From Formamide to Adenine: A Self-Catalytic Mechanism for an Abiotic Approach

ARTICLE *in* THE JOURNAL OF PHYSICAL CHEMISTRY B · OCTOBER 2013

Impact Factor: 3.3 · DOI: 10.1021/jp409296k · Source: PubMed

THE JOURNAL OF PHYSICAL CHEMISTRY B

Article

pubs.acs.org/JPCB

From Formamide to Adenine: A Self-Catalytic Mechanism for an Abiotic Approach

Jing Wang,[†] Jiande Gu,*[,†,‡] Minh Tho Nguyen,[§] Greg Springsteen,[∥] and Jerzy Leszczynski*[,†]

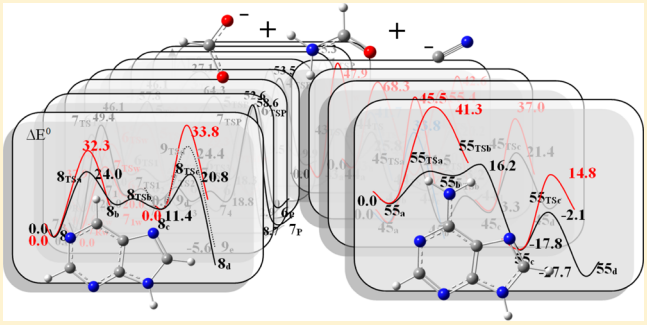
[†]Interdisciplinary Nanotoxicity Center, Department of Chemistry and Biochemistry, Jackson State University, Jackson, Mississippi 39217, United States

[‡]Drug Design and Discovery Center, State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, CAS, Shanghai 201203, People's Republic of China

[§]Department of Chemistry, University of Leuven, B-3001 Leuven, Belgium

[∥]Department of Chemistry, Furman University, 3300 Poinsett Hwy, Greenville, South Carolina 29613, United States

ABSTRACT: Mechanisms for abiotic reaction pathways from formamide (H_2NCHO) to adenine are presented herein. Formamide is a simple C1 building block hypothesized to be a precursor to many protometabolic compounds. On the basis of a step-by-step mechanism of the reaction pathways, formamide is suggested to be more reactive in addition reactions than HCN. In addition to its simplicity, the formamide self-catalyzed mechanism is energetically (kinetically) more viable than either a water-catalyzed mechanism or noncatalyzed processes. Moreover, this self-catalyzed mechanism accounts for the yields of purine and adenine previously observed in experiments. This mechanism may elucidate processes that were vital for the emergence of life on the early earth.



INTRODUCTION

Stanley Miller's experiment in 1953 revealed that amino acids can be derived from simple mixtures of water, methane, ammonia, and hydrogen under conditions that mimic the early earth.[1] Careful analyses suggest that a suite of simple organic molecules such as hydrogen cyanide (HCN) should exist as intermediates in such reactions leading to the formation of amino acids.[2] Another important molecule generated in the Miller's experiments is NH_2COH, formamide. The major pathway of the formation of this molecule involves the reaction of water with hydrogen cyanide.[3,4] Formamide has been identified as one of the most common carbon-containing molecules in the Universe; it has been detected in comets, satellites, and the interstellar medium.[5−7] Both hydrogen cyanide and formamide are promising raw materials in the abiotic synthesis of amino acids and nucleobases. By mixing hydrogen cyanide and ammonia in an aqueous solution Juan Oró found that in addition to amino acids, adenine, one of the bases of RNA and DNA, was generated from the mixture.[8,9] Formamide has also been suggested as a potential abiotic source of nucleobases and their analogues.[10−13] Purine has been generated in high yield simply by heating formamide.[14,15] All five nucleobases and their analogues have been synthesized from formamide under various conditions and catalysts.[4,16−23]

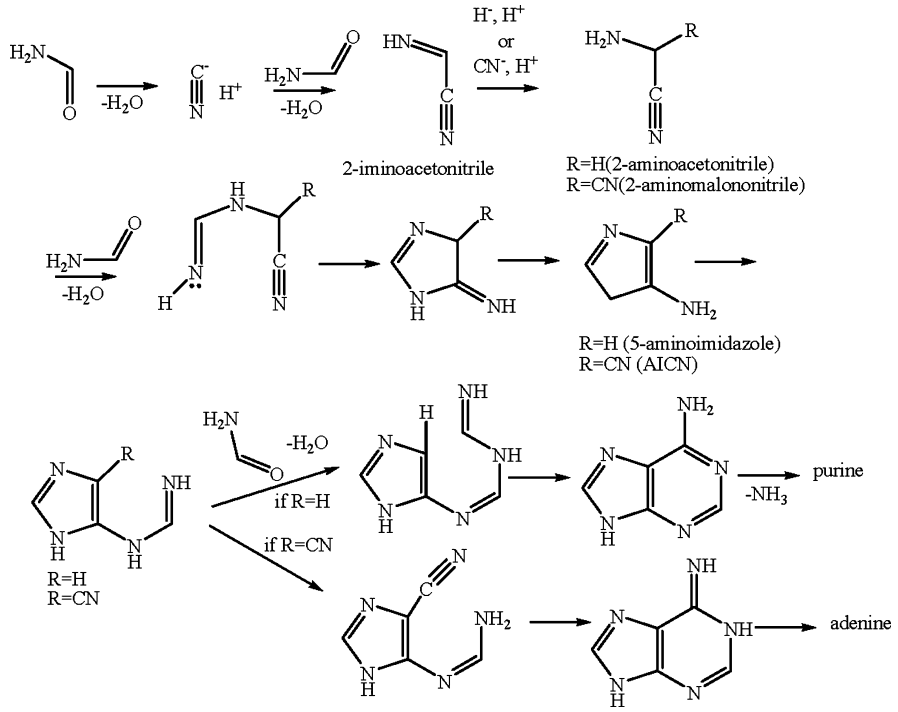
Different mechanistic pathways from formamide and hydrogen cyanide to purine and adenine have been proposed based on the experimental data.[4,19,23−30] One of the suggested mechanistic routes progresses through a pyrimidine intermediate to purine.[4,26−28] Interestingly, pathways from formamide to adenine have been theorized to progress though pyrimidine.[4,19] Another reaction route to purine suggests that a relatively stable complex diaminomaleonitrile (DAMN)[23] intermediate is an important species in this process. An analogous reaction route from HCN to adenine through DAMN and AICN (4-aminoimidazole-5-carboxamidine) intermediates has been outlined and analyzed based on the experimental investigations.[8,25,31−37] Using the outcome of recent experimental investigations, a unique route for the abiotic syntheses of both purine and adenine from formamide has been proposed.[24] In this pathway (Scheme 1), purine (or adenine) is formed through a five-membered ring intermediate, 5-aminoimidazole (for purine) or AICN (for adenine) .[24]

