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AM1 and PM3 Semi empirical Studies of the Reaction Mechanism of Formaldehyde and Glycine

[1]Hamza, U., [2]Uzairu, A., [2]Sallau, M.S., [2]Abechi, S,E.
[1]*Chemistry Department, Federal University of Education, Kano, Nigeria.*
[2]*Chemistry Department, Ahmadu Bello University, Zaria, Nigeria.*
Correspondence address: huzango@yahoo.com

Abstract: *A semi empirical study of the reaction of formaldehyde and glycine was conducted by using AM1and PM3 methods and the possible mechanism of the reaction was investigated. It was found that the glycine nitrogen attacks the carbonyl carbon of formaldehyde and forms a methylol intermediate that undergoes a condensation with another glycine to produce a methylenediglycine through a methylene bridge (cross-linking). The activation enthalpies of the reaction were also predicted. The reaction was found to be exothermic and second order.*

Key words: *Semi-empirical study, AM1, PM3, mechanism, methylol, intermediate and methylenediglycine.*

I. Introduction:

Amino acids are molecules containing an amine group, a carboxylic acid group, and a side-chain that is specific to each amino acid. The key elements of amino acids are carbon, hydrogen, oxygen and nitrogen.

Amino acids are organic compounds that combine to form proteins i.e. serve as the building blocks of proteins, which are linear chains of amino acids. Amino acids can be linked together in varying sequence to form a vast variety of proteins. Amino acids and proteins are the building block of life. As amino acids have both a primary amine group and a primary carboxyl group, these chemicals can undergo most of the reaction associated with these functional groups. These include nucleophilic addition, amide bond formation and imine formation for the amine group and esterification, amide bond formation and decarboxylation for the carboxylic acid group [1].

The amino functions of proteins are susceptible to modification by carbonyl moieties of aldehydes that are found in vivo, such as formaldehyde, glyceraldehydes, acetaldehyde and glucose. Small molecular weight aliphatic aldehydes present in vivo can form adducts with proteins [2].

Proteins undergo a large variety of chemical modification in the presence of formaldehyde, such as the formation of methylol groups, Schiff-bases and methylene bridges [3].

In humans, non-protein amino acids also have important roles as metabolic intermediates such as in the biosynthesis of neurotransmitter gamma-amino butyric acid. Many amino acids are used to synthesise other molecules, for example glycine is a precursor of porphyrins such as heme [4].

Formaldehyde is a colourless, flammable gas with a pungent, suffocating smell and soluble in water, acetone, benzene and some other organic solvents. The solid form of formaldehyde is called paraformaldehyde,, whereas the liquid form is known as formalin [5]. It is very reactive and condenses with numerous compounds to produce methylol or methylene derivatives [6]. It occurs in the air as a product of photo-oxidation of atmospheric hydrocarbons emitted in automobiles, truck and aircraft exhaust and released in indoor air from urea-formaldehyde foam insulation or from particle board that uses adhesives containing the urea- formaldehyde resin 6].

Formaldehyde is used as a sterilizing agent, disinfectant and preservative in many occupational areas and at home [7].

Formaldehyde posses many potentially detrimental effects to body system. Many animal studies showed that exposure to formaldehyde cause serious harm on respiratory system. Long term formaldehyde inhalation at a dose of 15ppm was induced squamous cell carcinomas in the nasal cavities of rats and mice [8].

Formaldehyde is found to be toxic to human at higher concentration as above 0.08% w/v of formaldehyde in human blood can cause irritation to the respiratory tract, eye and skin as reported by Chun et al (2007) [9]. Other examples of formaldehyde related cases of toxicity are: In the USA, the FEMA provided travel trailers and homes starting in 2006 for habitation by resident of the US gulf coast displaced by Hurricane Katrina and Rita. Some of the people who moved in the trailers complained of breathing difficulties, nose-bleeds and persistent headaches. Formaldehyde was used in the production of these homes [10]. Also in the U.S. problems arose again provided by FEMA to residents displaced by the IOWA flood of 2008[11]. A couple of month after moving to the trailers, occupants reported violent coughing, headaches, as well as asthma, bronchitis and other problems. It was reported by Erkrath et al (1981))[12], that high level of formaldehyde reacts with

protein and nitrogen atoms in the environment to form reversible and irreversible adducts in vitro, in vivo and clinically, the lethal dose of formaldehyde for human beings is found to be 0.08% w/v in the circulation. It also cause neurotoxicity in human by aggregation of neural tau protein [13].

