. The current is dependent on electrode area especially in the anode compartment and on the rate of stirring. Currents as high as 60 μA at a light intensity of 1.5×10^{-9} einstein/s were observed which were steady for 15 min or longer. The magnitude of the current is independent of wavelength (at 405, 453, 500, and 546 nm) when differences in absorbance and light intensity are taken into account. Variations in $[Co(C_2O_4)_3^{3-}]$ over the range 3×10^{-3} to 5×10^{-4} M had no effect on the maximum current over 15-min photolysis intervals, but the initial slopes of the current-time curves did decrease with decreasing $[Co(C_2O_4)_3^{3-}]$ as expected for a slower rate of quenching of $Ru(bpy)_3^{2+*}$.[6] One effect of the diffusion-limited nature of the photocurrents is that significant dark currents are observed when irradiation is stopped. The dark current falls exponentially as eq 4 goes to completion.

$$Ru(bpy)_3^{3+} + e^-(\text{electrode}) \rightarrow Ru(bpy)_3^{2+}$$
$$Fe^{2+} \rightarrow Fe^{3+} + e^-(\text{electrode}) \qquad (4)$$

Total integrated currents in the $Ru(bpy)_3^{2+}/Co(C_2O_4)_3^{3-}$-$Fe^{2+}/Fe^{3+}$ cell were used to calculate quantum yields for Fe^{3+} production. The yields for 3-min photolysis intervals were 0.82 ± 0.02 which is in good agreement with the value found by Demas and Adamson for eq 3 showing that the redox equivalents produced photochemically in the $Ru(bpy)_3^{2+}/Co(C_2O_4)_3^{3-}$ half-cell are transferred quantitatively through the cell to the Fe^{2+}/Fe^{3+} solution. The quantum yields fall with longer irradiation times (0.74 for 6 min) since the oxalic acid concentration increases with time (eq 3) and oxalic acid begins to compete effectively for $Ru(bpy)_3^{3+}$.

The experiments described here are of limited practical value because of the nature of the chemical systems involved. However, they do serve as prototypes for possible applications in (1) chemical actinometry, (2) photochemical fuel cells or photochemical synthesis cells based on more realistic net quenchers than $Co(C_2O_4)_3^{3-}$ (like O_2), and (3) photochemical water-splitting systems where the necessary catalytic oxidative and reductive parts of the redox chemistry can be dealt with separately and combined in an electrochemical cell. In such cells, it should be possible to obtain photocurrents and H_2 and O_2 evolution from separate cell compartments.

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Highly Discriminative Binding of Nucleoside Phosphates by a Lipophilic Diammonium Salt Embedded in a Bicyclic Skeleton

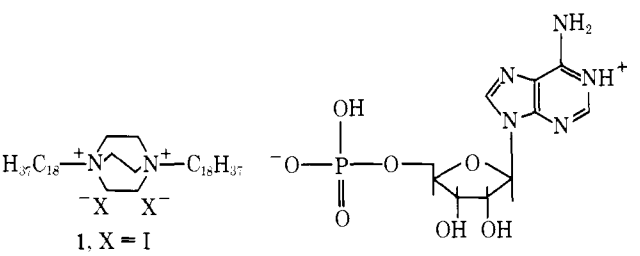
Sir:

Organized interconversions among nucleoside phosphates, AMP, ADP, and ATP are especially important in the sense that chemical energy supplied from outside is stored through the formation of high energy phosphate linkages. Their coupled cleavages provide energy sources for biosynthetic reactions which reverse catabolic pathways and which drive active transport through the cell membrane against electrochemical gradients.

The first crucial key to understanding these processes depends upon the knowledge of how enzymes recognize and discriminate between mono-, di-, and triphosphates. A specific or highly selective ion-ion interaction has been suggested to operate between phosphate and ammonium groupings at the binding site of ATPase[1] or various kinases.[2] The ion-ion and/or ion-dipole interaction is also one of the determining factors in the recognition of neurotransmitters.[3] Thus the investigation of the ion-ion interaction in a medium less polar than water is important to understand these biologically important phenomena.

