Theor Chem Account (2007) 118:973–978
DOI 10.1007/s00214-007-0380-9

REGULAR ARTICLE

Theoretical investigation of the decomposition mechanisms of *N*-(2-chloroethyl)-*N*-nitrosourea

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Received: 14 May 2007 / Accepted: 14 July 2007 / Published online: 2 August 2007

Abstract The initial reaction mechanisms of *N*-(2-chloroethyl)-*N*-nitrosourea (CENU) decomposition have been investigated at the MP2/6-311+G(d,p) level. The mechanistic processes considered were the hydrogen shifting from the nitrogen to the oxygen of the nitroso group, the oxygen of the nitroso and the carbonyl groups nucleophilic displacing the chlorine. The computational results showed that the energy barrier of retro-ene reaction was lower in the gas phase than that of substitution reactions. In the solvent, however, the energy values of each barrier in these three processes approach each other. It is concluded that the CENU decomposition in solvent can proceed via retro-ene reaction and intramolecular substitution reactions.

Keywords *N*-(2-chloroethyl)-*N*-nitrosourea (CENU) · Decomposition mechanism · MP2 method

1 Introduction

(2-Chloroethyl) nitrosoureas (CENUs), such as 1,3-bis (2-chloroethyl)-1-nitrosourea (BCNU) and 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU), are a class of anticancer agents which have a wide range of effects on various leukemias and solid tumors [1–6]. In addition, some other studies reported that many nitrosoureas can also induce tumors [7,8]. For their anticancer and carcinogenic bioactivities, nitrosoureas have long been a hot topic of pharmacological and toxicological investigations. Previous studies have pointed out that these anticancer and carcinogenic properties are due to alkylation of the DNA bases, and considerable evidence has been accumulated for a link between DNA modification and nitrosoureas decomposition [9–12]. In view of these points, understanding the decomposition mechanisms of nitrosoureas will lead to better explaining its biological activities and better development of anticancer nitrosoureas drugs.

For rich chemistry of nitrosourea, however, the decomposition processes are complex and have attracted great interests experimentally in the past decades [13–22]. Previously, experimental researchers observed the final products of nitrosoureas decomposition [13–18], and have proposed various mechanisms of nitrosoureas decomposition. Among them, the hydrogen transfer from the nitrogen to the oxygen of nitroso group [13] is generally believed to be the main route of CENUs decomposition. Also, the oxygen of the nitroso and the carbonyl groups nucleophilic displacement of chlorine were observed to play some roles in the decomposition of CENUs [14,15]. Later, a set of interesting experiments were carried out to determine the importance of these three pathways in different CENUs, and it was found that the structure of CENUs, especially the substituents on the N3-nitrogen, can influence the mode of decomposition [1,22]. However, since the intermediates have not been detected, the processes of nitrosourea decomposition remained unclear till now. Several quantum chemical calculations have been carried out to investigate the interaction of intermediates with DNA bases [19–21], however, no theoretical studies have been done to date on the initial reactions which are believed to be the rate-determining steps [14,23] in each mechanistic process.

In light of previous studies, this work explores these mechanistic processes by elucidating the rate-determining reactions of the nitrosourea decomposition from a theoretical perspective. *N*-(2-Chloroethyl)-*N*-nitrosourea, which is the simplest CENUs, was selected to investigate using quantum chemical methods.

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2 Theoretical methods

All the structures of tautomers, transition states and products in the gas phase were fully optimized using the MP2 [24] method, in conjunction with the 6-311+G(d,p) basis set. Vibrational frequencies were also calculated at the same level of theory to establish the nature of the stationary points and give Gibbs free energy at 298 K as well. The minimum-energy path (MEP) was obtained using intrinsic reaction coordinate (IRC) calculations [25] to confirm the connection of each transition state with the designated equilibrium species. All the calculations presented here were carried out with the GAUSSIAN-03 programs package [26].

The solvent effects of octanol and water on the reactions of CENU decomposition were also considered. A single point Polarizable Continuum Model (PCM) calculation [27] was performed using the polarizable dielectric model at the MP2/6-311+G(d,p) level based on each optimized geometry at the MP2/6-311+G(d,p) level, denoted as PCM- MP2/6-311+G(d,p). The dielectric constant of octanol was defined as 10.3 [28], and the default dielectric constant of water was taken from the GAUSSIAN-03 program.