Formaldehyde is a well-known cross.-linking agent that is often applied in the biological and pharmaceutical field to inactivate, stabilize, or immobilize proteins [3].

The reactions of formaldehyde with amino acids are essentially the reaction of amino group similar to those involving amine and amine derivatives. According to the mechanism proposed by Putchler et al, 1985[14], the formaldehyde reacts with uncharged amino ($-NH_2$) group but not with protonated amino ($-NH_3^+$) group as in equation1.

$$RNH_2 + CH_2O \rightarrow RNHCH_2OH \qquad \text{(Equation1)}$$

Highly reactive methylol compounds are formed. If steric conditions are favourable, methylol groups condensed with amide or other groups to yield a methylene bridge that cross-links polypeptide chain [15]. As in equation 2 below.

$$R.NH.CH_2.OH + NH_2COR' \rightarrow R.NH.CH_2.NH.CO.R' + H_2O \qquad \text{(Equation 2)}$$

The ratio of bound formaldehyde and amino groups is nearly 1:1. This suggests that each methylene bridge links an amino group to another functional group [16].

Therefore, in the present study, the reaction of glycine and formaldehyde is investigated using theoretical methods. In this study, we examined the transition states and intermediates of the amino acid glycine reaction with formaldehyde using a semi-empirical method (Spartan-AM1 and PM3) to afford the mechanistic insight of the reaction by discussion on the stability of the intermediates, energies of the transition states and calculating the energy barrier for the first time.

II. Materials and Methods:

All computations were made using the standard version of AM1 and PM3 semi-empirical MO methods in the Spartan 08 software package as run on a HP Pavilion computer, with Intel(R) Celeron(R) Dual CPU, 4.00GB of RAM.

The structures of the reactants, transition state, intermediates and products were built and minimized with the MM2 method in the Spartan 08. All the geometries were optimized first using Austin Model 1 (AM1) with 6-31G basis set followed by parametric method 3 (PM3) with 6-31G basis set in the Spartan 08 Global calculation environment work space. Heat of formation (ΔH_f) of the reactants, transition states, intermediate and products were all calculated. Infra-red (IR) and thermodynamic parameters were also calculated.

