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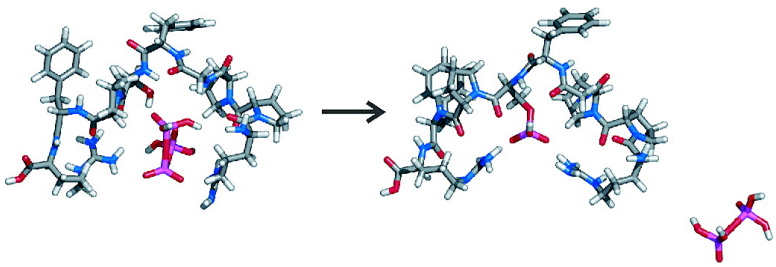

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Cluster-Phase Reactions: Gas-Phase Phosphorylation of Peptides and Model Compounds with Triphosphate Anions

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Abstract: Molecular clusters provide a unique environment in which chemical reactions between cluster components can occur. In the present study, electrospray ionization is used to examine the behavior of anionic clusters of triphosphate with choline, acetylcholine, and betaine, and the behaviors of cationic clusters of triphosphate with the peptides bradykinin (RPPGFSPFR) and ARRPEGRTWAQPGY. Phosphorylation of a hydroxyl group, when one is present, is shown to be a facile process when the cluster is subjected to collisional activation. Of particular interest is the selective phosphorylation of the hydroxyl substituent in serine and threonine residues of peptides. Less conclusive results are obtained with three peptides containing tyrosine, but the data obtained are consistent with phosphorylation on tyrosine residues. In the absence of residues with hydroxyl substituents, the C-terminus of a peptide is observed to be phosphorylated. The unique chemical reactions reported in this study represent the first examples of gas-phase phosphorylation of alcohols and are also interesting in that they occur at a site remote from charged functional groups in the same molecule. This facile process may have interesting implications for the synthesis of key molecules at the threshold of life.

Introduction

Phosphorylation of peptides is of great interest to the biological community. Approximately one-third of proteins in eukaryotic cells are thought to be phosphorylated at any given time, although the specific proteins that are phosphorylated are continually changing.[1] Phosphorylation is thought to play a major role in transmembrane signal tranduction[2] and has been observed to control metabolizing cytochromes P450 in a rapid switch-like fashion.[3] The ubiquitous nature of phosphorylation in cell signaling, and the fact that abnormal phosphorylation is often a cause or result of disease, makes it a key target for drug discovery,[4] and has increased interest in characterizing both the presence and the effects of site-specific phosphorylations.[5]

Recently, we have outlined criteria for the rational design of molecular aggregates to ensure that such aggregates react rather than dissociate in the gas phase.[6] Coulombic attraction between charged sites in cluster components is used to strongly bind components together. The cluster must retain a net charge for observation in a mass spectrometer, so a dianion and a singly charged cation, or a dication and a singly charged anion, are the simplest systems. Reactants are chosen so that simple proton transfer leading to neutralization and loss of one of the cluster components is not the most favorable reaction pathway. While numerous techniques are available for the preparation and study of well-characterized neutral and ionic molecular aggregates in the gas phase, such aggregates often dissociate to yield molecular constituents rather than undergoing complex reactions. By binding together cluster components using ionic interactions, we have successfully observed reactions between alkylammonium ions and triphosphate, as well as between alkylammonium ions and DNA.[6] Gronert and co-workers extensively explored the reactions of alkylammonium cations with carboxylate anions in several studies, using clusters that satisfy the proposed criteria for the rational design of reactive molecular aggregates.[7−10] Reactions between oligosaccharides and N-donor ligands have been observed by O'Hair et al.[11]

Phosphorylation has been previously observed in clusters. Julian and Beauchamp observed the abiotic synthesis of ATP from AMP via collisional activation of cationic and anionic trimers of AMP.[12] The AMP in the clusters is present as the sodium salt, with the addition or removal of a sodium ion yielding, respectively, the reactive cationic and anionic clusters. When the sodium ions are replaced by protons, the clusters are not observed to undergo the phosphorylation reaction. It appears that the additional cluster stability associated with the sodiated as compared to the protonated clusters leads to reaction rather

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(3) Oesch-Bartllomowicz, B.; Oesch, F. *Arch. Biochem. Biophys.* **2003**, *409*, 228−234.
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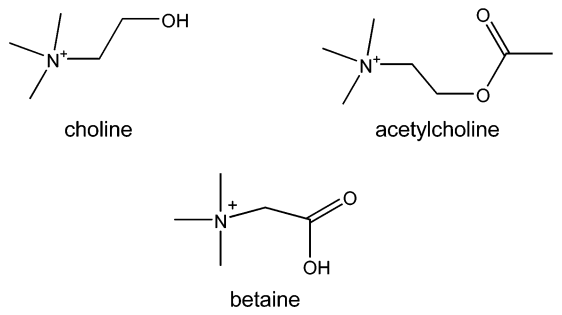
(12) Julian, R. R.; Beauchamp, J. L. *Int. J. Mass Spectrom.* **2003**, *227*, 147−159.

