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Intramolecular Charge-Assisted Hydrogen Bond Strength in Pseudochair Carboxyphosphate

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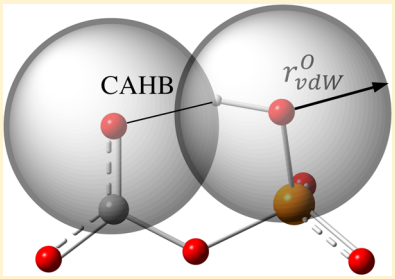
Supporting Information

ABSTRACT: Carboxyphosphate, a suspected intermediate in ATP-dependent carboxylases, has not been isolated nor observed directly by experiment. Consequently, little is known concerning its structure, stability, and ionization state. Recently, carboxyphosphate as either a monoanion or dianion has been shown computationally to adopt a novel pseudochair conformation featuring an intramolecular charge-assisted hydrogen bond (CAHB). In this work, additive and subtractive correction schemes to the commonly employed open−closed method are used to estimate the strength of the CAHB. Truhlar's Minnesota M06-2X functional with Dunning's aug-cc-pVTZ basis set has been used for geometry optimization, energy evaluation, and frequency analysis. The CHARMM force field has been used to approximate the Pauli repulsive terms in the closed and open forms of carboxyphosphate. From our additive correction scheme, differential Pauli repulsion contributions between the pseudochair (closed) and open conformations of carboxyphosphate are found to be significant in determining the CAHB strength. The additive correction modifies the CAHB prediction ($\Delta E_{\text{closed}-\text{open}}$) of −14 kcal/mol for the monoanion and −12 kcal/mol for the dianion to −22.9 and −18.4 kcal/mol, respectively. Results from the subtractive technique reinforce those from our additive procedure, where the predicted CAHB strength ranges from −17.8 to −25.4 kcal/mol for the monoanion and from −15.7 to −20.9 kcal/mol for the dianion. Ultimately, we find that the CAHB in carboxyphosphate meets the criteria for short-strong hydrogen bonds. However, carboxyphosphate has a unique energy profile that does not result in the symmetric double-well behavior of low-barrier hydrogen bonds. These findings provide deeper insight into the pseudochair conformation of carboxyphosphate, and lead to an improved mechanistic understanding of this intermediate in ATP-dependent carboxylases.



INTRODUCTION

Carboxyphosphate is an elusive intermediate in the ATP-dependent carboxylase superfamily of enzymes, which is involved in critical pathways related to obesity, diabetes, and microbial infections.[1−5] Recently, we found that carboxyphosphate exists in a unique "closed" or "pseudochair" conformation (Figure 1) stabilized by more than 14 kcal/mol as the monoanion and 12 kcal/mol as the dianion over the corresponding lowest energy "open" conformation.[6] Key to understanding the novel pseudochair stability resides in the balance between the stabilizing intramolecular charge-assisted hydrogen bond (CAHB), which is a special case of the intramolecular hydrogen bond (IMHB),[7] and associated destabilizing interactions. In addition, it is important to delineate carboxyphosphate's CAHB strength to understand its stability in nonpolar environments compared to its reactivity in aqueous solution.

The CAHB is suspected to be the key element of stabilization for the pseudochair conformation of carboxyphosphate, where the energy minimized pseudochair conformation reveals a hydrogen bond oxygen−oxygen distance of ca. 2.5 Å that is less than its van der Waals sum of 3.04 Å ($r_{\text{O}}^{\text{vdW}}$ = 1.52 Å)[8] and significant angle expansion across the bridging oxygen (ca. 127°) in both charged states. The geometric changes

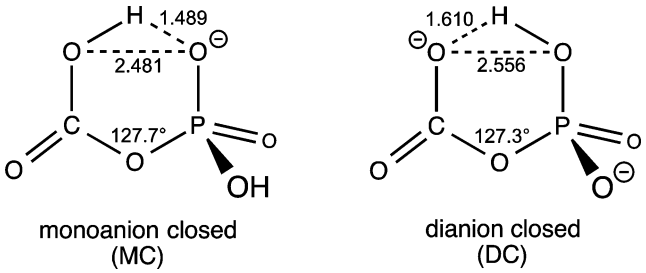


Figure 1. M06-2X/aug-cc-pVTZ key distances (Å) and angles of pseudochair carboxyphosphate.

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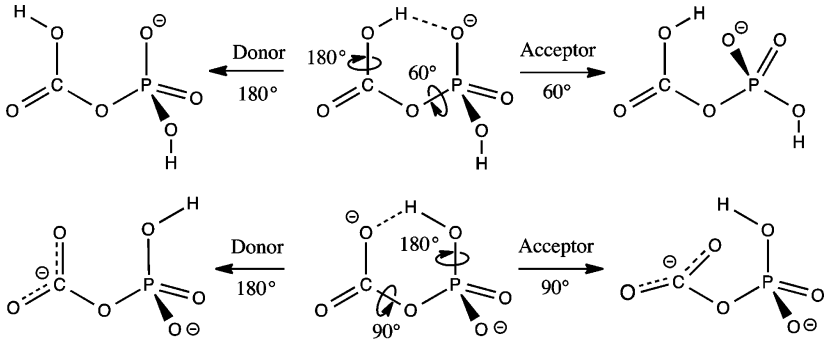

Figure 2. Possible conformational modification of pseudochair carboxyphosphate (center) to break the CAHB in the monoanion (top) and dianion (bottom).

suggest that Pauli repulsion and ring strain could be contributing factors to the overall destabilization of the pseudochair conformation. Such destabilizing interactions need to be addressed to determine the CAHB strength accurately.

Unlike the intermolecular hydrogen bond that has a convenient reference state, where infinite separation defines its absolute strength, measurement of IMHB strength must be determined relative to a nonzero reference system that makes its value ambiguous. Determining the strength of an IMHB is a nontrivial task due to the reference state selection; thus, many methods have been proposed and explored with varying degrees of success.[9−16] The challenge is to isolate an open structure (no IMHB), which may not necessarily be stationary,[9] without introducing new or removing essential geometric elements found in the closed conformation. To date, there is no one widely accepted method for identifying a reference structure or for estimating IMHB strength.