III. Result and Discussions

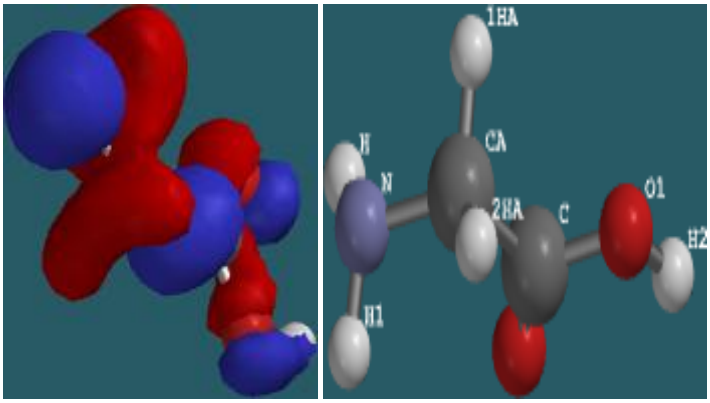


Fig 1 Glycine HOMO

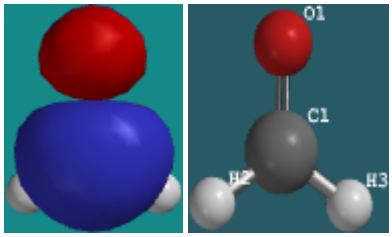
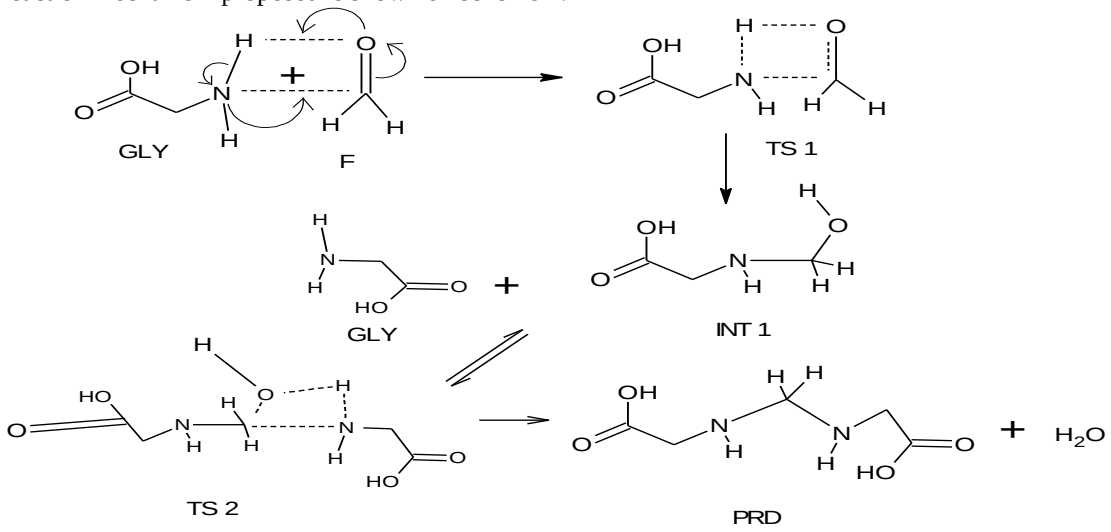


Fig 2 Formaldehyde LUMO

The optimized structures of glycine HOMO and formaldehyde LUMO were shown in figure 1 and 2 respectively.

In general the most likely interaction site for the two molecules is either the two atoms that have highest and opposite charges or two atoms that have a highest electron densities in their highest occupied or lowest unoccupied atomic molecular orbital's,(HOMO/LUMO) interaction [17,18]. In this reaction we considered the reaction between the HOMO of glycine and the LUMO of formaldehyde. For both the AM1 and PM3 success was achieved when the interaction of glycine N lone pair with the carbonyl centre of formaldehyde was performed. When considering both the charge interaction and most likely the HOMO/LUMO interactions led to the conclusion that glycine react with formaldehyde by nucleophilic attack of glycine nitrogen (N) lone pair on the carbonyl carbon C1 of formaldehyde.

The reaction mechanism proposed is shown on scheme 1.



(Scheme1)

The reaction is in two steps, first the amino acid reacts with the formaldehyde through its terminal amino group to form methylol substituent. In the second step the amino acid (which can be either same or different) then reacts with the methylol intermediate to form a methylene cross-linkage and water as products which confirms to the work of Gustavson (1956))[15]. In this research we restrict ourselves to use one amino acid (glycine) to model the reaction mechanism.

As shown from the scheme 1 glycine N attacks the C1 carbonyl centre of formaldehyde and a bonding occurred between the N and C1 with a concomitant transfer of hydrogen atom from N of glycine to a carbonyl O1 of the formaldehyde during the geometry optimization/energy minimization procedures to form intermediate1 that later react with another glycine to form the product which is a methylene cross-linkage. The glycine N attack the methylol carbon to form a bond between N and the methylol carbon with a transfer of hydrogen from N to the oxygen attach to the methylol carbon to form a transition in which a molecules of water is lost to form the methylenediglycine as a final product. This result is in agreement with that of Loeb (1913) [19].

Table 1: Optimised geometry of the reacting species.