Theoretical studies of various reaction mechanisms are able to provide details for not only products and reactants, but also intermediates and transition states along the reaction pathways. Usually these transition state species cannot be observed directly in experiments. Information revealed in quantum chemistry computational studies allows screening and assorting the most feasible route among various possible reaction pathways. However, the identification of a kinetically viable, thermodynamically realistic, step-by-step mechanism that can account for the formation of nucleobases is a considerable

Received: September 17, 2013
Revised: October 21, 2013
Published: October 22, 2013

   dx.doi.org/10.1021/jp409296k | J. Phys. Chem. B 2013, 117, 14039−14045

Scheme 1. Literature Proposed Pasthways Focusing on Progress from Formamide through a Five-Member Ring Intermediate Aminoimidazole to Purine and Adenine[24]



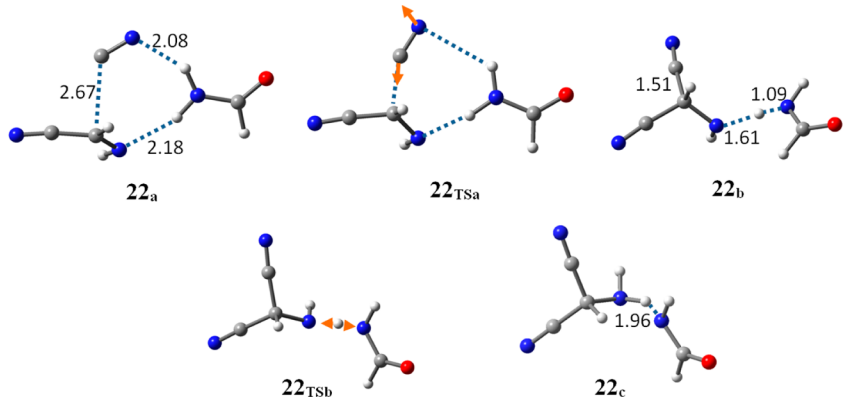


Figure 1. Optimized structures of the complexes in the formamide-assistant addition of cyanide nucleophile to 2-iminoacetonitrile and the transition state (at the B3LYP/6-311G(d,p) level of theory). Atomic distances are given in angstroms. Orange arrows represent the vibrational mode corresponding to the single imaginary frequency in the transition states. Color representations are red for oxygen, blue for nitrogen, gray for carbon, and white for hydrogen.

challenge for quantum chemistry computational researchers. Starting from AICN as an initial species of the reaction, the Schleyer group carefully examined the mechanism for the formation of adenine in abiotic conditions.[38] Using a formamide dimer and HCN as reactants, Sponer et al. suggested a possible mechanism for generating purine through pyrimidine.[30] Recently, following the route proposed by Hudson et al.,[24] we have detailed the step-by-step mechanism for the formation of purine based solely on formamide, as one single raw material.[39,40] The details revealed in these mechanisms have shed new light on understanding the chemical evolution of biomolecules at a primitive stage.

Herein, we report a study of a self-catalyzed mechanism for the formation of adenine from the ubiquitous formamide. Consistent with our previous investigations, this mechanism is focused on a chemical framework in which nucleobases are formed from simple C1 reactants. Together with the previous formamide self-catalyzed mechanism of purine synthesis, the present mechanism elucidates the ratio of purine:adenine yields observed in the experiments conducted by Hudson et al.[24]

COMPUTATIONAL METHODS

Consistent with the previous study of the mechanistic reaction pathways in the synthesis of purine,[39,40] the density functional theory (DFT) with functional B3LYP[41−43] was applied in this investigation. The basis set used was the standard polarization functions augmented valence triple-ζ basis set 6-311G(d,p).[44] The studied models have been fully optimized by analytical gradient techniques. The force constants were determined analytically in the analysis of harmonic vibrational frequencies for all of the complexes. An intrinsic reaction coordinate (IRC) analysis was carried out to ensure that each transition state

dx.doi.org/10.1021/jp409296k | J. Phys. Chem. B 2013, 117, 14039−14045

corresponds with the correct minima. The polarizable continuum model (PCM) self-consistent reaction field of Tomasi and co-workers[45] was employed to evaluate the solvent effects (with a dielectric constant of 108.9 to mimic the solvent formamide). The Gaussian-09 package of programs[46] was used for all computations.

RESULTS

The first step in the reaction route from formamide to adenine is the same as revealed in the route to purine, that is, generation of 2-iminoactonitrile through formiminylation of HCN, as shown in Scheme 1. The highest activation energy barrier of this reaction is 25.7 kcal/mol based on the formamide self-catalytic mechanism.[40] Therefore, the mechanism below begins with 2-iminoacetonitrile. Inclusion of solvent effects (employing the PCM model) in general reduces the reaction energy barrier by ca. 3 kcal/mol in formamide solutions. Therefore, discussions are focused on the results in gas phase calculations, except for cases when solvent effects cannot be ignored, as outlined below.