Despite its controversial nature, the most common IMHB estimation scheme involves the energy difference between the geometry optimized open and closed conformations,[17] otherwise known as the open−closed method. However, geometry optimization has the potential to remove essential energetic contributions in defining an accurate open reference state.[18−23] Subsequently, there has been effort in determining the effects of partially constrained versus full optimization of the open reference system, where significant differences have been reported.[22,24] Nevertheless, it is possible to take an alternative approach, where the energetic terms crucial to the IMHB configuration are estimated and then reinserted into the open−closed energy. The process of correcting the open and closed energy difference to estimate CAHB strength is referred to here as the *additive scheme*.

Jablonski proposed a partial optimization scheme to improve the open reference structure and the predicted accuracy of the IMHB energy depending upon the molecular system of interest.[24] An open reference system is created by eliminating the hydrogen bond. Only the position of the hydrogen bond donor or the hydrogen bond acceptor is modified, while retaining the closed geometry optimized structure. Such an open reference system is not a stationary state and higher in energy. Partial geometry optimization on selected degrees of freedom lowers the energy for a possibly more accurate open reference state. Since the energy is lowered with increasing relaxation, we coin this technique as the *subtractive scheme*. Systematic and partial relaxation of the starting conformation with the disengaged hydrogen bond leads to a series of partially optimized open reference geometries and energies. Selected dihedral angles determined in the closed structure, except the one to break the hydrogen bond, are typically held to their original closed values to prevent the introduction of new repulsive or attractive forces in the partially optimized open form. In this manner, comparison of the closed form to a partially optimized open reference form is suggested to yield an improved IMHB estimation.[24] However, selection of the partially optimized open structure is as arbitrary as selecting a completely geometry optimized open structure. Careful selection of the open reference state improves the predicted IMHB estimate, but the subtractive technique does not isolate, quantitate, nor clearly define any of the possible repulsive energies counterbalancing the attractive IMHB.

Many estimation schemes beyond the open−closed method[17] have been developed and implemented for their ability to estimate the strength of the IMHB. Specifically, isodesmic reactions have been used in order to determine the strength of the IMHB.[25] More recent schemes include the investigation of rotation barriers, as well as the relationship between rotamers.[12,22] The relationship between ^{1}H NMR shifts and hydrogen bond strength is well-known, and a direct correlation between the two has been discovered.[14,15] NBO analysis has been implemented to study the interactions of orbitals involved in the IMHB.[16,26] Topological analysis by QTAIM can be used to monitor changes in the electron density as the reference system is developed.[16,17,27,28]

Given the physiological importance and controversial understanding of carboxyphosphate's structure, stability, and role in the ATP-dependent carboxylase superfamily of enzymes, it is both appropriate and timely to investigate the intramolecular forces responsible for the novel pseudochair conformation of carboxyphosphate. The CAHB is estimated through additive corrections for Pauli repulsions to the open−closed method using CHARMM force field nonbond parameters,[29,30] and compared to a subtractive variant of the open−closed method. An improved understanding of the CAHB strength in relation to the overall stability of the pseudochair conformation in a vacuum is a starting point in dissecting the origin of instability in the aqueous phase and possible existence and meaningful contribution to the mechanism of ATP-dependent carboxylases. The importance of Pauli repulsion is demonstrated for the accurate prediction of the CAHB strength in carboxyphosphate.

■ COMPUTATIONAL DETAILS

Resources at the Center for Computational Sciences at Duquesne University,[31] the Gaussian 09 program,[32] and the CHARMM force field[29,30] have been used for all calculations.

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All electronic structure computations utilized Truhlar's Minnesota M06-2X functional[33] with Dunning's augmented correlation consistent polarized valence triple-ζ basis set (aug-cc-pVTZ).[34] This level of theory has been shown to capture the majority of energetic convergence giving the most consistent agreement with reference MP2 and CCSD(T) calculations, as shown in Figure S1 (Supporting Information). Frequency calculations were carried out to confirm all stationary points as minima on the potential energy surface. The atom types with epsilon and r_{min} values from the CHARMM force field[29,30] were used to compute the r^{-12} Pauli repulsion. All energy differences are reported as closed−open, $\Delta E_{c-o} = E_{closed} - E_{open}$, which delivers negative values for stabilized closed structures.

Borrowing from Jablonski's notation in the subtractive scheme,[24] several partial optimizations were performed to locate an accurate open reference system. All model structures were modified from the geometry optimized pseudochair structure by changing a single degree of freedom to maintain the original geometric features of the structure without the hydrogen bond, as defined in Figure 2.

For hydrogen bond donors, an idealized 180° rotation of the dihedral angle across the hydrogen bond donor to break the hydrogen bond was carried out with all other coordinates held fixed. Alternatively for hydrogen bond acceptors, the carboxylic acid was rotated by 90° (dianion) or the phosphoryl group by 60° (monoanion). The manner of generating open structures with a single conformational change isolated to either the donor or the acceptor results in differences with the lowest energy geometry optimized open structures computed. However, the two schemes are independent approaches so it is not necessary for the open structures to be identical. The modified structures without geometry optimization are referred to as SP. Next, only the bond lengths of SP were geometry optimized in order to yield a structure with a lower energy, which is labeled and referred to as B. Finally, geometry optimization of both bond lengths and bond angles with all dihedral angles held constant is carried out, and denoted as AB. The fully geometry optimized structure starting from SP is referred to as OPT. However, the lowest energy geometry optimized energy is used as a common reference throughout the study.