		Bond Length			
		Before reaction		After reaction	
	Bond	AM1	PM3	AM1	PM3
Breaking	N-H	1.001Å	0.998 Å	1.265 Å	1.325 Å
	O=C	1.348 Å	1.342 Å	1.416 Å	1.394 Å
Forming	N-C	1.528 Å	1.548 Å	1.457 Å	1.491 Å
	O-H	1.463 Å	1.457 Å	0.966 Å	0.950 Å

From table 1 it was found that the bonds between N-H in the course of reaction increase by 0.264 Å and 0.327 Å for AM1 and PM3 respectively. While for O=C it increases by 0.098 Å and 0.052Å for AM1 and PM3 respectively, all these is due to pulling of electron by the reacting atoms in the transition state (TS1) and the energy is also high.

For the bond formation N-C and O-H there is shortening of bond by 0.071 Å and 0.057 Å and 0.497 Å and 0.507 Å for both AM1 and PM3 respectively. This is due to formation of a stable molecule that has a strong bond and lower energy as compared with the reactants.

Table2: Heat of formation (ΔH_f) Kj/mol by the AM1and PM3 method for the species in scheme 1

Species	AM1	PM3
Formaldehyde	-131.75	-142.6
Glycine	- 424.82	- 401.52
TS1	-350.39	-350.39
INT	- 598.67	-583.35
TS2	- 782.77	-643.40
PRD	- 741.67	-755.98
Water	- 247.86	-223.54

The computed heat of formation .for species are shown in the table 2 above. It can be seen from the table that reactions that produce intermediates were both exothermic, by -42.1Kj/mol and -39.22Kj/mol for AM1 and PM3 respectively.

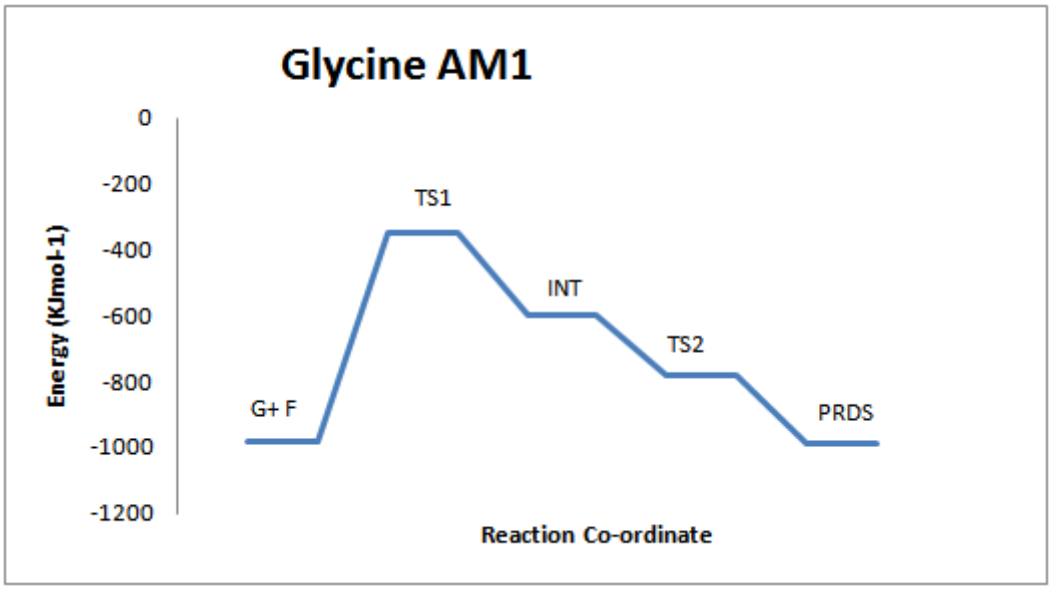


Figure: 3. The predicted AM1 reaction co-ordinate for interaction of glycine and formaldehyde according to scheme 1