1. Addition of CN^- to 2-Iminoacetonitrile. Competing with Leuckart reduction, addition of cyanide nucleophile to 2-iminoacetonitrile yields 2-aminomalononitrile (**22**$_b$, Figure 1) on the route to adenine. For the anionic CN^- ion, the energy barrier for the addition reaction is 8.3 kcal/mol. Presence of catalytic formamide further reduces this energy barrier to 4.1 kcal/mol (Figure 2). It should be noted that the resulting

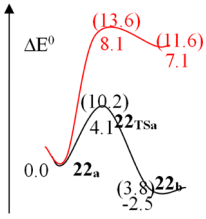


Figure 2. Schematic energy profile along the reaction pathway of the formamide-assisted addition of cyanide nucleophile to 2-iminoacetonitrile. ΔE^0 is the zero-point energy corrected relative energy (in kcal/mol). Black is for the formamide-catalyzed reaction. Red is for the noncatalyzed route. Numbers in parentheses are PCM corrected results.

species appears in anionic form (the 2-amino group is deprotonated) in the gas phase in the process carried out without the catalytic formamide. In the presence of formamide, the proton of the NH_2 group of formamide shifts toward this deprotonated 2-amino-malononitrile. Solvent effects in this reaction are important. The PCM corrected energy barrier is 10.2 kcal/mol for the formamide catalyzed process (13.6 kcal/mol under the noncatalyzed condition). On the other hand, addition of an HCN molecule in neutral form is difficult. The activation energy barrier corresponding to addition reaction of neutral HCN is found to be as high as 40.5 kcal/mol, even with the assistance of catalytic formamide (33.8 kcal/mol with PCM corrections), and ca. 100 kcal/mol without catalysts. Thus, we conclude that additional anionic CN^- ions are necessary for the formation of adenine in the present route. It is important to note that in the experiments conducted by Hudson et al. adenine is observed in sufficient quantities only when KCN was added to the reaction systems.[24]

2. Formiminylation of 2-Aminomalononitrile. The pattern of the involvement of formamide in catalyzing the process is unique; the catalytic formamide molecule bridges a proton transfer from the attacking nucleophile (2-amino group of 2-aminomalononitrile in here) to the electrophile being attacked (C═O group of reactant formamide in here). Thus, formamide increases the reactivity of both the nucleophile and the electrophile by moving electron density away from the later. In the formylation step, under the influence of a catalytic formamide molecule (in imidic acid form) the partly deprotonated NH_2 group of 2-aminomalononitrile attacks the carbon of the reactant formamide (in amino form), forming a formamide-2-aminomalononitrile adduct through an N—C single bond (**23**$_{TSa}$, Figure 3). After geometric relaxation,

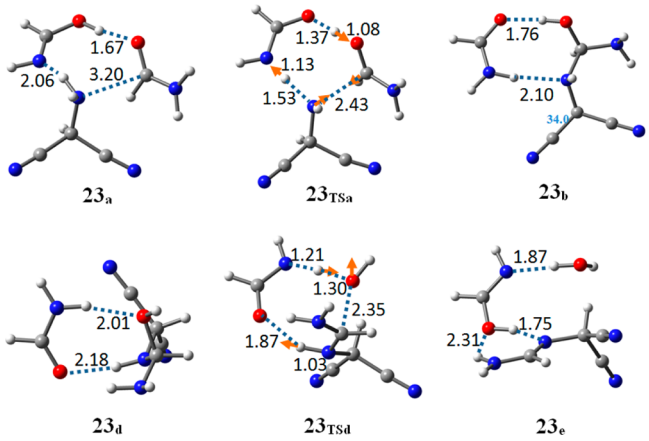


Figure 3. Optimized structures of the complexes in the formamide-catalyzed formiminylation of 2-aminomalononitrile and the transition states (at the B3LYP/6-311G(d,p) level of theory). Atomic distances are given in angstroms. Orange arrows represent the vibrational mode corresponding to the single imaginary frequency in the transition states. Color representations are red for oxygen, blue for nitrogen, gray for carbon, and white for hydrogen.

subsequent dehydration (with the assistance of catalytic formamide (in amino form) of **23**$_d$ results in formation of *N*-(dicyanomethyl)formamidine (**23**$_e$) and releases the imidic acid form of formamide as catalyst. The activation energy of the formylation step is 19.5 kcal/mol and that of dehydration is 13.2 kcal/mol in the formamide self-catalyzed process (Figure 4). It is found that formylation is kinetically inaccessible without the assistantance of a catalyst. The corresponding energy barrier is 47.9 kcal/mol in the gas phase.

3. Five-Membered Ring Closure. The catalytic formamide transfers its proton to the nitrogen of the one CN group of *N*-(dicyanomethyl)formamidine concertedly with the

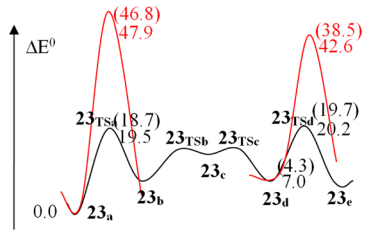


Figure 4. Schematic energy profile along the reaction process of the formiminylation of 2-aminomalononitrile. ΔE^0 is the zero-point energy corrected relative energy (in kcal/mol). Black is for the formamide-catalyzed reaction. Red is for the noncatalyzed route. Values in parentheses are PCM corrected results.