RESULTS AND DISCUSSION

Open−Closed Method. The open−closed method is one of the most widely used approaches for estimating CAHB strength, where the energy of the hydrogen-bonded (closed) form is compared to that of an open structure in which the CAHB is not present.[9,17,24,35−39] As previously discussed, the open−closed method typically involves rotation about one or more bonds starting from the closed form in order to disengage the hydrogen bond to produce an open structure. There are many variations to the open−closed method; however, the most basic procedure involves geometry optimization of the closed and opened forms with subsequent energetic comparison. Structural relaxation of the open form can either add or remove important interactions indigenous to the closed CAHB structure, thereby distorting the accuracy of the CAHB estimate, which has been recognized and criticized before.[18−23]

In the open−closed method, the open configuration of carboxyphosphate is selected to be the geometry optimized, lowest energy conformation without the intramolecular hydrogen bond. The M06-2X/aug-cc-pVTZ geometry optimized structures are shown in Figure 3. Stabilization of the pseudochair is defined to be the energy difference between

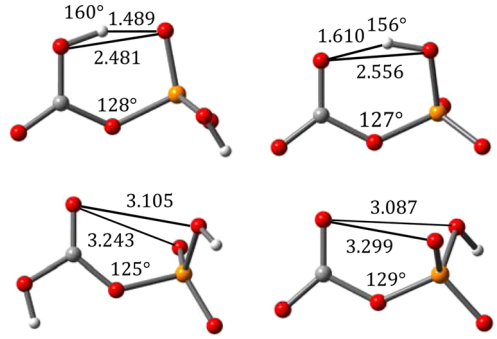

Figure 3. M06-2X/aug-cc-pVTZ open (bottom) and closed (top) monoanionic (left) and dianionic (right) conformations.

the closed and open conformations in both the monoanionic and dianion states. CAHB strength is commonly estimated from the open−closed method and defined by the computed stabilization energies of −14.5 kcal/mol for the monoanion and −12.0 kcal/mol for the dianion.

The open structures defined in Figure 2 are slightly different than the geometry optimized open structures using M06-2X/aug-cc-pVTZ shown in Figure 3. The differences are a consequence of how the two techniques generate the open structure to estimate independently the same CAHB strength. In this fashion, a more practical comparison is undertaken to represent approaches reported in the literature. Change in the lowest energy geometry optimized open structures affirms that essential geometric elements can differ from the closed conformation in the standard implementation of the open−closed method.

Additive Scheme. The geometry minimized pseudochair conformations, in comparison to the open structures, reveal that, in addition to the CAHB, Pauli repulsion interactions and ring strain are possible contributors to differences in energetics between the two forms, as shown in Figure 3. To gauge the effect of each type of contribution to the overall stability of the pseudochair, a series of model systems were developed and applied.

Ring Strain. In order to study the differential ring strain ($\Delta E^{rs}_{c-o} = E^{rs}_{closed} - E^{rs}_{open}$) between the closed and open forms of carboxyphosphate, dihydrogen phosphate was used as a model system for its ability to resemble the phosphate side and bridging oxygen of carboxyphosphate. Geometry optimized dihydrogen phosphate has an ∠HOP angle of 105.9° (M06-2X/aug-cc-pVTZ) and was used as the strain free reference, as shown in Figure 4.

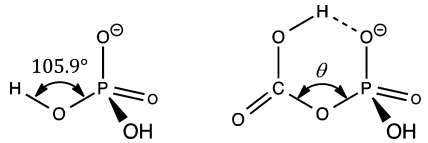

Figure 4. Angle representing ring strain in the dihydrogen phosphate model of carboxyphosphate.

To model the ring strain in the closed and open forms, the ∠HOP angle of 105.9° in dihydrogen phosphate was adjusted and fixed to that of the bridging oxygen in the different states and conformations of carboxyphosphate. The dihydrogen phosphate model systems were allowed to optimize constrained

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to the bridging oxygen angle. The relative energies (ΔE^{rs}_{c-o}) of the different constrained geometries are given in Table 1.

Table 1. Bridging Oxygen Angles and Contributions of Ring Strain (kcal/mol) in the Closed and Open States for Both the Monoanion and Dianion Using the H_2PO_4 Model

	open	closed	ΔE^{rs}_{c-o}
monoanion	125.3°	127.7°	−0.8
dianion	129.3°	127.3°	0.7

Even though ring strain from a ca. 20° expansion across the bridging oxygen is computed to be significant at ca. 4.0 kcal/mol, the angle and subsequent energy difference between the open and closed forms is found to be small. In fact, the computed energy difference is less than 1 kcal/mol. The angles between the open and closed structures are too small to generate appreciable ring strain differences, making model selection irrelevant. Consequently, ring strain is determined to play only a minor role in the accurate prediction of the CAHB from the open−closed method.

Pauli Repulsion. Comparison of the geometry optimized open and closed conformations using M06-2X/aug-cc-pVTZ reveals a significant shortening of the distance between the oxygen atoms (r_{OO}) and between the hydrogen bonded oxygen and hydrogen (r_{HO}) involved in the CAHB, as highlighted in Figure 5.

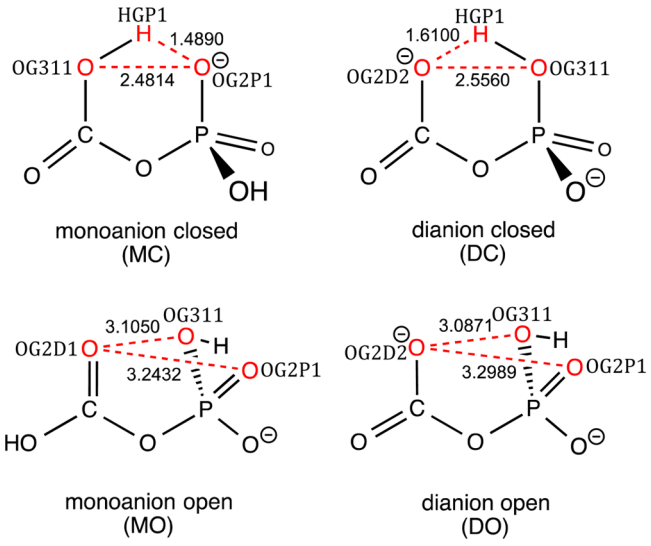

Figure 5. Specific nonbond interactions (red) computed to determine the Pauli repulsion in carboxyphosphate. CHARMM atoms types are included.