3 Results and discussion

The calculated relative energies without zero-point energy correction of each stationary point involved in the *N*-(2-chloroethyl)-*N*-nitrosourea decomposition reactions both in the gas phase and in the solvent of octanol and water, as well as the lowest harmonic vibrational frequencies (LHVF) calculated at MP2/6-311+G(d,p) in the gas phase, are listed in Table 1. All the related mechanistic pathways for the CENU decomposition in this paper are depicted in Fig. 1. The optimized structures and main parameters of Z and E tautomers and related transition state of isomerization, as well as corresponding transition states and intermediates in reactions of CENU decomposition are shown in Figs. 2 and 3, respectively. Figures 4 and 5 present the IRC plots for intramolecular substitution reactions. Figures 6 and 7 illustrate the schematic profiles of the potential energy surfaces of the CENU decomposition in the gas phase and in solvent, respectively.

Table 1 The relative energies without zero point energy correction both in the gas phase (RE) and in the solvent of octanol (REo) and water (RE_w) (in kcal/mol) as well as the lowest harmonic vibrational frequencies (LHVF) (in cm^{-1}) of each equilibrium specie in CENU decomposition calculated at the MP2/6-311+G(d,p) level

Species	RE	RG[a]	REo	REw	LHVF
Z	0.00	0.00	0.00	0.00	58.4
TSr	18.37	17.70	17.90	17.90	183.7*i*
E	−3.90	−3.97	−4.22	−4.24	46.8
TS1	23.07	20.82	24.72	25.02	318.6*i*
IM1	9.63	3.83	9.39	9.50	21.4
TS2	46.44	46.01	20.29	16.05	295.4*i*
IM2	46.33	45.96	16.66	12.02	30.9
TS3	48.67	47.93	20.31	16.11	153.6*i*
IM3	48.66	47.63	19.06	14.73	48.3

[a] For comparison, relative Gibbs free energies in the gas phase (RG) calculated at the MP2/6-311+G(d,p) level are also presented

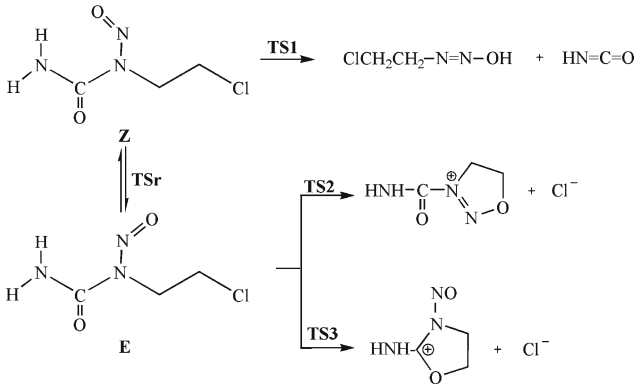

Fig. 1 Three mechanistic pathways for CENU decomposition

Considering the importance of entropy in these fragmental reactions, the relative Gibbs free energies (RG) were also shown in Table 1. Obviously, the values of relative Gibbs free energies are in accordance with that of relative energies (RE). Therefore, all energies discussed in the following parts are the calculated relative energies.

3.1 The reactions in the gas phase

In previous research, it was found that the initial reactions in each decomposition mechanism are rate-determining steps [14,23]. Therefore, we only need to compare the thermodynamic values of the initial steps for each mechanism, i.e., the hydrogen of the amido group transfer to the oxygen of the nitroso group as well as displacement of the chlorine with the oxygen of nitroso and carbonyl groups, outlined in Fig. 1 [1].