10.1021/ja0452673 CCC: $30.25

than dissociation. In all cases, monophosphate species reacted to form polyphosphate species. The formation of deoxyribose nucleotide diphosphates from dimers of deoxyribose nucleotide monophosphates was also observed by Williams et al.[13]



In this work, we examine the phosphorylation of alcohols via cluster-phase reactions involving triphosphate and molecules possessing hydroxyl functional groups. Choline is used as a model system, as it contains a quaternary ammonium ion to provide charge and a hydroxyl group that is observed to be phosphorylated by triphosphate in an anionic cluster. Results from anionic clusters of d_9-choline, acetylcholine, and betaine with triphosphate are compared to the choline results. Of particular interest, we demonstrate that two peptides containing amino acids with alcohol groups in their side chains (specifically, serine, and threonine) undergo phosphorylation when a cluster containing the peptide and triphosphate is activated. Less conclusive results are obtained with three peptides containing tyrosine, but the data obtained are consistent with phosphorylation on tyrosine residues.

Methods

Experimental Methods. Experiments were performed on either a Finnigan LCQ Classic ion trap mass spectrometer or a Finnigan LCQ Deca ion trap mass spectrometer, both in positive and in negative ion mode. Clusters were formed by electrospray ionization of methanol solutions of 50 μM pentasodium triphosphate and 50−60 μM of choline, acetylcholine, or betaine. These compounds were purchased from Sigma-Aldrich (St. Louis, MO) and used without further purification. Deuterated choline (trimethyl-d_9 choline chloride, 98%) was obtained from Cambridge Isotopes (Andover, MA) and used without further purification.

Peptide samples (American Peptide Co., Sunnyvale, CA) were typically sprayed from solutions of 90:10 methanol:water with 0.1% acetic acid to promote protonation of the peptides. These solutions were 50−70 μM in peptide and 50 μM in pentasodium triphosphate. In all cases, neutral products of collisional activation are inferred from the charged products produced and not directly observed.

Computational Methods. Several likely candidate structures were initially evaluated at the PM5 level using CAChe 5.04 (Fujitsu, Beaverton, OR). The lowest-energy structure was then further optimized using density functional theory (DFT). The DFT calculations were carried out using Jaguar 4.1 (Schrödinger, Inc., Portland, OR). Full geometry optimization was performed at the B3LYP/6-31++G** level, which has been shown to be an appropriate basis set for hydrogen-bonded complexes.[14,15] Zero-point energy corrections were applied at conditions of 298 K and 1 atm.

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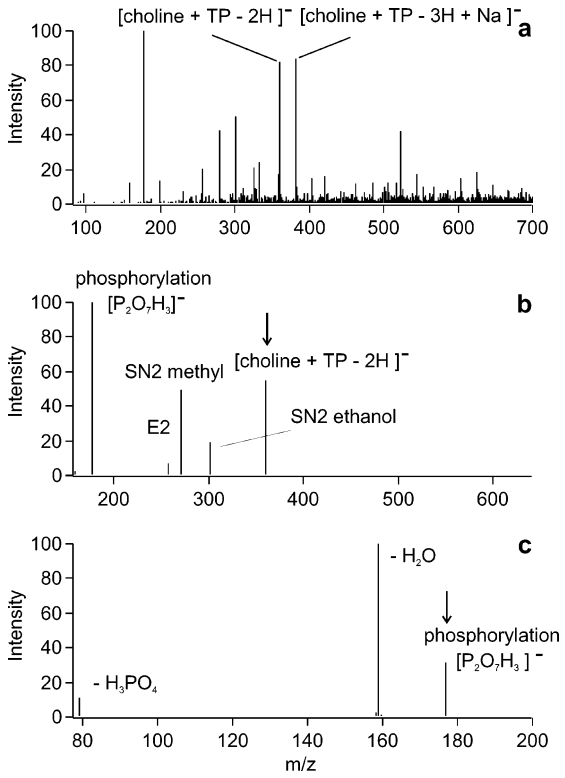
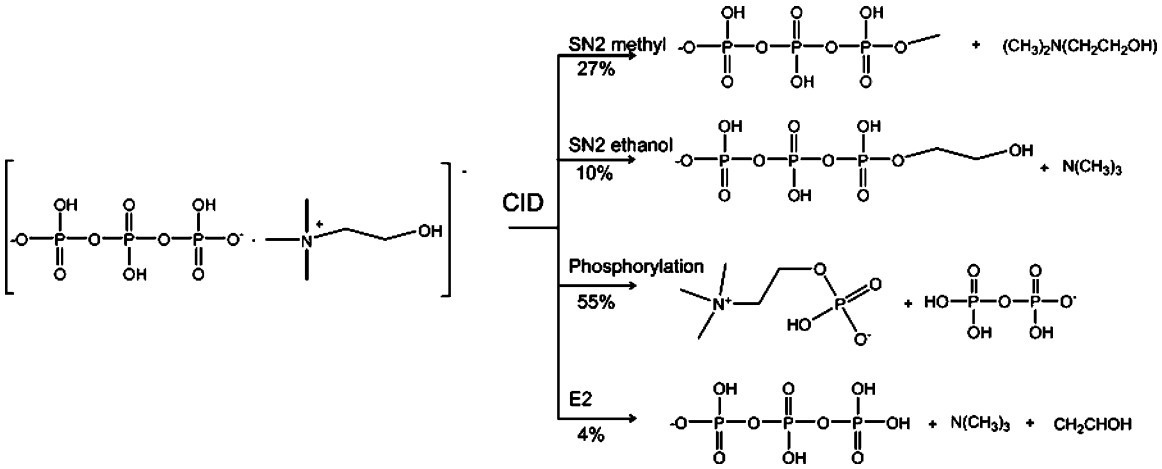


Figure 1. (a) Negative mode ESI mass spectrum of a solution of choline and sodium triphosphate. "TP" stands for neutral triphosphate, $P_3O_{10}H_5$. (b) Collisionally activated spectrum of the cluster [choline + TP − 2H]$^-$ from (a). Products resulting from alkylation of triphosphate are observed and labeled in the figure, as well as the E2 elimination product. The predominant product of collisional activation is diphosphate anion. (c) Collisionally activated spectrum of diphosphate anion from (b). The fragmentation pattern is consistent with the assignment of diphosphate to the peak.