In the geometry optimized closed conformations of both charge states, the r_{OO} distance of ca. 2.5 Å is less than its van der Waals sum of 3.04 Å (r^{vdW}_{O} = 1.52 Å).[8] In addition, the hydrogen bond r_{HO} distances are 1.49 and 1.61 Å for the monoanion and dianion, respectively, which are shorter than the idealized van der Waals sum of 2.61 Å (r^{vdW}_{H} = 1.09 Å),[40] as given in Table 2.

In the geometry optimized open conformations, the hydrogen bond is broken, and the distances between atoms change to relieve intramolecular nonbond interactions. The distances are computed to become greater than the van der Waals sum, as shown in Figure 5. However, two r_{OO} distances

Table 2. r_{OO} and r_{HO} Distances (Å) in the Open and Closed Forms of the Monoanion and the Dianion and the Difference Δr_{OO}

	open		closed		
	r^{A}_{OO}	r^{B}_{OO}	r_{OO}	r_{OH}	Δr_{OO}
monoanion	3.105	3.243	2.481	1.489	0.624
dianion	3.087	3.299	2.556	1.610	0.531

in the open structures are close to the van der Waals sum, as given in Table 2. Thus, two interactions for each conformation were computed to determine the difference in Pauli repulsion between the closed and open conformations, as defined in Figure 5.

The difference in the Pauli repulsion between the closed and open conformations of carboxyphosphate was estimated by using the sum of the repulsive r^{-12} part of the Lennard-Jones approximation, as given in eq 1.

$$E_{Pauli} = \epsilon_{ij}\left(\frac{r^{min}_{ij}}{r_{ij}}\right)^{12} \quad (1)$$

The atomic Lennard-Jones parameters ϵ and r^{min} are combined with the appropriate mixing rules for a pair of atoms i and j. In the current CHARMM force field, the parameters are $\epsilon_{ij} = (\epsilon_i\epsilon_j)^{1/2}$ (geometric mean) and $r^{min}_{ij} = (r^{min}_i + r^{min}_j)/2$ (arithmetic mean), as listed in Table 3.

Table 3. CHARMM Force Field Lennard-Jones Parameters (ϵ, r^{min})[29,30]

atom type	description	ϵ	$r_{min}/2$
OG311	hydroxyl oxygen	−0.1921	1.7650
OG2P1	=O in phosphate	−0.1200	1.7000
OG2D1	carbonyl oxygen	−0.1200	1.7000
OG2D2	carboxylate oxygen	−0.1200	1.7000
HGP1	polar hydrogen	−0.0460	0.2245

The CHARMM atom types defining the parameters used in eq 1 for the computation of the Pauli repulsion ($\Delta E^{Pauli}_{c-o} = E^{Pauli}_{closed} - E^{Pauli}_{open}$) are shown in Figure 5 and Table 3. The computed Pauli repulsion for the closed and open forms of the dianion are 6.48 and 0.78 kcal/mol, respectively. Therefore, the difference in the Pauli energies for the dianion is 5.70 kcal/mol. Likewise, the computed Pauli repulsions for the closed and open forms of the monoanion are 9.96 and 0.78 kcal/mol, respectively. Thus, the difference in the Pauli energies for the monoanion is 9.18 kcal/mol.

Intramolecular Charge-Assisted Hydrogen Bond. Analyses of the differential ring strain and Pauli repulsions in the open and closed forms uncover contributions that impact the estimated CAHB strength of the open−closed method, as described in eq 2.

$$\Delta E_{c-o} = \Delta E_{CAHB} + \Delta E^{Pauli}_{c-o} + \Delta E^{rs}_{c-o}$$
$$\Delta E_{CAHB} = \Delta E_{c-o} - \Delta E^{Pauli}_{c-o} - \Delta E^{rs}_{c-o} \quad (2)$$

The results of eq 2 are given in Table 4 in order to estimate the range of CAHB strength. According to the additive correction for the Pauli repulsion to the open−closed method, the CAHB strength is −22.9 kcal/mol for the monoanion, whereas the CAHB strength is −18.4 kcal/mol for the dianion.

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Table 4. Components of the CAHB Energy (kcal/mol) of Dianionic and Monanionic Carboxyphosphate

	ΔE_{c-o}^{rs}	ΔE_{c-o}^{Pauli}	ΔE_{c-o}	ΔE_{CAHB}
monoanion	−0.8	9.2	−14.5	−22.9
dianion	0.7	5.7	−12.0	−18.4

The additive corrective terms for Pauli repulsion significantly increase the predicted CAHB strength compared to that predicted by the standard implementation of the open−closed method.

Subtractive Scheme. It has been reported that geometry optimization of the open form may lead to an inappropriate reference system.[18−23] Jablonski has shown that the calculated strength of an IMHB with the open−closed method is highly dependent on the degree of optimization.[24] As previously discussed, the idea is to create an open structure by modifying the closed geometry optimized structure with minimal structural changes to break the IMHB and improve the CAHB estimate. Typically, adjustment of a dihedral angle to reposition the hydrogen shared in the IMHB is made (Figure 2). A single point energy evaluation (SP) is carried out, giving an upper bound to the IMHB estimation. At the other extreme, geometry relaxation starting from the SP structure gives a geometry optimized (OPT) structure. Ideally, two pathways to break the IMHB can be taken by modifying either the hydrogen bond donor or the hydrogen bond acceptor. Both result in a different reference and range of IMHB values.