3.1.1 Isomerization of CENU

CENU has Z and E tautomers, i.e., the nitroso group is *syn* and *anti* to the carbonyl group, respectively. From Fig. 2, we can see that the dihedrals of C(9)–N(2)–N(5)–C(1) in Z and E tautomers were calculated to be 177.6° and 175.9°, respectively. It indicates that the hybridization of N(2) atom is sp^2. The N(5)–N(2)–C(1) angles in Z and E tautomers were calculated as 130.4° and 117.6°, respectively. Compared to the optimal angle in sp^2 hybridization with value of 120°, Z tautomer involves a larger energy of tension. Therefore, the E

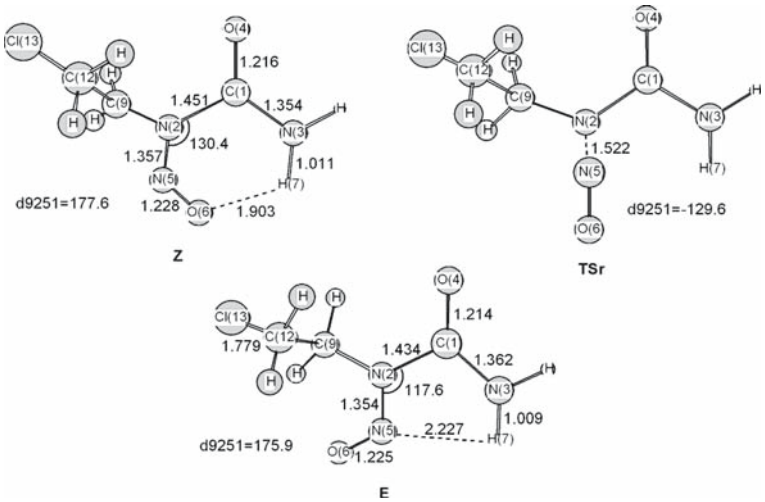

Fig. 2 The structures and main parameters of Z and E tautomers and of corresponding transition state of isomerization (distances in Å and angles as well as dihedrals in degree)

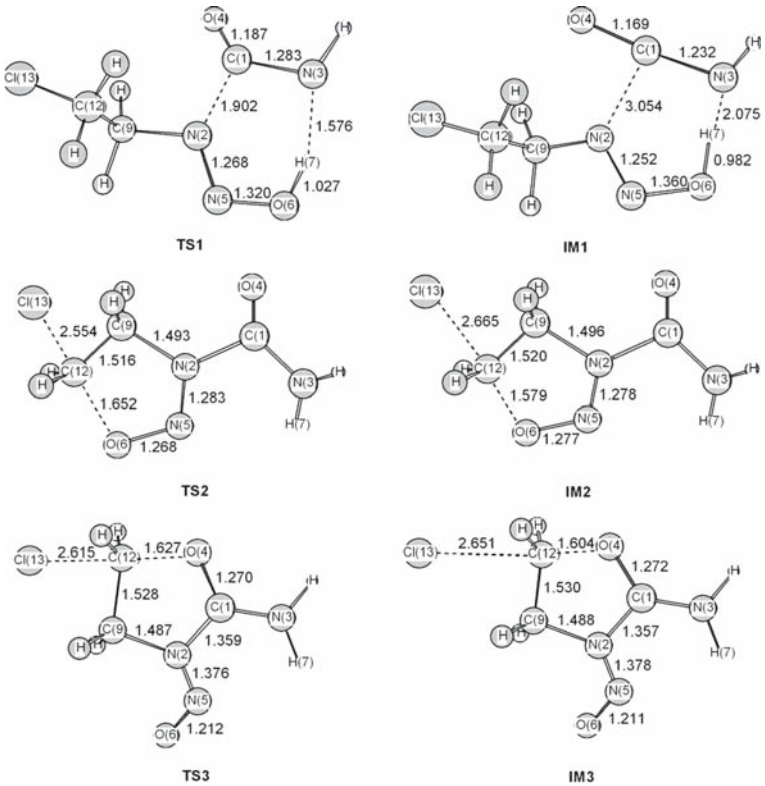

Fig. 3 The structures and main parameters of transition states and intermediates in the CENU decomposition reactions (distances in Å)

tautomer was more stable than Z tautomer by 3.90 kcal/mol in the gas phase (Table 1), which is in good agreement with previous results of some other nitroso compounds containing carbonyl group [29,30].