Here we wish to report that a lipophilic diammonium salt embedded in a rigid bicyclic structure can specifically bind ADP under the condition of no appreciable binding of AMP. This is the first example of artificial molecules which successfully discriminate among nucleoside phosphates.

The diammonium salt was obtained by heating 1,4-diazabicyclo[2.2.2]octane, Dabco (1.0 g, 8.9 mmol), and stearyl iodide (20 g, 53 mmol), in 50 mL of DMF at 70 °C for 2 days. After removing excess stearyl iodide and monoammonium salt, the remaining solid was recrystallized from dimethylformamide to give white crystals of *N,N'*-distearyldiammonium diiodide of Dabco in 83% yield (6.5 g), mp 234–237 °C.[4] The iodide was converted to the corresponding chloride **2** using silver oxide followed by treatment with hydrochloric acid.[5]



1, X = I
2, X = Cl

5

An aqueous solution of adenosine phosphates (0.2 and 1.0×10^{-4} M, 8 mL) was shaken at 25 °C with a chloroform solution (8 mL) of **2**[6] (2.5 molar equivalent) or trioctylmethylammonium chloride (Adogen 464) **3** (5.0 molar equiv) at specified pH values, 3.0, 5.0, and 8.0 where AMP (or ADP) is dissociated to the mono(di)anionic species containing protonated adenine, **5**, the mono(di)anionic, and di(tri)anionic forms, respectively, as the predominant species in aqueous solution.[7] The concentrations of phosphate remaining in aqueous solution (and/or extracted into the chloroform layer) were determined by UV spectroscopy from which apparent equilibrium constants were calculated and listed in Table I.

The diammonium salt **2** exhibits a remarkably high selectivity toward the binding of ADP compared with that of AMP. The selectivity ratios of binding, K_{ADP}/K_{AMP}, amounted to 79 and 28 at pH 3 and 5, respectively, at relatively higher concentrations of the diammonium salt and the phosphate. These results strongly support our basic concept that two cationic centers rigidly spaced by embedding them in the rigid bicyclic skeleton should be complementary to vicinal **6** (ADP

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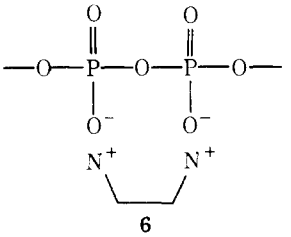
Table I. Relative Values of Equilibrium Constants K^a for the Binding of AMP and ADP by **2** or **3**

Compd	concn, 10^{-4} M		pH 3.0		pH 5.0		pH 8.0	
	Phosphate	**2** or **3**	AMP	ADP	AMP	ADP	AMP	ADP
2	0.2	0.5	(1)	7.7	17	61	27	34
3	0.2	1.0	0.7	0.4	0.0	0.4	0.7	1.4
2	1.0	2.5	(1)	79	12	335	270	1010[b]
3	1.0	5.0	0.6	6	0.9	9	0	6

[a] Relative values of equilibrium constants for extraction: $K = ([AXP]_{CHCl_3}/[AXP]_{aq})_{pH\ i}/([AMP]_{CHCl_3}/[AMP]_{aq})_{pH\ 3}$, for two different phosphate concentrations independently, where X = M or D and i = 3, 5, 8. [b] See note 9.

at pH 3 and 5[8]) or geminate **7** (AMP at pH 8) dianions of phosphate, although the latter interaction occurs to a lesser extent.[9] In marked contrast to the diammonium salt **2**, the monoammonium salt **3** is far less effective at lipophilizing **6** or **7**. Furthermore, the ability of **3** to extract **6** and **7** into chloroform does not depend on the extent of dissociation of phosphates. Thus, the aggregation of two (or three) monoammonium groups is not induced significantly to form the ion pair **8** with the phosphate anion (di- or tri-), probably owing to the unfavorable entropy effect involved.