Analogous to the partial optimizations of Jablonski, the SP, B, AB, and OPT computations were carried out. First, the CAHB was removed by an idealized 180° rotation of the hydrogen bond donor (∠HOCO for the monoanion and ∠HOPO for the dianion) starting from the closed geometry optimized structure. Our second method of breaking the CAHB also started with the closed geometry optimized structure but with a rotation of the hydrogen bond acceptor (60° for ∠OPOC for the monoanion and 90° for ∠HOPO for the dianion). However, only modification of the donor structure leads to an appropriate open state that removes the CAHB. Modification of the acceptor leads to a structure that connects closed states (Figures S2 and S3, Supporting Information), which does not represent a desirable open reference state and is referred to as *inappropriate* in Table 5. As such, analysis is carried out only on the CAHB estimation from the donor-modified structure. As reported in Table 5, the SP calculations were found to give the highest estimations and the OPT geometry optimizations the lowest. Removal of the bond and angle constraints (B and AB) reduced the energies as compared to the SP value.

Table 5. Computed Energies (kcal/mol) for the Subtractive Method

	monoanion donor	monoanion acceptor	dianion donor	dianion acceptor
SP	−25.4	−18.2[a]	−20.9	−16.8[a]
B	−21.2	−16.2[a]	−18.8	−14.8[a]
AB	−17.8	−14.8[a]	−15.7	−13.5[a]
−TS	−19.5	−9.0[a]	−12.1	−13.0[a]
OPT	−14.5	0.0	−12.0	0.0

[a]Inappropriate reference state that is not open.

According to the subtractive method, the CAHB strength is between −17.8 and −25.4 kcal/mol for the monoanion, whereas the CAHB strength is between −15.7 and −20.9 kcal/mol for the dianion. The estimated ranges from the subtractive scheme are in good agreement with the additive corrections to the open−closed method.

Transition Structures. The transition structures for each process in breaking the CAHB were computed and verified using M06-2X/aug-cc-pVTZ, as shown in Table 5 as TS. The computed transition structure is at the highest point on the potential energy surface along the reaction coordinate[41] to break the CAHB separating the closed and open conformations. As such, the activation energy defined from the pseudochair structure represents the energy to break the CAHB. The computed activation energy for breaking the monoanion CAHB through the donor is 19.5 kcal/mol, while it was 9.0 kcal/mol for a different process through the acceptor, where the computed transition structure is a CAHB switch between the two equivalent phosphate oxygens. As a result, the 9.0 activation energy is inappropriate for CAHB strength assessment, as with the subtractive scheme of Table 5, since the transition structure does not connect the pseudochair and open conformations (Figure S2, Supporting Information). Therefore, the predicted CAHB strength for the monoanion is 19.5 kcal/mol. The computed activation energy for breaking the dianion CAHB through the acceptor is 13.0 kcal/mol, and 12.1 kcal/mol through the donor (Figure S3, Supporting Information). Analogously, the transition structure interconnecting the pseudochair and open structures is through the donor change in geometry. Thus, the predicted CAHB strength for the dianion is 12.1 kcal/mol.

CAHB Model Comparison. The geometries of the computed transition structures, the AB minimized structures suggested by Jablonski, and the geometry optimized closed structures (Figure 3) were compared, as shown in Figure 6.[24] It is of interest to analyze the key geometric features of the closed structure and the change observed in the CAHB models.

The M06-2X/aug-cc-pVTZ geometry optimized closed structures (monoanion and dianion) have the hydrogen bond proton positioned directly in between the donor and the acceptor (Figure 3). The structural consequence is that there is reduced lone-pair congestion between the donor and acceptor oxygens. In addition, the closed structures involve two nonbond interactions that could lead to strong Pauli repulsion, involving the hydrogen bond donor and acceptor oxygens, and the hydrogen bond hydrogen and noncovalent oxygen. The dianion models (TSDd and ABDd) maintain the eclipsing hydrogen bond donor and acceptor, as found in the closed structure. However, the main geometric difference in the dianion models is in the position of the hydrogen. TSDd directs the lone pairs away from the acid group (∠HOPO = 63°), whereas ABDd directs the lone pairs toward the acid (∠HOPO = 151°). Thus, the dianion ABDd model should overestimate the repulsion in the estimation of the CAHB. The additional repulsion is seen by the lengthening of the hydrogen bond donor and acceptor oxygens from 2.48 Å in the closed dianion to 3.07 Å (TSDd) and 2.87 Å (ABDd). The monoanion models (TSDm and ABDm) result in different orientations between the hydrogen bond donor and acceptor. TSDm rotates the phosphate group by ca. 60° from the closed structure, lengthening the donor and acceptor oxygen difference from 2.48 Å in the closed to 2.85 Å and generating a second weak interaction at 3.31 Å. The hydrogen rotates to ∠HOCO = 114°

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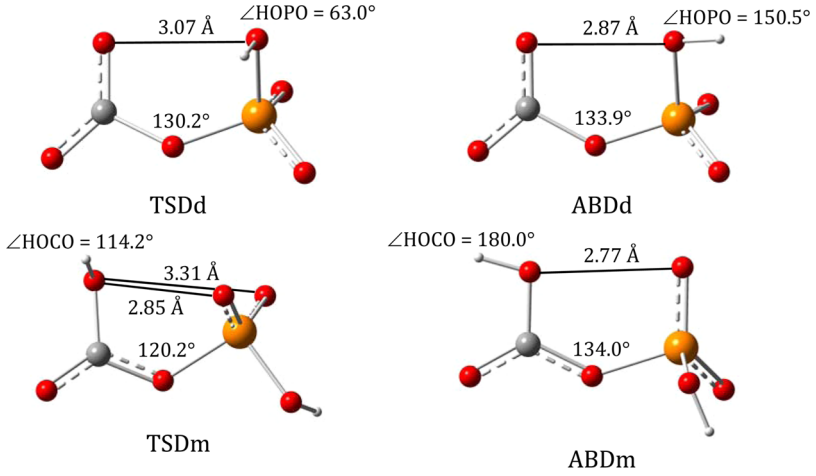