dx.doi.org/10.1021/jp409296k | J. Phys. Chem. B 2013, 117, 14039−14045

formation of the new C—N bond between the partly deprotonated amine nitrogen at the formamidine end and the electron-deficient CN carbon. This process leads to ring closure, resulting in the five-membered ring intermediate **24**$_b$ 4,5-dihydro-5-imino-1*H*-dimiazole-4-carbonitrile. H-transfer from C4 to N of 5-imino follows with assistance of the catalytic formamide, producing 5-aminoimidazole-4-cyanide AICN and releasing the imidic acid form of formamide as a catalyst (see Figure 5). The energy barrier for the ring closure

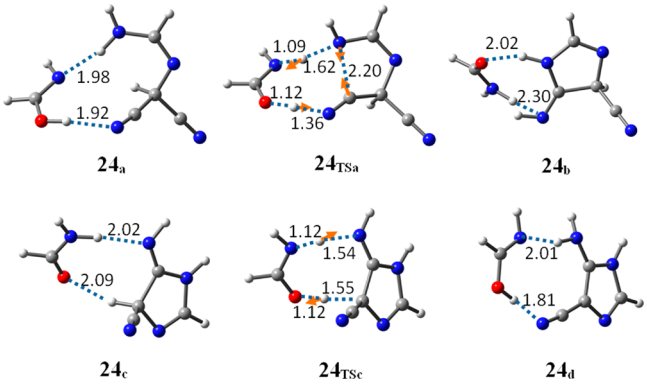


Figure 5. Optimized structures of the complexes in the formamide-catalyzed five-membered ring closure and the transition states (at the B3LYP/6-311G(d,p) level of theory). Atomic distances are given in angstroms. Orange arrows represent the vibrational mode corresponding to the single imaginary frequency in the transition states. Color representations are red for oxygen, blue for nitrogen, gray for carbon, and white for hydrogen.

step is 25.8 kcal/mol and that for the H-transfer is 15.3 kcal/mol in the catalyzed process (Figure 6). The energy barriers of

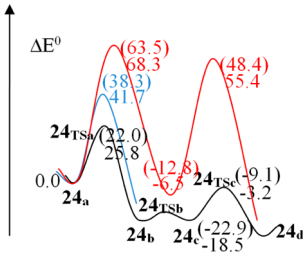


Figure 6. Schematic energy profile along the reaction process of the five-membered ring closure. ΔE^0 is the zero-point energy corrected relative energy (in kcal/mol). Black is for the formamide-catalyzed reaction. Blue is for the water-catalyzed process. Red is for the noncatalyzed route. Values in parentheses are PCM corrected results.

these two steps in the noncatalytic process are 68.3 and 61.9 kcal/mol, respectively. In the case of a water molecule catalyzing the ring closure, the activation energy is calculated to be 41.7 kcal/mol.

4. Formiminylation of AICN. A new C—N bond forms between the amine nitrogen of AICN and the electron-deficient carbon of a formamide during the formylation of AICN, generating a formamide-AICN adduct (**25**$_b$). This reaction is catalyzed by increasing the nucleophilicity of the attacking nitrogen (through donation of a proton from the amino group) and enhancing the electron deficiency of carbon of the formamide (through accepting a proton to its neighboring oxygen, as shown in **25**$_{TSa}$, Figure 7). The activation energy barrier for this reaction is 21.5 kcal/mol when an imidic acid

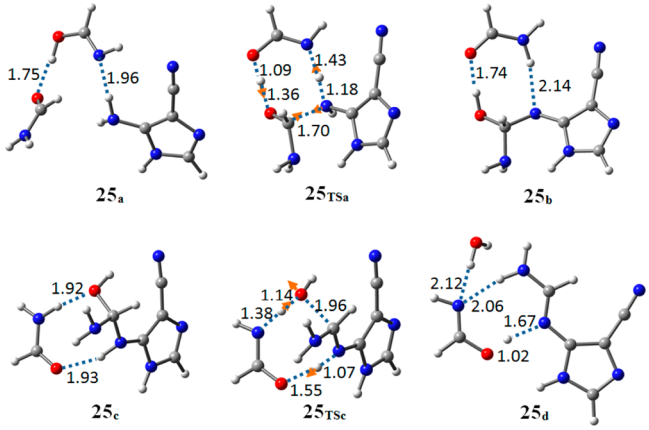


Figure 7. Optimized structures of the complexes in the formamide-catalyzed formiminylation of AICN and the corresponding transition states (at the B3LYP/6-311G(d,p) level of theory). Atomic distances are given in angstroms. Orange arrows represent the vibrational mode corresponding to the single imaginary frequency in the transition states. Color representations are red for oxygen, blue for nitrogen, gray for carbon, and white for hydrogen.

formamide molecule serves as catalyst, and 45.5 kcal/mol without a catalyst. Water molecules can also act as a catalyst, but they are less effective. The corresponding energy barrier is 33.8 kcal/mol when one water molecule catalyzes the reaction. Dehydration of the formamide-AICN adduct requires an activation energy of 18.1 kcal/mol with the assistance of catalytic formamide (in the keto form) and 33.7 kcal/mol without a catalyst (Figure 8). As opposed to the other steps, the

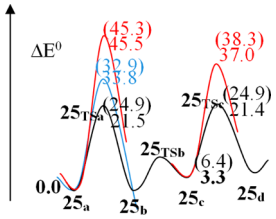


Figure 8. Schematic energy profile along the reaction process of the formiminylation of AICN. ΔE^0 is the zero-point energy corrected relative energy (in kcal/mol). Black is for the formamide-catalyzed reaction. Blue is for the water-catalyzed process. Red is for the noncatalyzed route. Values in parentheses are PCM corrected results.

energy barrier of the formylation of AICN is higher in formamide solutions than in gas phase (24.9 vs 21.5 kcal/mol). Relatively large dipole moment variation between **25**$_a$ (8 Deby) and **25**$_{TSa}$ (5 Deby) likely accounts for this increase in energy barrier.