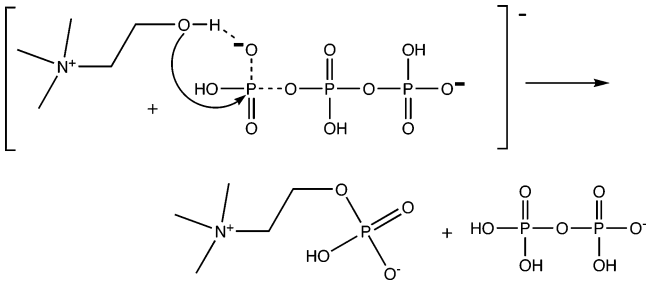
Results and Discussion

1. Model Compounds. A. Choline. Figure 1a shows the full negative ion mass spectrum of a mixture of choline and sodium triphosphate. Singly negatively charged clusters composed of doubly deprotonated triphosphate anions and choline, [choline + TP − 2H]$^-$, are observed, as well as similar singly negatively charged clusters with one of the remaining triphosphate protons replaced by a sodium ion, [choline + TP − 3H + Na]$^-$. Both of these species are observed at high intensity (both at approximately 80% of base peak). In Figure 1b, the cluster [choline + TP − 2H]$^-$ is isolated and collisionally activated. As one would expect, based on our previous study of cluster reactions of alkylammonium ions[6] as well as work by Gronert et. al.,[7−9] both S_N2 products and E2 products are observed. Scheme 1 shows the S_N2 products that would be expected from the reaction of deprotonated triphosphate with choline. The substituted methyl product and the substituted ethanol product are observed at 27% and 10% of the total product ion intensity, respectively. The E2 product could result from β-attack on the ethanolic moiety, resulting in the formation of trimethylamine, vinyl alcohol, and singly deprotonated triphosphate. The E2 product is seen in relatively low abundance (4% of total product ion intensity). The most prominent product is a peak at *m*/*z* 177 (55% of product ion intensity), which we have assigned as the singly deprotonated diphosphate anion. Further support for this assignment is provided by the collisional activation of this species as shown in Figure 1c, where the product is seen to

Scheme 1. Reactions between Choline and Triphosphate Leading to S_N2 and Phosphorylation Products



Scheme 2. Proposed Mechanism of Choline Phosphorylation



lose H_2O (86% of product ion intensity) and H_3PO_4 (10% of product ion intensity).

A possible reaction that would result in the formation of singly deprotonated diphosphate is shown in Scheme 2. Because the neutral products are not detected, a variety of possible products were considered. However, theoretical calculations indicated that the products postulated in Scheme 1 for the phosphorylation reaction were, by far, the lowest energy reaction products. In this process, the alcoholic oxygen attacks one of the phosphorus atoms in triphosphate, resulting in the formation of phosphorylated choline (in zwitterionic form) and singly deprotonated diphosphate. This mechanism might be favored by the presence of the charged group in the choline, which has the effect of making the alcohol group more acidic. The enthalpy of deprotonation of choline is 1152 kJ/mol. This is significantly less than the 1563 kJ/mol enthalpy of deprotonation of 2-dimethylamino alcohol, an analogue of choline that does not contain a quaternary ammonium ion. These values are calculated at the B3LYP/6-31++G** level with zero-point energy corrections. The charge site in choline increases the acidity of the alcohol group and facilitates the attack of the alcoholic oxygen on a phosphorus atom and transfer of a proton to the oxygen of the phosphate. This might occur in a four-center reaction mechanism, as depicted in Scheme 2.

B. Acetylcholine. To see if the observed phosphorylation reaction is specific to alcohols, several additional singly negatively charged clusters were examined. The behavior of a singly negatively charged cluster of triphosphate and acetylcholine, $[ACh + TP - 2H]^-$, which does not have a free alcohol group, is shown in Figure 2. Figure 2a shows the full negative ion mass spectra of a mixture of acetylcholine with sodium triphosphate. The anionic cluster $[ACh + TP - 2H]^-$ (67% of the base peak in Figure 2a) is isolated and collisionally activated, and the resulting mass spectrum is shown in Figure 2b. While two S_N2 products are observed (37% and 58% of the product ion intensity), analogous to those found when the $[choline + TP - 2H]^-$ cluster is collisionally activated, and a small amount of E2 product (2% of product ion intensity) is observed, no diphosphate is formed from the collisional activation of the acetylcholine−triphosphate cluster. This suggests that the hydroxyl group is necessary if the reaction producing diphosphate is to be observed.