Figure 6. Dianionic transition structure computed using M06-2X/aug-cc-pVTZ for the breaking of the CAHB donor (TSDd) and the partially minimized dianion donor (ABDd) reference structure. Monoanionic transition structure for the breaking of the CAHB donor (TSDm) and the partially minimized monoanion donor (ABDm) reference structure.

reorienting its lone pairs away from the phosphate group. However, ABDm maintains the eclipsing hydrogen bond donor and acceptor interaction, since it is constrained to that of the closed structure. The hydrogen bond oxygen to oxygen distance lengthens to 2.77 Å, and the hydrogen adopts a position of ∠HOCO = 180° that increases the congestion of lone pairs between the donor and the acceptor. The net result should overestimate the repulsion in the estimation of the CAHB, as in the dianion model. However, the overestimation is somewhat mitigated when the energy difference is determined in reference to the OPT structures with open geometries.

In a final comparison of the activation energies with the subtractive and additive corrections to the open−closed method (Table 6), an agreement to the estimated CAHB strength is observed, which is greater than the stabilization energy of pseudochair carboxyphosphate from the standard implementation of the open−closed method.

Table 6. Comparison of the Three Highlighted Methods of CAHB Estimated in This Study (kcal/mol)

	additive	subtractive	−TS	open−closed
monoanion	−22.9	−17.8 to −25.4	−19.5	−14.5
dianion	−18.4	−15.7 to −20.9	−12.1	−12.0

As expected, the predicted CAHB values from the computed transition structures are on the lower end of the additive and subtractive schemes for the monoanion, and below the lower bounds for each open−closed method describing the dianion. The dihedral angle constraints, or release of other degrees of freedom, in the partial optimization procedure of the subtractive method are a likely source of error causing an overestimation, as highlighted in the discussion above. The additive corrections to the open−closed method are possibly in error due to the parametrization of nonbond terms, where the parameters were developed for phosphate esters for intermolecular interactions. Despite overestimating the CAHB strength compared to those predicted by the transition structures, the importance of Pauli replusion interactions is highlighted for the first time by the additive corrections to the open−closed method.

On the basis of the above estimation schemes, the CAHB can be classified as strong (greater than 12 kcal/mol).[42−44] The class of hydrogen bonds known as short-strong hydrogen bonds (SSHBs) is often associated with those that are also low barrier (LBHBs).[43,45−48] Although hydrogen bonds may be both short-strong and low barrier, these classifications are made on the basis of separate criteria.[48,49] Classification as SSHB is based on the distance between the heteroatoms (less than 2.5 Å for an OHO hydrogen bond). However, classification as an LBHB is based on the equivalence of the pK_a's of the two donor atoms, resulting in a low barrier to proton transfer between the two.

The estimated CAHB strengths from each of the three independent estimation schemes result in a bond strength greater than 12 kcal/mol used to classify a short-strong hydrogen bond.[42−44] The heteroatom distance is 2.48 Å in the monoanion and 2.56 Å in the dianion. Thus, considering the sources of error, carboxyphosphate meets the geometric and energetic criteria for short-strong hydrogen bonding. The pK_a values of the donor and acceptor are expected to be different. Thus, there is no double-well potential found, and the bond cannot be considered low barrier. In summary, the bond is considered to be short-strong but not low barrier in both charge states of pseudochair carboxyphosphate.

CONCLUSION

The CAHB strength of the pseudochair conformation of monoanionic and dianionic carboxyphosphate in a vacuum has been estimated by independent additive and subtractive corrections to the open−closed method. Our additive correction scheme shows differential Pauli repulsion contributions between the pseudochair (closed) and open conformations of carboxyphosphate that are significant in estimating the CAHB strength. Our CAHB estimate is −22.9 kcal/mol for the monoanion and −18.4 kcal/mol for the dianion. Results from the subtractive technique reinforce those from our additive procedure, where the predicted CAHB strength ranges from −17.8 to −25.4 kcal/mol for the monoanion and from −15.7 to −20.9 kcal/mol for the dianion. Ultimately, we find the CAHB in carboxyphosphate meets the criteria for short-strong hydrogen bonds. However, carboxyphosphate has a unique energy profile that does not result in the symmetric double-well behavior of low-barrier hydrogen bonds. The CAHB in carboxyphosphate is short-strong but cannot be considered to be low barrier, as the proton is not shared equally between the

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donor and acceptor groups. The strong hydrogen bond coupled with the fact that the hydrogen prefers to be on the phosphate group provides a previously unrecognized mechanism by which protons can be shuttled from the carboxylic acid side of carboxyphosphate to the phosphate. Such a proton shuttling mechanism has previously unconsidered implications for the catalytic mechanism of the carboxylase enzymes. The present analysis delivers a fundamental report on the strength of the CAHB in the ionized forms of carboxyphosphate, which has the potential to realign and improve the mechanistic understanding of this intermediate in ATP-dependent carboxylases.

■ ASSOCIATED CONTENT

Supporting Information

All computed structures, Figures S1−S3, discussion of MP2, CCSD(T) and M06-2X comparison, and extended energy profile including the hydrogen bond acceptor geometry change. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Thoden, J. B.; Holden, H. M.; Firestine, S. M. Structural Analysis of the Active Site Geometry of N5-Carboxyaminoimidazole Ribonucleotide Synthetase from Escherichia Coli. *Biochemistry* **2008**, *47*, 13346−13353.

(2) Firestine, S. M.; Davisson, V. J. Carboxylases in de Novo Purine Biosynthesis. Characterization of the Gallus Gallus Bifunctional Enzyme. *Biochemistry* **1994**, *33*, 11917−11926.