The corresponding transition state of isomerization was TSr, as shown in Fig. 2. The N(2)–N(5) bond was elongated from 1.357 Å in Z tautomer to 1.522 Å in TSr. It indicates that the partial double bond of N(2)–N(5) in Z tautomer is elongated into a weak single bond in the transition state TSr, which makes rotation of the nitroso group around the N(2)–N(5) bond possible. The energy barrier of 18.37 kcal/mol for the isomerization of Z to E is more likely used to destroy the partial double bond of N(2)–N(5) in Z tautomer. Therefore, a mixture of both Z and E tautomers is present in the system.

3.1.2 Retro-ene reaction

As can be seen from Fig. 3, the conformer Z decomposition to 2-chloroethanediazohydroxide and isocyanate proceeds via the retro-ene reaction, which involves an intramolecular transfer of a γ-hydrogen atom to an unsaturated center via a six-electron cyclic transition state as stated in Refs [31–33]. The transition state of this process is TS1, a moiety of which is composed of a planar hexagon C(1)–N(2)–N(5)–O(6)–H(7)–

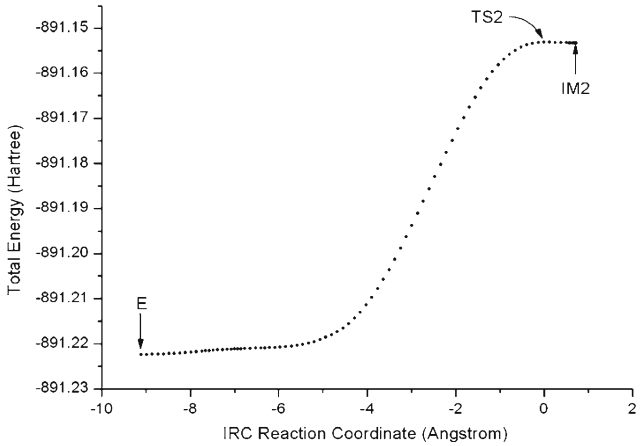

Fig. 4 The IRC plots for intramolecular substitution reaction of the nitroso group displacing the chlorine

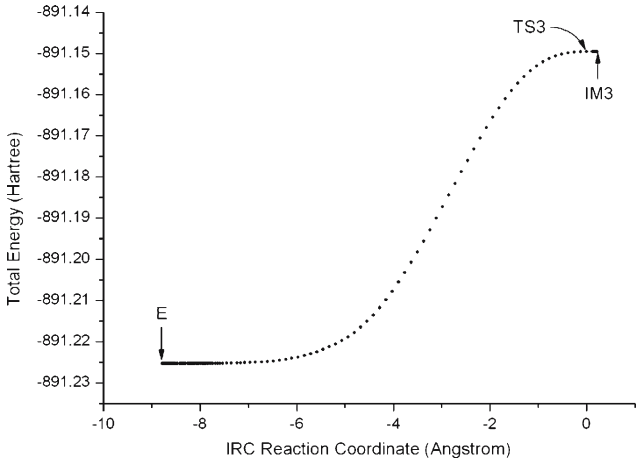

Fig. 5 The IRC plots for intramolecular substitution reaction of the carbonyl group displacing the chlorine

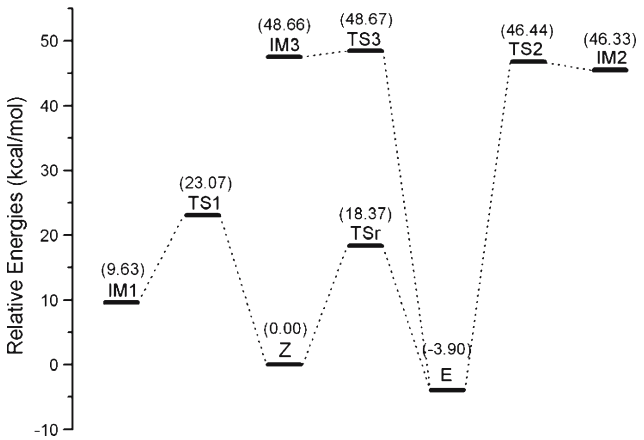

Fig. 6 The schematic profiles of the potential energy surfaces of the CENU decomposition in the gas phase

N(3). TS1 had only one imaginary frequency of $318.6i\,\mathrm{cm}^{-1}$, corresponding to the hydrogen shift on the infrared vibrational spectrum. Table 1 showed that the energy barrier of this