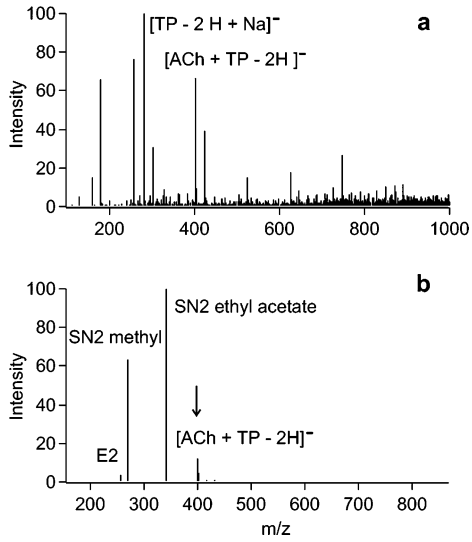


Figure 2. (a) Negative ion mass spectrum of a mixture of sodium triphosphate and acetylcholine (ACh). (b) Collisionally activated spectrum of the cluster $[ACh + TP - 2H]^-$ from (a). The S_N2 and E2 products are observed. Note the absence of any diphosphate product at *m*/*z* 177 (the *m*/*z* scale of this figure extends to *m*/*z* 157).

C. Betaine. Betaine was also studied to observe how a carboxylic acid group might interact with the triphosphate. The singly charged anionic cluster of betaine and triphosphate was isolated and collisionally activated. The dominant product in the resulting spectrum (95% of product ion intensity) appears at *m*/*z* 257, corresponding to singly deprotonated triphosphate. The data are not shown. It is likely that the betaine is in a zwitterionic conformation, due to betaine's low gas-phase basicity,[16] and the triphosphate retains a single negative charge.

(16) Patrick, J. S.; Yang, S. S.; Cooks, R. G. *J. Am. Chem. Soc.* **1996**, *118*, 231−232.

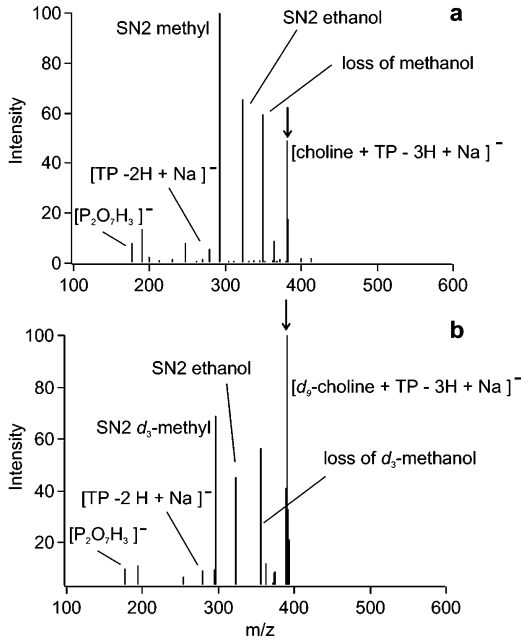


Figure 3. (a) Collisionally activated negative ion mass spectrum of the cluster [choline + TP − 3H + Na]$^-$ from Figure 1a. S_N2 and E2 products analogous to those found in Figure 1b are observed, as is a small amount of diphosphate. Another major peak, corresponding to the loss of methanol, appears in this spectrum. (b) Collisionally activated negative ion mass spectrum of the cluster [d_9-choline + TP − 3H + Na]$^-$. The peak corresponding to loss of methanol is shifted by three mass units, indicating that the methyl group lost comes from one of the three methyl groups found on choline.

In this case, the cluster is not held together sufficiently strongly by Coulombic interactions to prevent the dissociation of zwitterionic betaine from singly deprotonated triphosphate.

D. Sodiated Clusters. The singly negatively charged cluster [choline + TP − 3H + Na]$^-$, labeled in Figure 1a, was also isolated and collisionally activated. The products observed from collisional activation of the negatively charged sodiated cluster of choline and triphosphate are shown in Figure 3a. Again, S_N2 (34% and 22% of product ion intensity) and E2 products (2%) are observed. The diphosphate peak, corresponding to phosphorylation of the alcohol group, is significantly reduced in intensity (3% $[P_2O_7H_3]^-$; 5% $[P_2O_7H_2Na]^-$) as compared to the nonsodiated cluster reaction. A new peak is also seen in this spectrum (20% of product ion intensity), corresponding to the loss of methanol from the cluster. Figure 3b shows the products resulting from collisional activation of an analogous singly negatively charged cluster containing d_9-choline, in which the methyl groups of the choline are deuterated; the peak corresponding to loss of methanol has shifted by six mass units, indicating that the methyl groups of choline are involved in the formation of the methanol lost by the cluster. The only products resulting from collisional activation of a sodiated anionic cluster of acetylcholine and triphosphate, [ACh + TP − 3H + Na]$^-$, are products of S_N2 reactions. The predominant product (87% of product ion intensity) is a substituted ethyl acetate product, and a small amount of methyl phosphate is observed (8% of product ion intensity).

In our previous work,[6] we found that sodiated clusters of triphosphate and alkylammonium ions were more likely to undergo E2 reactions than S_N2 reactions. However, the effect of sodium on clusters of triphosphate and choline seems to be more complex. The loss of methanol from the sodiated triphosphate−choline cluster indicates that sodiation of these clusters does not have a causal link with E2 elimination, as previously observed. The structure and charge distribution of gas-phase clusters that incorporate sodium ions may bear little resemblance to clusters in which sodium is absent. This has also been observed in studies of H/D exchange of sodiated peptides.[17]

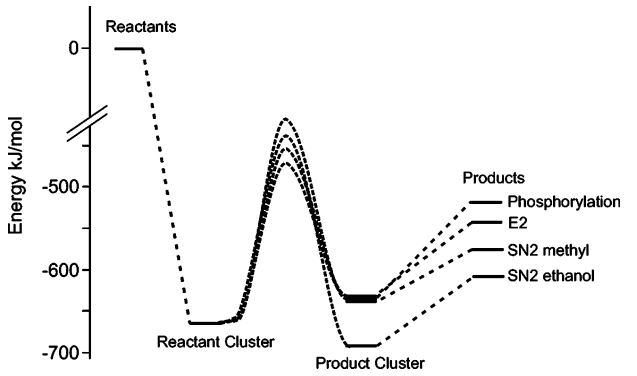


Figure 4. Energy level diagram showing the relative energies of the separated reactants, reactant clusters, product clusters, and products for the four reactions observed between doubly deprotonated triphosphate and choline. Energy values are given in kJ/mol and are calculated at the B3LYP/6-31++G** level. The zero-point energy is taken to be the energy of the separated reactants. Note the break in the energy scale. Barrier heights are not known and are shown for schematic purposes only.