(3) Firestine, S. M.; Poon, S.-W.; Mueller, E. J.; Stubbe, J.; Davisson, V. J. Reactions Catalyzed by 5-Aminoimidazole Ribonucleotide Carboxylases from Escherichia Coli and Gallus Gallus: A Case for Divergent Catalytic Mechanisms? *Biochemistry* **1994**, *33*, 11927−11934.

(4) Firestine, S. M.; Misialek, S.; Toffaletti, D. L.; Klem, T. J.; Perfect, J. R.; Davisson, V. J. Biochemical Role of the Cryptococcus Neoformans ADE2 Protein in Fungal de Novo Purine Biosynthesis. *Arch. Biochem. Biophys.* **1998**, *351*, 123−134.

(5) Fawaz, M. V.; Topper, M. E.; Firestine, S. M. The ATP-Grasp Enzymes. *Bioorg. Chem.* **2011**, *39*, 185−191.

(6) Pakkala, V. S. *Computational Investigation of the Mechanism of N5-Carboxyaminoimidazole Ribonucleotide Synthetase*; Duquesne University: Pittsburgh, PA, 2012.

(7) Gilli, G.; Gilli, P. Towards an Unified Hydrogen-Bond Theory. *J. Mol. Struct.* **2000**, *552*, 1−15.

(8) Bondi, A. van der Waals Volumes and Radii. *J. Phys. Chem.* **1964**, *68*, 441−451.

(9) Schuster, P. LCAO-MO Investigations of Molecular Structures. IV. LCAO-MO Studies on Intramolecular Hydrogen Bonding. *Monatsh. Chem.* **1969**, *100*, 2084−2095.

(10) Hehre, W. J.; Ditchfield, R.; Radom, L.; Pople, J. A. Molecular Orbital Theory of the Electronic Structure of Organic Compounds. V. Molecular Theory of Bond Separation. *J. Am. Chem. Soc.* **1970**, *92*, 4796−4801.

(11) Pople, J.; Radom, L.; Hehre, W. Molecular Orbital Theory of the Electronic Structure of Organic Compounds. VII. Systematic Study of Energies, Conformations, and Bond Interactions. *J. Am. Chem. Soc.* **1971**, *13*, 289−300.

(12) Nowroozi, A.; Raissi, H.; Farzad, F. The Presentation of an Approach for Estimating the Intramolecular Hydrogen Bond Strength in Conformational Study of β-Aminoacrolein. *J. Mol. Struct.* **2005**, *730*, 161−169.

(13) Buemi, G.; Zuccarello, F.; Venuvanalingam, P.; Ramalingam, M.; Salai, C. A. S. Ab Initio Study of Formazan and 3-Nitroformazan. *J. Chem. Soc., Faraday Trans.* **1998**, *94*, 3313−3319.

(14) Kumar, G. A.; McAllister, M. A. Theoretical Investigation of the Relationship between Proton NMR Chemical Shift and Hydrogen Bond Strength. *J. Org. Chem.* **1998**, *63*, 6968−6972.

(15) Scheiner, S. In *Hydrogen Bonding: A Theoretical Perspective*; Truhlar, D. G., Ed.; Oxford University Press: New York, 1997.

(16) Lopes, J. A. J.; Redinha, J. S. Charge-Assisted Intramolecular Hydrogen Bonds in Disubstituted Cyclohexane Derivatives. *J. Phys. Chem. A* **2011**, *115*, 14069−14077.

(17) Grabowski, S. J. Hydrogen Bonding Strength - Measures Based on Geometric and Topological Parameters. *J. Phys. Org. Chem.* **2004**, *17*, 18−31.

(18) Craw, J. S.; Bacskay, G. B. Quantum-Chemical Studies of Hydrogen Bonding Involving Thioxoketones, Thienols, Thioformaldehyde and Hydrogen Sulfide with Specific Reference to the Strength of Intramolecular Hydrogen Bonds. *J. Chem. Soc., Faraday Trans.* **1992**, *88*, 2315−2321.

(19) Scheiner, S.; Kar, T.; Cuma, M. Excited State Intramolecular Proton Transfer in Anionic Analogs of Malonaldehyde. *J. Phys. Chem. A* **1997**, *101*, 5901−5909.

(20) Chung, G.; Kwon, O.; Kwon, Y. Theoretical Study on 1,2-Dihydroxybenzene and 2-Hydroxythiophenol: Intramolecular Hydrogen Bonding. *J. Phys. Chem. A* **1997**, *101*, 9415−9420.

(21) Cuma, M.; Scheiner, S.; Kar, T. Effect of Adjoining Aromatic Ring upon Excited State Proton Transfer, O-Hydroxybenzaldehyde. *J. Mol. Struct.* **1999**, *467*, 37−49.

(22) Buemi, G.; Zuccarello, F. Is the Intramolecular Hydrogen Bond Energy Valuable from Internal Rotation Barriers? *J. Mol. Struct.* **2002**, *581*, 71−85.

(23) Jablonski, M.; Kaczmarek, A.; Sadlej, A. J. Estimates of the Energy of Intramolecular Hydrogen Bonds. *J. Phys. Chem. A* **2006**, *110*, 10890−10898.

(24) Jablonski, M. Full vs. Constrain Geometry Optimization in the Open-Closed Method in Estimating the Energy of Intramolecular Charge-Inverted Hydrogen Bonds. *Chem. Phys.* **2010**, *376*, 76−83.

(25) Varnali, T.; Hargittai, I. Geometrical Consequences of Resonance-Assisted Hydrogen Bonding in 2-Nitrovinyl Alcohol and Indication of a Slight Attractive O···H Interaction in 2-Nitroethanol. An Ab Initio Molecular Orbital Investigation. *J. Mol. Struct.* **1996**, *388*, 315−319.

(26) Weinhold, F.; Landis, C. R. *Valency and Bonding: A Natural Bond Orbital Donor-Acceptor Perspective*; Cambridge University Press: Cambridge, U.K., 2005.

(27) Bader, R. F. W. *Atoms in Molecules-A Quantum Theory*; Oxford University Press: Oxford, U.K., 1990.