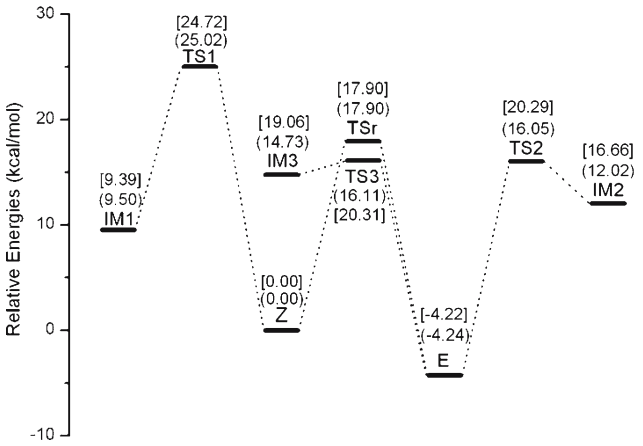

Fig. 7 The schematic profiles of the potential energy surfaces of the CENU decomposition in ocatanol (numbers given with *square brackets*) and in water (numbers given with *parenthesis*)

reaction was 23.07 kcal/mol in the gas phase. In TS1, the broken C(1)–N(2) and N(3)–H(7) bonds were significantly elongated by 0.451 and 0.565 Å, respectively, while the C(1)–N(3) bond and the forming O(6)–H(7) bond were shortened by 0.071 and 0.876 Å, respectively, compared to those values in Z conformer.

3.1.3 Intramolecular substitution reactions

For the reaction of the oxygen of the nitroso group displacing the chlorine, a transition state TS2 involving a planar pentagon composed of N(2)–N(5)–O(6)–C(12)–C(9), was found. As can be seen in Fig. 3, in TS2, the broken C(12)–Cl(13) bond was elongated by 0.775 Å, while the distance of C(12)–O(6) was decreased to 1.652 Å, compared with the corresponding values in E. To confirm TS2 connects E tautomer and intermediate IM2, the IRC calculation was further performed and it was shown in Fig. 4. The results from IRC and frequency calculations indicate that intermediate IM2 is a true minimum. However, as presented in Fig. 4, the PES between TS2 and IM2 is very flat like those of other reactions with departing leaving groups [34]. Thus, as shown in Fig. 3, IM2 is similar in structure to TS2. This reaction would give rise to charge-separated species in the encounter transition state (that is, the 3-carbamoyl-[1,2,3]oxadiazolidine carried a positive charge and the chlorine atom carried a negative charge), which is disfavored. Therefore, this reaction is an energy demanding process, with derived activation energy of 50.34 kcal/mol in the gas phase.

Another intramolecular substitution reaction, in which the oxygen of the carbonyl group displaces the chlorine, produces an intermediate 2-(imino)-3-nitrosooxazolidine, as shown in Fig. 3. The corresponding transition state TS3 was similar to the TS2 in geometry, in which there was also a planar pentagon composed of C(1)–N(2)–C(9)–C(12)–O(4).

In TS3, the C(12)–Cl(13) bond was elongated to 2.615 Å while the distance of C(12)–O(4) was decreased to 1.627 Å, which indicated that the oxygen of the carbonyl group displaced the chlorine. Like IM2, as shown in Figs. 3 and 5, complex IM3 is also a minimum on a flat PES and has a similar structure to TS3. As shown in Table 1, the activation energy of this reaction was calculated to be 52.57 kcal/mol in the gas phase.