5. Six-Membered Ring Closure. Two major steps are included in this reaction, ring closure and intramolecular proton transfer. One formamide molecule in its imidic acid tautomeric form catalyzes the ring closure step by bridging the proton-transfer from the amino group to the cyano group of the dehydrated formamide-AICN adduct. This proton-transfer leads to an intermediate (**26**$_b$) that facilitates creation of a new C—N bond between the deprotonated amine nitrogen and the electron-deficient CN carbon (due to the protonation of the nitrogen of cyano group). Subsequent N1—N6 proton-transfer bridged by a formamide molecule completes the formation of adenine. Concurrently, formamide is restored to its imidic acid

dx.doi.org/10.1021/jp409296k | J. Phys. Chem. B 2013, 117, 14039−14045

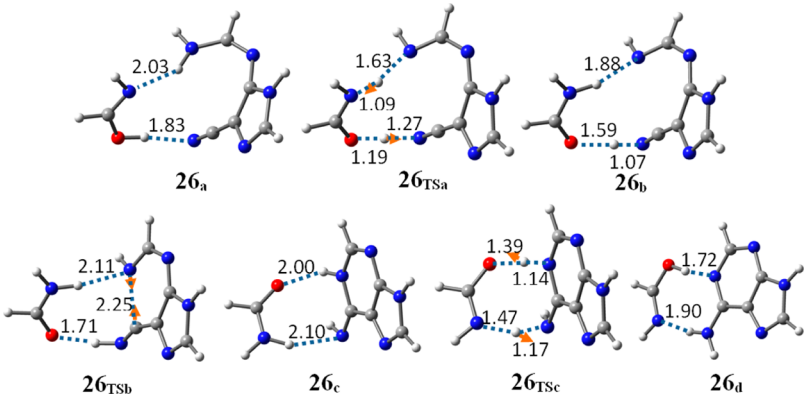


Figure 9. Optimized structures of the complexes in the formamide-catalyzed six-membered ring closure and the corresponding transition states (at the B3LYP/6-311G(d,p) level of theory). Atomic distances are given in angstroms. Orange arrows represent the vibrational mode corresponding to the single imaginary frequency in the transition states. Color representations are red for oxygen, blue for nitrogen, gray for carbon, and white for hydrogen.

tautomeric form (see $\mathbf{26_d}$ in Figure 9). Figure 10 displays the energy profile along the reaction process of the six-membered

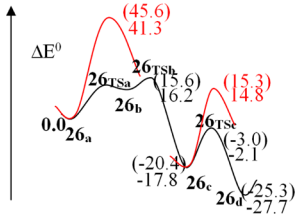


Figure 10. Schematic energy profile along the reaction process of the six-membered ring closure. ΔE^0 is the zero-point energy corrected relative energy (in kcal/mol). Black is for the formamide-catalyzed reaction. Red is for the noncatalyzed route. Values in parentheses are PCM corrected results.

ring closure and the formation of adenine. The energy barrier for the ring closure is computed to be 16.2 kcal/mol and that for the intramolecular proton-transfer is 15.7 kcal/mol in the formamide-catalyzed mechanism. In the noncatalytic mechanism, ring closure needs an activation energy of around 43.1 kcal/mol to proceed and the intramolecular proton-transfer must overcome an energy barrier of 32.6 kcal/mol.

Similar to the route from formamide to purine,[40] the five-membered ring-closing is the rate-determining step in this formamide self-catalyzed mechanistic pathway (without PCM corrections). With PCM corrections, the rate-control step is the formylation of AICN in formamide solutions. However, the energy barrier of this rate-control step (24.9 kcal/mol) is very close to the energy barrier of the rate-control step in formamide solutions along the route from formamide to purine (25.2 kcal/mol for formamide self-catalyzed five-membered ring closure).[40]

Although HCN can be generated via formamide self-catalyzed decomposition, the reaction of HCN addition to 2-iminoacetonitrile is prohibited due to its high activation energy requirement, 40.5 kcal/mol, even with the assistance of catalytic formamide. Thus this mechanistic route depends on the addition of raw materials that contain anionic cyanide ion.

Comparison with Other Mechanism Pathways. In abiotic routes leading to the formation of either purine or adenine, the number of carbon atoms in the intermediates is increased by one at each step. HCN or CN^- was used to expand the carbon skeleton of compounds in the pathway from pyrimidine to purine, as suggested by Sponer's study.[30] HCN was suggested by Schleyer's group to append the last carbon atom into AICN in the mechanistic route to adenine.[38] In the formamide-based mechanisms, formamide serves as a source of carbon atoms (with the exception of the addition of CN anion to 2-iminoacetonitrile along the route to adenine). The activation energy barrier of HCN addition to the intermediates is reported to be 61.9 kcal/mol (in reaction of the formamide dimer with HCN)[30] and 60.4 kcal/mol (in addition of a HCN to AICN)[38] in noncatalyzed processes in the gas phase. Under the same conditions, the typical activation energy barrier of formylation is around 50 kcal/mol and that of the subsequent dehydration is below 53 kcal/mol (refs 39, 40, and present study). In the presence of catalytic water, the energy barrier for addition of HCN to formamide-dimer is 43.8 kcal/mol and that for addition of HCN to AICN is 38.0 kcal/mol.[30,38] Comparably, the energy barrier amounts to 33.8 kcal/mol for the formylation of AICN in the water-catalyzed process (in gas phase). Thus, formamide is more active than HCN for the addition of carbon under abiotic conditions.