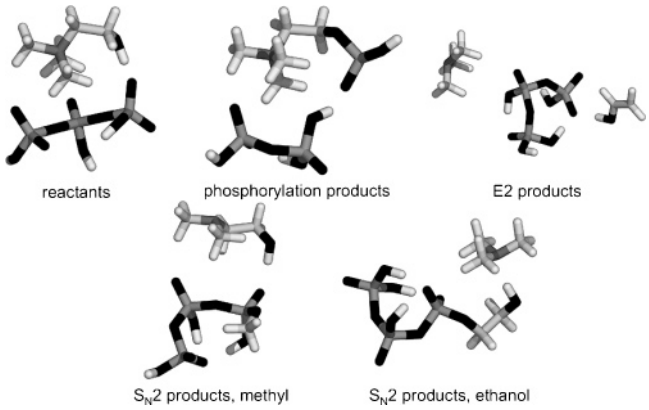


Figure 5. B3LYP/6-31++G** optimized structures of the reactant ion cluster and the product ion clusters obtained from the collisional activation of the reactant ion cluster.

E. Theoretical Results. Calculations were conducted on the doubly deprotonated triphosphate and choline system at the B3LYP/6-31++G** level with zero-point energy corrections to better understand the phosphorylation reaction. Figure 4 shows the relative energies of the reactant cluster, the product cluster, and the separated products for the four observed reaction channels. All of the reaction channels share the same lowest-energy structure for the reactant cluster, which is 663 kJ/mol lower in energy than the separated reactants. The product cluster for the S_N2 reaction at ethanol is the lowest energy product cluster. The remaining product clusters are 56 kJ/mol (for the E2 reaction), 51 kJ/mol (S_N2 methyl reaction), and 54 kJ/mol (phosphorylation reaction) higher in energy than the S_N2 product cluster.

Figure 5 shows the optimized structures for the reactant cluster and the four product clusters corresponding to the four

(17) Cox, H. A.; Julian, R. R.; Lee, S.-W.; Beauchamp, J. L. *J. Am. Chem. Soc.* **2004**, *126*, 6485−6490.

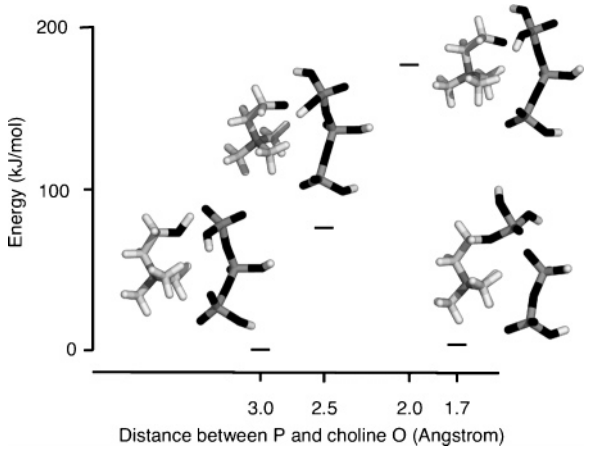


Figure 6. Energy level diagram showing minimized structures at several values of the P−O separation associated with the phosphorylation reaction. The optimized reactant cluster structure shown in Figure 5 has a P−O separation of 3.7 Å and lies lower in energy than the 3.0 Å structure shown by 31 kJ/mol. The optimized product cluster structure shown in Figure 5 has a P−O separation of 1.7 Å, but the diphosphate group has undergone rearrangement to make the final product cluster structure shown in Figure 5 7 kJ/mol lower in energy than that shown here.

observed reaction pathways. The reaction leading to the phosphorylation product involves a process requiring less rearrangement than the other three product clusters. The minimized intermediates shown in Figure 6 indicate that the reaction is likely to proceed via a four-center reaction intermediate, comprised of a phosphorus atom, a negatively charged oxygen atom attached to the phosphorus atom, and the alcoholic oxygen and hydrogen on choline. The prediction (shown in Figure 6) of a facile reaction pathway (from examination of the reactant and product cluster structures) and the limited amount of rearrangement necessary to reach the optimal structure implies that the phosphorylation reaction has a lower barrier than the other reactions observed.

2. Peptides. The studies of model compounds outlined in the previous section indicate that the hydroxyl group in choline is readily phosphorylated. In addition, the hydroxyl moiety being phosphorylated is remote from the charge site in choline. This suggests the possibility of selectively phosphorylating hydroxylated residues in peptides. Several peptides were chosen for study, including bradykinin (RPPGFSPFR) and ARRPEGRTWAQPGY. Negative ion spectra could be obtained of these peptides clustered with triphosphate, but the products of collisional activation of these clusters were difficult to assign, and so positively charged clusters were examined instead. The investigation of positively charged clusters was possible because, unlike the model systems, both bradykinin and ARRPEGRTWAQPGY can easily be formed with more than one positive charge.