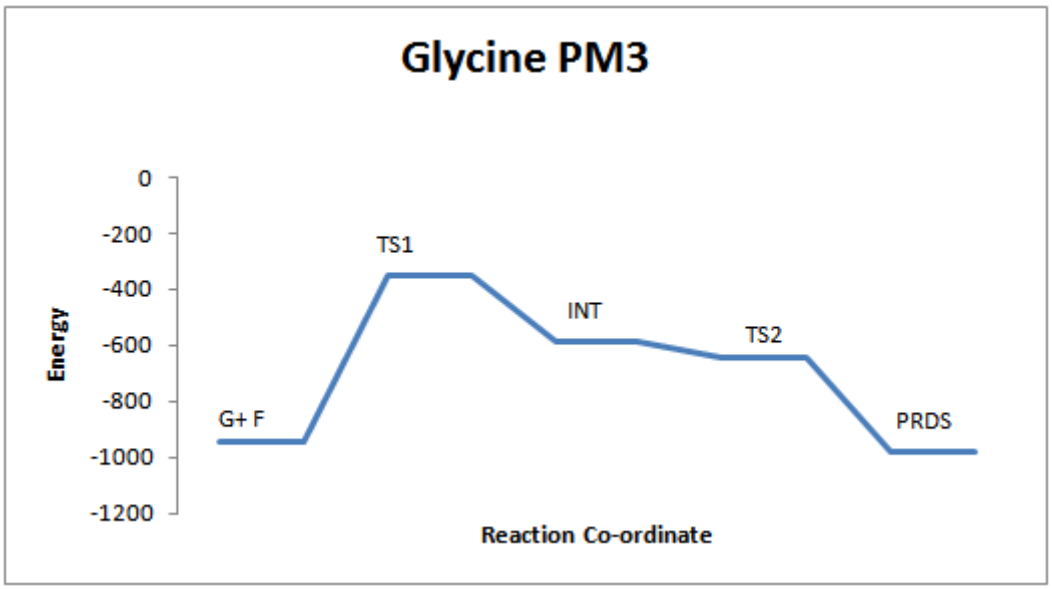
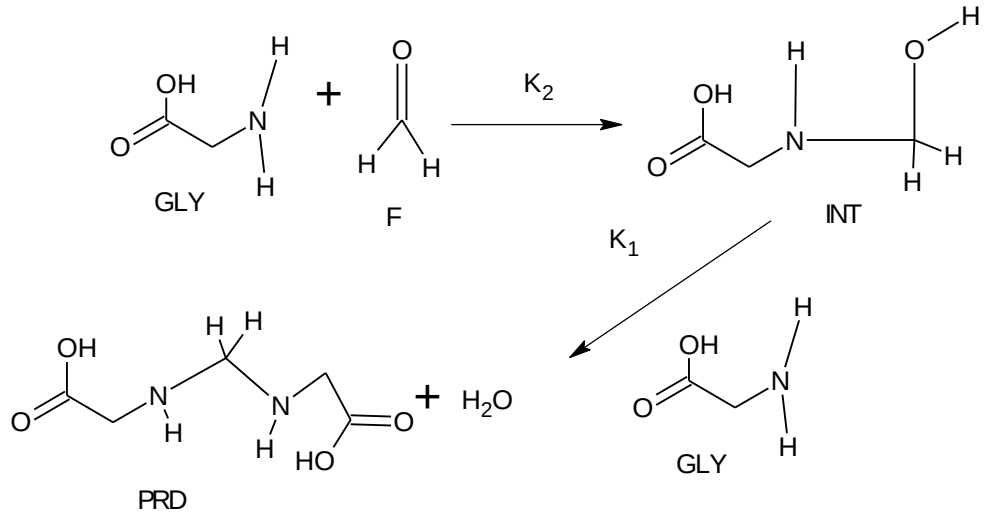


Figure: 4: The predicted PM3 reaction co-ordinate for interaction of glycine and formaldehyde according to scheme 1

From figure 3 and 4 the transition (TS1) represent a high energy species in which the activation energies are -631Kj/mol and -595.27Kj/mol for AM1 and PM3 respectively. While for TS2 activation energies are - 184.1Kj/mol and -60.05Kj/mol for the AM1 and PM3 methods respectively. This shows that the reaction is exothermic. The AM1 having lower activation energy is more favoured.