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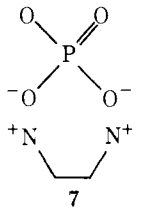

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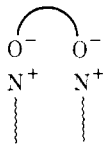

8

The conventional micellar reagent, stearyltrimethylammonium chloride **4** exhibited binding characteristics similar to the new phase transfer reagent **2** reported here. However, **4** and **2** form structurally different ion pairs with the phosphates. Thus, the ADP concentration in the aqueous phase decreased from 1.0 to 0.40 × 10^{-4} M at pH 8 by treatment with the chloroform solution of **4** (5.0 × 10^{-4} M); no trace of ADP was detected in the chloroform solution. The ADP appeared bound at the water–chloroform interface where a thick and opaque third phase was observed. In contrast to **4**, when **2** was employed, the phosphate that disappeared from the aqueous phase was found in the chloroform solution in a quantitative amount. These facts indicate that **2** acts as a typical phase transfer reagent rather than as a micellar reagent.

The highly effective binding of **2** to ADP relative to that of AMP in phase transfer suggests that this novel ammonium salt might be used as a specific carrier of ADP in transport through a liquid membrane. A significant rate difference already has been observed, and the details are currently under investigation.

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(8) These pH's should be valid only in bulk aqueous solution. The presence of an ammonium grouping in close proximity in nonpolar media should shift the pK_a of bound phosphates to a lower value.
(9) When concentrations of diammonium ion and phosphate were high at pH 5 and 8, a relatively slow and minor extraction of phosphate was observed to follow after a very rapid and major uptake of phosphate into chloroform. The final equilibrium value listed in Table I (3rd row) was obtained after long incubation time (usually 3 h). This slow rate process may be correlated with an assumed complex formation between a certain number of ammonium and phosphate ions when both concentrations are relatively high. The trianionic form of ADP could be accommodated in such structured aggregates.

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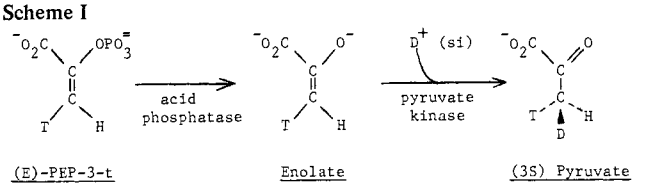
Stereochemistry of Ketonization of Enolpyruvate by Pyruvate Kinase. Evidence for Its Role as an Intermediate[1]

Sir:

Enolpyruvate and enolic forms of other substrates have been proposed as intermediates in many enzymatic reaction mechanisms,[2-8] but alternate proposals such as concerted displacement mechanisms are in active consideration.[9-13] We have recently shown[14] that treatment of phosphoenolpyruvate (PEP) with phosphatase in the presence of large amounts of lactate dehydrogenase gives rise to a transient intermediate which is believed to be enolpyruvate. Judging from the kinetics of this two-enzyme system, the conversion of the intermediate to pyruvate is slow, especially in D_2O ($t_{1/2}$ in $D_2O \simeq 10$ min at 15 °C, pD 6.4; $v_{H_2O}/v_{D_2O} = 6$). If the reaction in D_2O was terminated in acid–H_2O during the steady-state period, the expected amount of pyruvate was found. Mass analysis indicated that formation of the methyl group of the pyruvate occurred subsequent to the inactivation of the enzymes in the primarily H_2O medium as expected if the intermediate was enolpyruvate. It was also observed that pyruvate kinase catalyzed the conversion of the intermediate to pyruvate. The present communication shows that this catalysis is stereospecific and has the same stereospecificity that is observed when pyruvate is generated by pyruvate kinase by the overall reaction: PEP + ADP → pyruvate + ATP.

(*E*)-PEP-*3-t* was prepared as described earlier[4] and converted to enolpyruvate-*3-t* by action of acid phosphatase in D_2O in presence or absence of pyruvate kinase. Scheme I is

Scheme I



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