A. Bradykinin (RPPGFSPFR). Figure 7a shows the products of collisional activation of a cluster at *m*/*z* 1318.2, corresponding to a singly positively charged cluster of bradykinin (RPPGFSPFR) and triphosphate, $[BK + TP + H]^+$. It is likely that the bradykinin carries two charges, one on each arginine residue, and that the triphosphate has a single negative charge. The fragmentation products correspond to a loss of phosphoric acid (and attachment of diphosphate to the peptide), phosphorylation of the peptide (the most abundant product at 42% of the total product yield), and dehydration of the peptide.

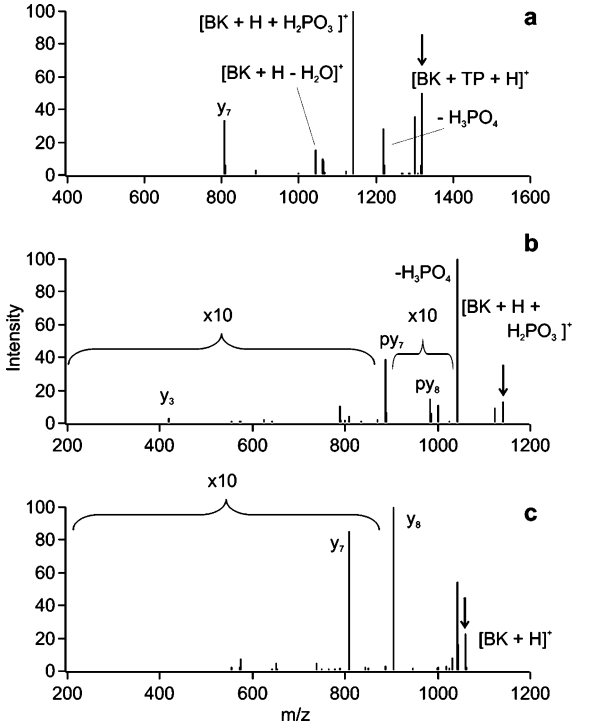
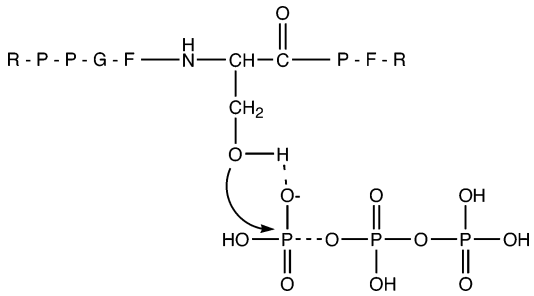


Figure 7. (a) Products of collisional activation of the cluster $[BK + TP + H]^+$. BK stands for bradykinin. (b) Products of collisional activation of the phosphorylated peptide from (a). Identifiable peptide fragments are labeled in the figure. (c) Products of collisional activation of $[BK + H]^+$. Identifiable fragments are labeled on the figure.

Scheme 3. Possible Reaction of Bradykinin with Triphosphate[a]



[a] It is assumed that the guanidinium groups of both arginine (R) groups are protonated.

The dehydrated peptide is probably produced by secondary fragmentation of the phosphorylated peptide. A small amount of fragmentation of the peptide (the y_7 peak, 24% of total product yield) is also observed. Phosphorylation of the peptide is shown schematically in Scheme 3.

In Figure 7b, the phosphorylated peptide $[BK + H + H_2PO_3]^+$ produced by the above process is collisionally activated, leading to several products. The major product corresponds to a loss of H_3PO_4, previously noted as a dominant product in the spectra of phosphorylated peptides.[18,19] Other products include phosphorylated py_7 and py_8 fragment ions, consistent with phosphorylation at the serine residue, and a nonphosphorylated y_3 fragment peak. Although of low intensity, this peak is completely reproducible. The appearance of the nonphosphorylated y_3 peak rules out phosphorylation on the

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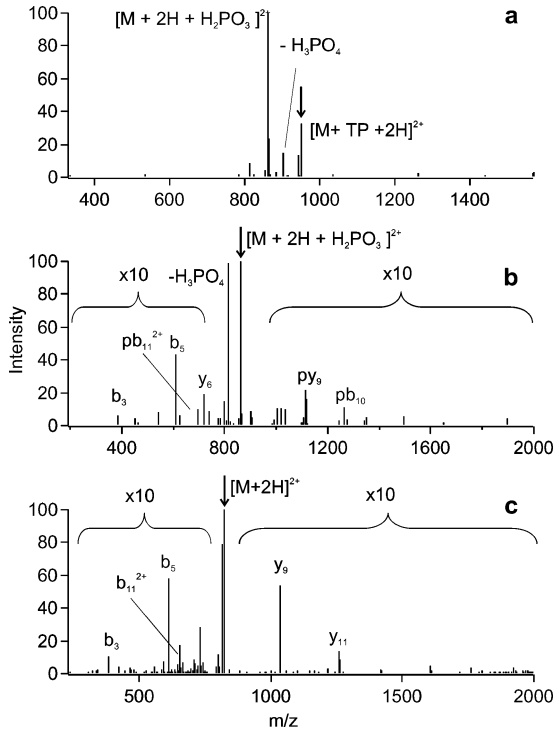


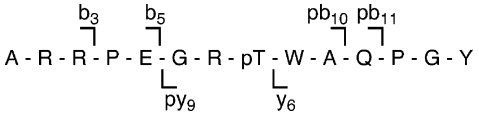
Figure 8. (a) Products of the collisional activation of a doubly charged cluster containing ARRPEGRTWAQPGY (M), $[M + TP + 2H]^+$. Peaks corresponding to the phosphorylation of the peptide, $[M + 2H + H_2PO_3]^{2+}$, and to the addition of a diphosphate to the peptide, $-H_3PO_4$, are observed. (b) Products of the collisional activation of the phosphorylated peptide from (a). Identifiable fragments are labeled on the figure. All observed fragments are consistent with a structure in which the threonine (T) is phosphorylated by triphosphate. (c) Products of collisional activation of the doubly charged, doubly protonated peptide. Identifiable fragments are labeled on the figure.