(28) Schiøtt, B.; Iversen, B. B.; Madsen, G. K. H.; Bruice, T. C. Characterization of the Short Strong Hydrogen Bond in Benzoylacetone by Ab Initio Calculations and Accurate Diffraction Experiments. Implications for the Electronic Nature of Low-Barrier Hydrogen Bonds in Enzymic Reactions. *J. Am. Chem. Soc.* **1998**, *120*, 12117−12124.

(29) Vanommeslaeghe, K.; Hatcher, E.; Acharya, C.; Kundu, S.; Zhong, S.; Shim, J.; Darian, E.; Guvench, O.; Lopes, P.; Vorobyov, I.; et al. CHARMM General Force Field: A Force Field for Drug-like

DOI: 10.1021/jp506796r
J. Phys. Chem. B 2015, 119, 1184−1191

Molecules Compatible with the CHARMM All-atom Additive Biological Force Fields. *J. Comput. Chem.* **2010**, *31*, 671−690.
(30) MacKerell, A. D.; Bashford, D.; et al. All-Atom Empirical Potential for Molecular Modeling and Dynamics Studies of Proteins. *J. Phys. Chem. B* **1998**, *5647*, 3586−3616.
(31) Center for Computational Sciences at Duquesne University Supported by National Science Foundation: CHE-0723109 and CHE-1126465 and United States Department of Education: P116Z080180 and P116Z050331.
(32) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.; et al. *Gaussian 09*, revision D.01; Gaussian, Inc.: Wallingford, CT, 2013.
(33) Zhao, Y.; Truhlar, D. G. The M06 Suite of Density Functionals for Main Group Thermochemistry, Thermochemical Kinetics, Noncovalent Interactions, Excited States, and Transition Elements: Two New Functionals and Systematic Testing of Four M06-Class Functionals and 12 Other Function. *Theor. Chem. Acc.* **2008**, *120*, 215−241.
(34) Dunning, T. H., Jr. Gaussian Basis Sets for Use in Correlated Molecular Calculations. I. The Atoms Boron through Neon and Hydrogen. *J. Chem. Phys.* **1989**, *90*, 1007−1023.
(35) Schuster, P. Energy Surfaces for Hydrogen Bonded Systems. In *The Hydrogen Bond - Recent Developments in Theory and Experiment*; Schuster, P., Zundel, G., Sandorfy, C., Eds.; North-Holland Publ. Co.: Amsterdam, The Netherlands, 1976; Vol. *1*, pp 25−163.
(36) Hajiabadi, H.; Nowroozi, A.; Hasani, M.; Jahani, P. M.; Raissi, H. A Comparative Study of Open-close and Related Rotamers Methods to Evaluate the Intramolecular Hydrogen Bond Energies in 3-imino-propen-1-ol and Its Derivatives. *Int. J. Quantum Chem.* **2012**, *112*, 1384−1391.
(37) Nowroozi, A.; Hajiabadi, H. How to Estimate the Intramolecular Hydrogen-Bond Energy of Complex RAHB Systems? A Theoretical Study. *Struct. Chem.* **2013**, *25*, 215−220.
(38) Nowroozi, A.; Hajiabadi, H.; Akbari, F. OH···O and OH···S Intramolecular Interactions in Simple Resonance-Assisted Hydrogen Bond Systems: A Comparative Study of Various Models. *Struct. Chem.* **2013**, *25*, 251−258.
(39) Nowroozi, A.; Raissi, H.; Hajiabadi, H.; Jahani, P. M. Reinvestigation of Intramolecular Hydrogen Bond in Malonaldehyde Derivatives: An Ab Initio, AIM and NBO Study. *Int. J. Quantum Chem.* **2011**, *111*, 3040−3047.
(40) Rowland, R.; Taylor, R. Intermolecular Nonbonded Contact Distances in Organic Crystal Structures: Comparison with Distances Expected from van Der Waals Radii. *J. Phys. Chem.* **1996**, *100*, 7384−7391.
(41) Acevedo, O.; Evanseck, J. D. Trqnsition States and Transition Structures. In *Computational Medicinal Chemistry for Drug Discovery*; Bultinck, P., De Winter, H., Langenaeker, W., Tollenaere, J. P., Eds.; Marcel Dekker, Inc.: New York, New York, 2004; pp 323−344.
(42) Emsley, J. Very Strong Hydrogen Bonding. *Chem. Soc. Rev.* **1980**, *9*, 91−124.
(43) Perrin, C. L.; Nielson, J. B. Strong" Hydrogen Bonds in Chemistry and Biology. *Annu. Rev. Phys. Chem.* **1997**, *48*, 511−544.
(44) Jeffrey, G. A. *An Introduction to Hydrogen Bonding*; Oxford University Press: New York, 1997.
(45) Kreevoy, M. M.; Liang, T. M.; Chang, K. C. Structures and Isotopic Fractionation Factors of Complexes AHA-1. *J. Am. Chem. Soc.* **1977**, *99*, 5207−5209.
(46) Kreevoy, M. M.; Liang, T. M. Structures and Isotopic Fractionation Factors of Complexes, A1HA2-. *J. Am. Chem. Soc.* **1980**, *102*, 3315−3322.
(47) Cleland, W. W. Low-Barrier Hydrogen Bonds and Low Fractionation Factor Bases in Enzymic Reactions. *Biochemistry* **1992**, *31*, 317−319.
(48) Perrin, C. L. Are Short, Low-Barrier Hydrogen Bonds Unusually Strong? *Acc. Chem. Res.* **2010**, *43*, 1550−1557.
(49) Remer, L. C.; Jensen, J. H. Toward a General Theory of Hydrogen Bonding: The Short, Strong Hydrogen Bond [HOH···OH]-. *J. Phys. Chem. A* **2000**, *104*, 9266−9275.

DOI: 10.1021/jp506796r
J. Phys. Chem. B 2015, 119, 1184−1191