The rate-control step along the route through a pyrimidine intermediate can be identified as the six-membered ring closure catalyzed with one water molecule. The corresponding activation energy barrier is 41.5 kcal/mol in aqueous solutions (43.4 kcal/mol in formamide solutions).[30] Following the mechanistic route through AICN to adenine reported by Schleyer's group, the reaction rate is determined by the addition of HCN to AICN, which requires an activation energy of 33.9 kcal/mol in aqueous solutions.[38] In the formamide self-catalyzed mechanism, the rate-control step is recognized as the five-membered ring closure. The corresponding activation energy barrier is 27.2 kcal/mol for the formation of purine,[40] which is similar to the 25.8 kcal/mol for the formation of adenine (present study). Inclusion of solvent effects of the polarizable medium with the PCM model further reduces these energy barriers. Therefore, the formamide self-catalyzed mechanisms are kinetically favored as compared with other proposed mechanisms.

Comparison with Experiments. Three important conclusions can be derived from the experiment conducted by Hudson et al. (1) No significant yields of adenine will be generated by heating formamide without adding KCN. (2) The yield of adenine is roughly proportional to the amount of KCN

dx.doi.org/10.1021/jp409296k | J. Phys. Chem. B 2013, 117, 14039−14045

added. (3) The yield of purine is approximately independent of added KCN (in the range of 2−14 mg, see Table 2 in ref 24) or ammonium formate (in the range of 5−15 mol/%, see Table 1 in ref 24). Along with our previously reported mechanism for the route from formamide to purine, the formamide self-catalytic mechanism detailed here enables a better understanding of these experimental results.

It is important to note that the addition of 2-iminoacetonitrile to HCN (formation of 2-aminomalononitrile) is difficult even with catalytic formamide. The activation energy barrier corresponding to the addition reaction is 40.5 kcal/mol in the formamide catalyzed reaction (33.8 kcal/mol with PCM correction). Thus, although HCN can be effectively produced through the decomposition of formamide through a self-catalytic process, it will not result in the intermediate 2-aminomalononitrile that leads the formation of adenine. Meanwhile, formate can effectively reduce 2-iminoacetonitrile, resulting in 2-aminoacetonitrile (with formamide as a catalyst the corresponding activation energy barrier is 14.0 kcal/mol in the gas phase and 13.8 kcal/mol in formamide solutions, see ref 40 and present study). Consistently, previous experimental studies revealed that adenine has not been detected effectively without the presence of CN^- anions.[14,15,24]

It is important to note that the activation energy barrier of generating adenine (24.9 kcal/mol in formamide solutions) is very close to that of producing purine (25.2 kcal/mol in formamide solutions[40]). Therefore, the yields of adenine and purine are determined by the competition between the formation of 2-aminomalononitrile (for adenine) and the formation of 2-aminoacetonitrile (for purine). With anionic CN^-, the energy barrier for the addition reaction of 2-iminoacetonitrile is 10.2 kcal/mol in the catalytic reaction in formamide solutions (13.8 kcal/mol in noncatalytic reaction). This energy barrier is still slightly lower than the activation energy required for Leuckart reduction (13.8 kcal/mol in formamide solutions). Therefore, increasing concentration of CN^- ion does lead to the increase of the yield of 2-aminomalononitrile and thus increases the yield of adenine in the products. Moreover, the yield of adenine in the formamide self-catalyzed route is expected to be roughly proportional to the quantity of CN^- ion in the reaction system. This is consistent with the results shown in the formamide-heating experiment by controlled addition of KCN.[24]

A previous study demonstrated that formate can be effectively generated from reactions between formamide and water in self-catalytic mechanism.[40] Therefore, adding ammonium formate should not be expected to change the rate of formation of 2-aminoacetonitrile and the yield of purine. Moreover, because the rate of Leuckart reduction is compatible to that of addition of CN^- in formamide solutions, as long as there is sufficient amount of reactant (2-iminoacetonitrile) increases in the concentration of CN^- ion will not substantially affect the yield of 2-aminoacetonitrile, and therefore, not significantly alter the yield of purine.

CONCLUDING REMARKS

The formamide self-catalyzed mechanism presented in this report represents the simplest and most complete reaction pathway for the formation of adenine in formamide. Except for anionic cyanide ion, all the reactants and catalysts needed in the reaction are available from a single compound, formamide. The energy barrier required for the rate-control step of the whole process is reasonable for the reaction conditions. Moreover, combined with our previous reported mechanism for the route from formamide to purine, this mechanism elucidates the KCN dependency of adenine yield observed in abiotic synthesis. Formamide is found to be more active as compared to HCN in the synthesis of purine and adenine. Compared with other mechanisms proposed, the formamide based self-catalytic mechanism is more kinetically viable, more complete, and requires fewer raw materials. In terms of these validations, this proposed mechanism best explains the abiotic synthesis of nucleobases from formamide and cyanide.

AUTHOR INFORMATION

Corresponding Authors

*E-mail: jiande@icnanotox.org (J.G.).
*E-mail: jerzy@icnanotox.org (J.L.).

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was jointly supported by NSF and the NASA Astrobiology Program under the NSF Center for Chemical Evolution, CHE1004570. We would like to thank the Mississippi Center for Supercomputing Research for a generous allotment of computer time.