C-terminus. The observed fragmentation pattern suggests that phosphorylation has occurred at the hydroxyl group of the serine residue. The other possibilities given the experimental data would be reaction at glycine, proline, or phenylalanine, all of which are very unlikely to be phosphorylated. Figure 7c shows the products of collisional activation of singly protonated bradykinin, $[BK + H]^+$, for comparison. The y_7 and y_8 fragment ions are produced by collisional activation of the bradykinin species. Note that the distributions seen in Figure 7b and c are fairly similar in terms of the types of fragment ions produced.

Collisional activation of the peak labeled $-H_3PO_4$ in Figure 7b yields $y_7 - H_2O$ and $y_8 - H_2O$ fragments. These are 18 amu lower in mass than the fragments seen when nonphosphorylated bradykinin is fragmented. These results are consistent with the collisional activation of bradykinin with a phosphorylated serine residue, with loss of H_3PO_4 to produce a dehydroserine residue. The formation of dehydroserine is common in phosphorylated peptides.[19] These data are also consistent with the conclusion that the peak $[BK + H + H_2PO_3]^+$ corresponds to phosphorylated bradykinin.

B. ARRPEGRTWAQPGY. A second peptide, ARRPEGRTWAQPGY, was studied in the same manner. In this case, the doubly protonated cluster $[M + TP + 2H]^{2+}$, where M refers to the peptide, was fragmented as shown in Figure 8a. This cluster is likely to consist of a triply protonated peptide, where all three arginine residues are protonated, and a singly deprotonated triphosphate. Again, products corresponding to loss of phosphoric acid (addition of diphosphate to the peptide) and

Scheme 4. Fragments Observed from Collision-Induced Dissociation of the Peak Assigned as $[M + 2H + H_2PO_3]^{2+}$ [a]



[a] Threonine, the phosphorylated residue, is denoted pT.

phosphorylation of the peptide $[M + 2H + H_2PO_3]^{2+}$ were obtained. When the phosphorylated peptide was collisionally activated, as shown in Figure 8b, the fragments generated (shown in Scheme 4) are consistent with the hypothesis that the peptide is phosphorylated on the hydroxyl of the threonine (T) residue. The presence of the b_5 fragment precludes phosphorylation on the aspartic acid residue, while the presence of the y_6 fragment indicates that the C-terminus is not phosphorylated. The residues other than threonine that could possibly be phosphorylated given these data are glycine and arginine, which are unlikely candidates for phosphorylation. Figure 8c shows the collision-induced dissociation of $[M + 2H]^{2+}$. Again, similar fragmentation patterns are observed for the phosphorylated and nonphosphorylated peptides.

Results from doubly charged clusters containing one sodium and one proton, and results of collisionally activating the product in which diphosphate is bound to ARRPEGRTWAQPGY, also indicate phosphorylation of the threonine residue. Data for the sodiated clusters show the b_4, b_5, py_9, and py_{11} fragment ions, where the phosphorylated fragments are sodiated and the nonphosphorylated fragments do not include sodium. The fragments generated from the attachment of diphosphate to the peptide include the b_5 fragment ion. Also observed are ppy_9 and ppy_{11} fragment ions that have diphosphate attached.

C. Other Peptides. Results with tyrosine-containing peptides GFQEAYRRFYGPV and YGGFLRKYRPK showed evidence that would support phosphorylation on the tyrosine residue. Positively charged clusters of peptide and triphosphate, such as $[M + TP + H]^+$ and $[M + TP + 2H]^{2+}$, were isolated, and the products $[M + H + H_2PO_3]^+$ and $[M + 2H + H_2PO_3]^{2+}$ were observed in good abundance and dissociated. However, very few peptide fragments were observed (for example, the $[M + H + H_2PO_3]^+$ of YGGFLRKYRPK yielded pb_7 and pb_9 fragments). While observed fragments for these species were consistent with phosphorylation on a tyrosine residue, there were not enough fragments to determine the site of phosphorylation with any certainty.

A control peptide INLKAKAALAKKLL (mastoparan 17), which does not contain tyrosine, threonine, or serine, was also studied. Figure 9a shows the spectrum resulting from collisional activation of the cluster $[M + TP + 2H]^{2+}$, which probably consists of a singly deprotonated triphosphate and a triply protonated peptide. A peak corresponding to a phosphorylated peptide is seen. The most abundant product is $[M + 2H - H_2O]^{2+}$, and we also see a peak corresponding to the doubly protonated peptide, $[M + 2H]^{2+}$. This peak is significantly larger than that seen in the dissociation of bradykinin or ARRPEGRTWAQPGY.