The consecutive step of this reaction can be illustrated as G + F $\xrightarrow{K_2}$ INT $\xrightarrow{K_1}$ PRD + H_2O is shown in scheme 2 below. Kinetically,the second is the rate determining step with lower rate constant value of k_2 1.07793 x $10^3 s^{-1}$.



Consecutive step **(scheme 2)**

Thermodynamically, the consecutive step are all irreversible in nature. The step from the reactants (G+F) to lntermediate INT have a Gibb's free energy change ΔG^o_{rxn} of 16.4kj/mol and that from INT to products has a value of 40.22kj/mol, which indicate the irreversibility of the reaction. Likewise for the PM3 method the free energy change ΔG^o_{rxn} values are 18.66 and 8.98kj/mol respectively.
For the transition state, the activation energy values indicate that the steps are reversible due to low energy barrier as opposed to entropy change.

Table 3: Kinetic parameters for step1 and 2 of the consecutive reaction

Step 2	E_a 2448.419 A 289442 K_2 1.07793 x 10^3
Step 1	E_a 2449.659 A 422061 K_1 1.57104 x 10^3

Kinetics of the Reaction:
The reaction mechanism consists of the following steps:

(1) $A + F \underset{K_{-1}}{\overset{K_1}{\rightleftharpoons}} T_1$

(2) $T_1 \xrightarrow{K_2} I_1$

(3) $I_1 + A \underset{K_{-3}}{\overset{K_3}{\rightleftharpoons}} T_2$

(4) $T_2 \xrightarrow{K_4} P + H_2O$

For step 1 the rate equation is
$R_f = k_1 [A] [F]$
$R_b = k_{-1}[T1]$
At equilibrium $R_f = R_b$
Therefore, $k_1 [A] [F] = k_{-1}[T1]$
For step 2

$R_2 = k_2[T1]$
For step 3
$R_f = k_3[I][A]$
$R_b = k_{-3}[T2]$
At equilibrium $R_f = R_b$
$k_3[I][A] = k_{-3}[T2]$
For step 4
$R_4 = k_4[T4]$
Applying steady-state approximation
For [T1]
$k_1[A][F] - k_{-1}[T1] - k_2[T1]$
$[T1] = \frac{k1[A][F]}{k_{-1} + k_2}$
For [I]
$k_2[T1] - k_3[A][I] = 0$
$[I] = \frac{k2[T1]}{k3[A]}$
$= \frac{K_2 K_1 [A][F]}{K_3 [A](K_{-1}+K_2)}$
For T2
$k_3[A][I] - k_4[T4] = 0$
$[T2] = \frac{k3[A][I]}{k_4}$
$[T2] = \frac{k1[A]}{K_4} (\frac{K_2K_1[A][F]}{K_3[A](K_{-1}+K_2)})$
$= \frac{K_2K_1[A][F]}{K_4K_{-1}}$
But step 4 is the slowest and hence rate determining step
$R_4 = k_4[T2]$
Substitute the value of [T2]
$R_4 = k_4 \frac{K_2K_1[A][F]}{K_4K_{-1}} [A][F]$
$= \frac{K_2K_1[A][F]}{K_{-1}}$
But $\frac{K_1}{K_{-1}}$ is the equilibrium constant for step 1, so $\frac{K_1}{K_{-1}} = K_{eq}$
Therefore, $R_4 = K_{eq}\, k_2 [A][F]$
Let $k = k_{eq}\, k_2$
$R_4 = k [A] [F] = -4.0494 \times 10^{13}\ dm^3mol^{-1}sec^{-1} [A] [F]$.
k is the experimental rate constant.
This indicate that the reaction between amino acid and formaldehyde is a second order overall, first order with respect to each reactant.

IV. Conclusion:

It was concluded that the most favoured interaction is the HOMO/LUMO interaction. The reaction is nucleophilic in nature in which the nitrogen lone pair of glycine attacks the carbonyl carbon (C1) of formaldehyde to form glycine-formaldehyde adduct (methylol) which later combine with another amino acid (glycine) to produce a methylene bridge compound and water. The two methods are in good agreement with one another since they produced two transition states and one intermediate. The reaction was found to be second order overall and exothermic in nature.

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