REFERENCES

(1) Miller, S. L. A Production of Amino Acids Under Possible Primitive Earth Conditions. *Science* **1953**, *117*, 528−529.
(2) Miller, S. L.; Harold, C. U. Organic Coumpounds Synthesis on the Primitive Earth. *Science* **1959**, *130*, 245−251.
(3) Saladino, R.; Crestini, C.; Costanzo, G.; Di Mauro, E. On the prebiotic synthesis of nucleobases, nucleotides, oligonucleotides, pre-RNA and pre-DNA molecules. In *Topics in Current Chemistry, "Prebiotic Chemistry*; Walde, P., Ed.; Springer Verlag: New York, 2005; Vol. *259*, pp 29−68.
(4) Saladino, R.; Crestini, C.; Ciciriello, F.; Costanzo, G.; Di Mauro, E. Formamide Chemistry and the Origin of Informational Polymers. *Chem. Biodiversity* **2007**, *4*, 694−720.
(5) Despois, D.; Crovisier, J.; Bockelee-Morvan, D.; Biver, N. *Comets and prebiotic chemistry: the volatile component*. From European Space Agency, [special publication] SP, SP-518. Proceedings of the Second European Workshop on Exo-Astrobiology; September 16−19, 2002, Graz, Austria; pp 123−127.
(6) Parnell, J.; Baron, M.; Lindgren, P. Potential for irradiation of methane to form complex organic molecules in impact craters: implications for Mars, Titan and Europa. *J. Geochem. Explor.* **2006**, *89* (1−3), 322−325.
(7) Flygare, W. H.; Benson, R. C.; Tigelaar, H. L.; Rubin, R. H.; Swenson, G. W.; Gordon, M. A Chemical and rotational state stability of interstellar formamide. *Mol. Galactic Environ., Proc. Symp.* **1973**, 173−179.
(8) Oró, J.; Kimball, A. P. Synthesis of purines under possible primitive earth conditions. I. Adenine from hydrogen cyanide. *Arch. Biochem. Biophys.* **1961**, *94*, 217−227.
(9) Oró, J.; Kamat, S. S. Amino-acid Synthesis from Hydrogen Cyanide under Possible Primitive Earth Conditions. *Nature* **1961**, *190*, 442−443.
(10) Solomon, P. M. Interstellar molecules. *Phys. Today* **1973**, *26*, 32−40.
(11) Crovisier, J. The Molecular Complexity of Comets in Astrobiology: Future Perspectives. In *Astrophysics and Space Science Library*; Ehrenfreund, P. et al., Eds.; Kluwer Academic Publishers: Dordrecht, 2004; Vol. 305, pp 179−203.
(12) Bockelee-Morvan, D.; Lis, D. C.; Wink, J. E.; Despois, D.; Crovisier, J.; Bachiller, R.; Benford, D. J.; Biver, N.; Colom, P.; Davies, J. K.; et al. New molecules found in comet C/1995 O1 (Hale-Bopp).