Figure 9b shows a spectrum resulting from the dissociation of $[M + 2H + H_2PO_3]^{2+}$. The only product observed is the doubly protonated peptide, $[M + 2H - H_2O]^{2+}$, which results from loss of H_3PO_4. Dissociation of $[M + 2H - H_2O]^{2+}$ yields many fragments, including only dehydrated y-type fragment

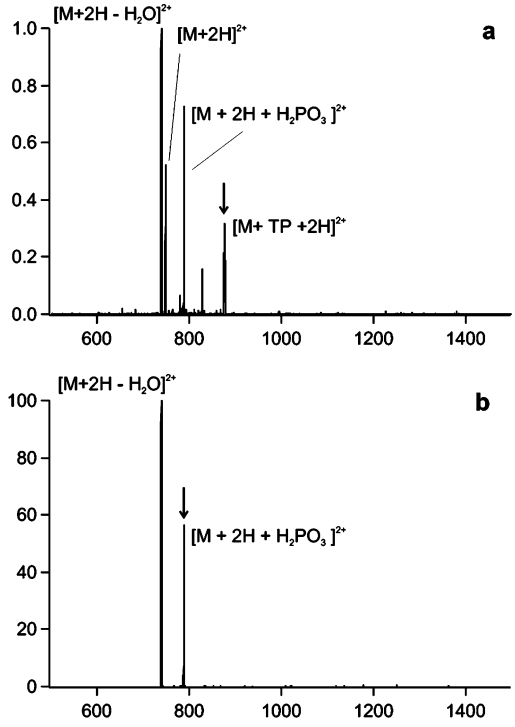


Figure 9. (a) Products of the collisional activation of $[M + TP + 2H]^{2+}$ for the control peptide INLKAKAALAKKLL (M) which does not contain tyrosine, threonine, or serine residues. (b) Collisional activation of the species $[M + 2H + H_2PO_3]^{2+}$. The loss of phosphate to yield $[M + 2H - H_2O]^{2+}$ is observed.

ions. This implies that the C-terminus is phosphorylated in this system. This contrasts with the behavior of the anionic cluster of betaine and triphosphate, noted in section 1C. Zwitterionic betaine can easily dissociate from the anionic betaine−triphosphate complex. However, in the case of the control peptide, the triphosphate and the peptide are held together by Coulombic attraction, and the carboxylic acid on the C-terminus is sufficiently reactive with triphosphate that the phosphorylated peptide is observed.

While the C-terminus of a peptide is observed to be phosphorylated in the absence of a residue with hydroxyl substituents, no indication of phosphorylation on the C-terminus is found in the peptides studied here that contain serine or threonine residues. We conclude that the phosphorylation reaction preferentially occurs on serine or threonine over the C-terminus, but that the C-terminus is likely to be phosphorylated if these residues are absent from a peptide.

Conclusions

Molecular clusters represent a unique chemical environment in which specific chemical reactions between cluster components can occur. The behavior of anionic clusters of triphosphate with choline, acetylcholine, and betaine has been examined both experimentally and theoretically. Phosphorylated choline compounds such as phosphatidyl choline are major components of cell membranes. The facile gas-phase phosphorylation of choline by triphosphate may be useful in understanding prebiotic syntheses of membrane lipid components.

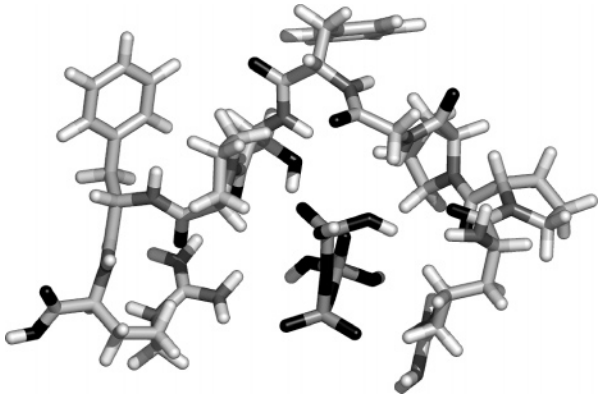


Figure 10. PM5 optimized structure of doubly protonated bradykinin complexed with the deprotonated triphosphate anion. The triphosphate anion is held in close proximity to the serine residue, with which it reacts when the cluster is collisionally activated.

The behavior of cationic clusters of triphosphate with several peptides has also been scrutinized. Phosphorylation of an alcohol group, when one is present, is shown to be a facile process when the cluster is subjected to collisional activation. Of particular interest is the selective gas-phase phosphorylation of the hydroxyl substituent in serine and threonine residues of peptides. While the C-terminus can be phosphorylated, our data indicate that when a side chain with a hydroxyl group is present, it is phosphorylated preferentially over the C-terminus.

In peptides, phosphorylation occurs remote from charged sites, although intramolecular solvation of the charged sites may result in a compact structure with closely interacting groups. In favorable circumstances, the interaction of charged sites might lead to alignment of centers in a pair of molecules and facilitate a desired transformation. Figure 10 illustrates this point with a PM5-minimized structure of the cationic cluster bradykinin with triphosphate, where the guanidinium groups are protonated and the triphosphate is singly deprotonated. The triphosphate anion is held in close proximity to the serine residue. Cluster-phase reactions observed previously typically involved reaction close to a charged site, at the α or β positions relative to the charged functional group.[7−9] This study expands the set of cluster-phase reactions to include those which can occur remotely (more than two atoms away) from a charge site in the same molecule. Just as supramolecular cavities can preorganize reactants, arranging them such that specific reactions are catalyzed,[20] charged sites are capable of organizing molecular clusters to favor specific reactions in the gas phase.

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