3.2 The effects of solvent

It is well known that the uptake, distribution, biotransformation, and elimination of a wide variety of chemicals take place in the condition of an emulsion of fat/water in vivo. As a model, the octanol/water was selected to simulate the fat/water medium in the early studies [35,36]. For this reason the solvents effect of octanol and water on the CENU decomposition reactions has also considered using the PCM-MP2/6-311+G(d,p) method. As can be seen from Table 1, the inclusion of solvent effects slightly increased the energy barrier of retro-ene reaction but largely decreased that of intramolecular substitution processes. The energy barriers at TS1, TS2 and TS3, which were calculated as 23.07, 50.34 and 52.57 kcal/mol, respectively, in the gas phase, became 24.72, 24.51 and 24.53 kcal/mol, respectively, in octanol, and became 25.02, 20.29 and 20.35 kcal/mol, respectively, in water. These numbers indicate that the charge-separated structures (TS2 and TS3) are more stable in solvent than in the gas phase, and that, in other words, the cyclization mechanisms of CENU decomposition are ready to occur in polar solvent. Moreover, with the dielectric constants of solvent, the activation energies showed positive correlation for cyclization reactions whereas negative correlation for retro-ene reaction. Briefly, the CENU decomposition could proceed via retro-ene reaction and cyclization reactions in solvent. Based on these theoretical results here, it is possible to explain that N3-monosubstituted CENUs without hindrance of adjacent carbon, like CENU studied in this paper, may decompose through all the modes investigated above, and that N3,N3-disubstituted CENUs without γ-H may decompose only via intramolecular substitution [1,22].

3.3 Comparison of decomposition mechanisms

From Fig. 6, we can see that the E conformer is the most stable stationary point in this reaction system, and can decompose via three different pathways: E → Z → IM1, E → IM2, and E → IM3. For the processes of E → Z → IM1, the energy barrier of Z → IM1 was slightly higher than that of E → Z by 0.8 kcal/mol; therefore, this Z → IM1 process is the rate-determining step. Respecting the E → IM2 and E → IM3 processes, the energy barriers are nearly the same, both higher than that of Z → IM1 process by 27–30 kcal/mol in the gas phase. Thus, E conformer decomposition in the gas phase proceeds preferentially via a stepwise mechanism, i.e., E → Z → IM1. Obviously, the Z → IM1 process is also the chief route for Z conformer decomposition. Therefore, it can be concluded that CENU decomposition in the gas phase predominantly proceeds via the retro-ene reaction.

In octanol and water, the inclusion of solvents effect did not change the energy barriers of E → Z isomerization, and only slightly increased the energy barriers of retro-ene reaction by about 2 kcal/mol. Therefore, the retro-ene reaction is still the rate-determining step in octanol and water for the processes of E → Z → IM1. For the E → IM2 and E → IM3 processes, however, inclusion of solvents effect significantly decreased the energy barriers to 24.51 and 24.53 kcal/mol in octanol, respectively, and to 20.29 and 20.35 kcal/mol in water, respectively. Comparing the retro-ene reaction to intramolecular substitution reactions, the latter lied lower than the former in energy barriers (see Fig. 7). Therefore, it is reasonable to conclude that the major pathway of CENU decomposition in polar solvents proceeds via intramolecular substitution reactions rather than retro-ene reaction.

4 Conclusions

The initial reaction mechanisms of *N*-(2-chloroethyl)-*N*-nitrosourea decomposition have been investigated at the MP2/6-311+G(d,p) level. Three mechanistic processes were considered. One process was the hydrogen transfer from the nitrogen to the oxygen of the nitroso group, and the other two were the oxygen of the nitroso and the carbonyl groups nucleophilic substitution the chlorine. The computational results showed that the energy barrier of retro-ene reaction was 23.07 kcal/mol in the gas phase, lower than that of substitution reactions by about 27 kcal/mol. Thus, CENU decomposition proceeds predominantly via retro-ene reaction in the gas phase.

The solvent effects of octanol and water on the reactions of CENU decomposition were also taken into account. The inclusion of solvent effects slightly increased the energy barrier of retro-ene reaction by 1–2 kcal/mol, whereas inclusion largely decreased that of substitution processes by around 30 kcal/mol. Importantly, the energy values of each barrier in these three processes approach each other. Therefore, the CENU decomposition in solvent could proceed through retro-ene reaction and intramolecular substitution reactions. This conclusion is in good agreement with the previous experimental results, which proposed that all these three pathways contribute to CENUs decomposition based on the products derived from 2-chloroethanediazohydroxide, substituted [1,2,3]oxadiazolidine and 2-(alkylimino)-3-nitrosooxazolidine intermediates.

Acknowledgments The authors would like to thank Peter King. P.Eng. of Beijing University of Technology for English language polishing. This work was supported by the National Natural Science Foundation of China (No. 20672011) and National Natural Science Foundation of Beijing (No. 8072006) and PHR (IHLB).

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