Investigating the link between cometary and interstellar material. *Astron. Astrophys.* **2000**, *353*, 1101−1114.
(13) Schutte, W. A.; Boogert, A. C. A.; Tielens, A. G. G. M.; Whittet, D. C. B.; Gerakines, P. A.; Chiar, J. E.; Ehrenfreund, P.; Greenberg, J. M.; van Dishoeck, E. F.; de Graauw, T. Weak ice absorption features at 7.24 and 7.41 MU M in the spectrum of the obscured young stellar object W 33A. *Astron. Astrophys.* **1999**, *343*, 966−976.
(14) Bredereck, H.; Effenberger, F.; Rainer, G.; Schosser, H. P. Säureamid-Reaktionen, XXXI. Eine einfache synthese des Purins. *Justus Liebigs Ann. Chem.* **1962**, *659*, 133−138.
(15) Yamada, H.; Okamoto, T. A One-step Synthesis of Purine Ring from Formamide. *Chem. Pharm. Bull.* **1972**, *20*, 623−624.
(16) Saladino, R.; Botta, G.; Pino, S.; Costanzo, G.; Di Mauro, E. From the one-carbon amide formamide to RNA all the steps are prebiotically possible. *Biochimie* **2012**, *94*, 1451−1456.
(17) Saladino, R.; Crestini, C.; Pino, S.; Costanzo, G.; Di Mauro, E. Formamide and the origin of life. *Phys. Life Rev.* **2012**, *9*, 84−104.
(18) Saladino, R.; Crestini, C.; Costanzo, G.; Negri, R.; Di Mauro, E. A Possible Prebiotic Synthese of Purine, Adenine, Cytosine, and 4(3H)-Pyrimidinone from Formamide: Implications for the Origin of Life. *Bioorg. Med. Chem.* **2001**, *9*, 1249−1253.
(19) Saladino, R.; Crestini, C.; Costanzo, G.; Di Mauro, E. Advances in the Prebiotic Synthesis of Nucleic Acids Bases: Implications for the Origin of Life. *Curr. Org. Chem.* **2004**, *8*, 1425−1443.
(20) Saladino, R.; Ciamecchini, U.; Crestini, C.; Costanzo, G.; Negri, R.; Di Mauro, E. One-Pot TiO2-Catalyzed Synthesis of Nucleic Bases and Acyclonucleosides from Formamide: Implications for the Origin of Life. *ChemBioChem* **2003**, *4*, 514−521.
(21) Saladino, R.; Crestini, C.; Ciambecchini, U.; Ciciriello, F.; Costanzo, G.; Di Mauro, E. Synthesis and Degradation of Nucleobases and Nucleic Acids by Formamide in the Presence of Montmorillonites. *ChemBioChem* **2004**, *5*, 1558−1566.
(22) Senanayake, S. D.; Idriss, H. Photocatalysis and the origin of life: Synthesis of nucleoside bases from formamide on TiO_2(001) single surfaces. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 1194−1198.
(23) Barks, H. L.; Buckley, R.; Grieves, G. A.; Di Mauro, E.; Hud, N. V.; Orlando, T. M. Guanine, Adenine, and Hypoxanthine Production in UV-Irradiated Formamide Solutions: Relaxation of the Requirements for Prebiotic Purine Nucleobase Formation. *ChemBioChem* **2010**, *11*, 1240−1243.
(24) Hudson, J. S.; Eberle, J. F.; Vachhani, R. H.; Rogers, L. C.; Wade, J. H.; Krishnamurthy, R.; Springsteen, G. A Unified Mechanism for Abiotic Adenine and Purine Synthesis in Formamide. *Angew. Chem., Int. Ed.* **2012**, *51*, 5134−5137.
(25) Yamada, H.; Hirobe, M.; Higashiyama, K.; Takahashi, H.; Suzuki, K. T. Detection of I3C-'5N Coupled Units in Adenine Derived from Doubly Labeled Hydrogen Cyanide or Formamide. *J. Am. Chem. Soc.* **1978**, *100*, 4617−4618.
(26) Ochiai, M.; Marumoto, R.; Kobayashi, S.; Shimazu, H.; Morita, K. A facile one-step synthesis of adenine. *Tetrahedron* **1968**, *24*, 5731−5737.
(27) Yamada, H.; Hirobe, M.; Higashiyama, K.; Takahashi, H.; Suzuki, K. T. Reaction mechanism for purine ring formation as studied by ^{13}C-^{15}N coupling. *Tetrahedron Lett.* **1978**, *19*, 4039−4042.
(28) Yamada, H.; Hirobe, M.; Okamoto, T. Formamide Reaction. III. Studies on the Reaction Mechanism of Purine Ring Formation and the Reaction of Formamide with Hydrogen Cyanide. *Yakugaku Zasshi* **1980**, *100*, 489−492.
(29) Shuman, R. F.; Shearin, W. E.; Tull, R. J. Chemistry of HCN. 1. Formation and Reactions of *N*-(AL minomethylidene)-diaminomaleonitrile,a n HCN Pentamer and Precursor to Adenine. *J. Org. Chem.* **1979**, *44*, 4532−4536.
(30) Sponer, J. E.; Mladek, A.; Sponer, J.; Fuentes-Cabrera, M. Formamide-Based Prebiotic Synthesis of Nucleobases: A Kinetically Accessible Reaction Route. *J. Phys. Chem. A* **2012**, *116*, 720−726.
(31) Oró, J. Synthesis of adenine from ammonium cyanide. *Biochem. Biophys. Res. Commun.* **1960**, *2*, 407−412.
(32) Oró, J. Mechanism of synthesis of adenine from hydrogen cyanide under possible primitive Earth conditions. *Nature* **1961**, *191*, 1193−1194.
(33) Sanchez, R. A.; Ferris, J. P.; Orgel, L. E. Studies in Prebiodic Synthesis: II. Synthesis of purine precursors and amino acids from aqueous hydrogen cyanide. *J. Mol. Biol.* **1967**, *30*, 223−253.
(34) Ferris, J. P.; Orgel, L. E. An Unusual Photochemical Rearrangement in the Synthesis of Adenine from Hydrogen Cyanide. *J. Am. Chem. Soc.* **1966**, *88*, 1074.
(35) Sanchez, R. A.; Ferris, J. P.; Orgel, L. E. Studies in prebiotic synthesis: IV. Conversion of 4-aminoimidazole-5-carbonitrile derivatives to purines. *J. Mol. Biol.* **1968**, *38*, 121−128.
(36) Ferris, J. P.; Orgel, L. E. Aminomalononitrile and 4-Amino-5-cyanoimidazole in Hydrogen Cyanide Polymerization and Adenine Synthesis. *J. Am. Chem. Soc.* **1965**, *87*, 4976−4977.
(37) Ferris, J. P.; Orgel, L. E. Studies in Previotic Synthesis. I. Aminomalononitrile and 4-Amino-5-cyanoimidazole. *J. Am. Chem. Soc.* **1966**, *88*, 3829−3831.
(38) Roy, D.; Najafian, K.; von R. Schleyer, P. Chemical evolution: The mechanism of the formation of adenine under prebiotic conditions. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 17272−17277.
(39) Wang, J.; Gu, J.; Nguyen, M. T.; Springsteen, G.; Leszczynski, J. From Formamide to Purine: An Energetically Viable Mechanistic Reaction Pathway. *J. Phys. Chem. B* **2013**, *117*, 2314−2320.
(40) Wang, J.; Gu, J.; Nguyen, M. T.; Springsteen, G.; Leszczynski, J. From Formamide to Purine: A Self-Catalyzed Reaction Pathway Provides a Feasible Mechanism for the Entire Process. *J. Phys. Chem. B* **2013**, *117*, 9333−9342.
(41) Becke, A. D. Density-functional thermochemistry. III. The role of exact exchange. *J. Chem. Phys.* **1993**, *98*, 5648−5652.
(42) Lee, C.; Yang, W.; Parr, R. G. Development of the Colle-Salvetti correlation-energy formula into a functional of the electron density. *Phys. Rev. B* **1988**, *37*, 785−789.
(43) Miehlich, B.; Savin, A.; Stoll, H.; Preuss, H. Results obtained with the correlation energy density functionals of becke and Lee, Yang and Parr. *Chem. Phys. Lett.* **1989**, *157*, 200−206.
(44) Hehre, W. J.; Radom, L.; Schleyer, P. R.; Pople, J. A. *Ab Initio Molecular Orbital Theory*; Wiley: New York, 1986.
(45) Cossi, M.; Barone, V.; Cammi, R.; Tomasi, J. Ab initio study of solvated molecules: a new implementation of the polarizable continuum model. *Chem. Phys. Lett.* **1996**, *255*, 327−335.
(46) 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.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, H. P.; Izmaylov, A. F.; Bloino, J.; Zheng, G.; Sonnenberg, J. L.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Montgomery, J. A., Jr.; Peralta, P. E.; Ogliaro, F.; Bearpark, M.; Heyd, J. J.; Brothers, E.; Kudin, K. N.; Staroverov, V. N.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, N. J.; Klene, M.; Knox, J. E.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Zakrzewski, V. G.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Dapprich, S.; Daniels, A. D.; Farkas, Ö.; Ortiz, J. V.; Cioslowski, J.; Fox, D. J. *Gaussian 09*, revision A.1; Gaussian, Inc.: Wallingford, CT, 2009.