Short Research Article

Computational Study of *n*-Acetylglutamate Hydrolysis Under Acidic and Basic Conditions

ABSTRACT

Aims: To study N-acetylglutamate hydrolysis under acidic and basic conditions, using molecular modeling techniques.
Study design: Hydrolysis of N-acetylglutamate was studied under acidic and basic conditions to establish the differences in chemical properties and conditions of favorability; this was performed using the Mulliken charges and the geometric parameters as descriptors, as well as proton affinity, Gibbs free energy, and equilibrium constants.
Place and Duration of Study: Grupo de Investigación Max Planck, Facultad de Química y Farmacia, Universidad del Atlántico, between February 2014 and March 2015.
Methodology: Structures of the hydrolysis reaction under acidic and basic conditions were optimized using molecular mechanics prior to calculating various molecular descriptors. The Hartree–Fock (HF) method was used with the 3-21G and 6-31G* basis sets. Some useful parameters for analyzing the reactions are proton affinity, Frontier Molecular Orbitals, Gibbs free energy, and equilibrium constants.
Results: In general, reaction profiles demonstrated that the two reactions are favorable; however, in agreement with our preliminary equilibrium constant findings, a greater favorability for basic hydrolysis was shown.
Conclusion: The calculated equilibrium constants are in agreement with the favorability of hydrolysis under basic conditions, which is consistent with the biochemical process.

Keywords: Hydrolysis conditions, N-Acetylglutamate hydrolysis, Hartree Fock calculations.

1. INTRODUCTION

N-acetylglutamate (NAG) is the first intermediate in the arginine biosynthetic pathway in prokaryotes, eukaryotes, and lower plants [1, 2]. The acetylated form of glutamate, which is amidic [3], is deacetylated to glutamate to undergo hydrolysis. Glutamate is a physiologically important neurotransmitter, and is responsible for brain signalling. This signalling acts on glutamate receptors, which are localized on the cell surface [4].

The hydrolysis of amides is very important in biochemistry as a model for bond cleavage in living systems and has been studied experimentally and in theory [5-8]. The hydrolysis of amides under basic and acidic conditions has received much attention in theoretical studies because of their role in several biological processes [3]. The degradation of amides by this route produces a carboxylic acid and an amine. Heating is often required even with acidic or basic conditions, so water is not sufficient to hydrolyze most amides. Under acidic conditions, protonation of the oxygen atom occurs Fig.1.

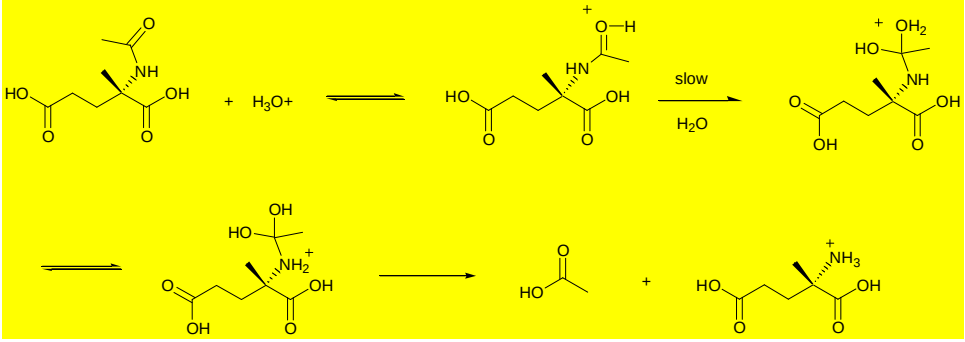
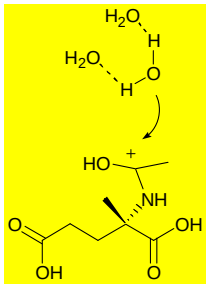


Fig. 1. Acid hydrolysis of N-acetylglutamate

Kinetic data have shown three molecules of water are involved in the rate determining step [9], suggesting that additional water molecules take part in the process as follows:



In the base-catalyzed pathway, the hydroxyl ion acts as a nucleophile on the amidic carbonyl carbon atom, producing a tetrahedral intermediate Fig. 2.

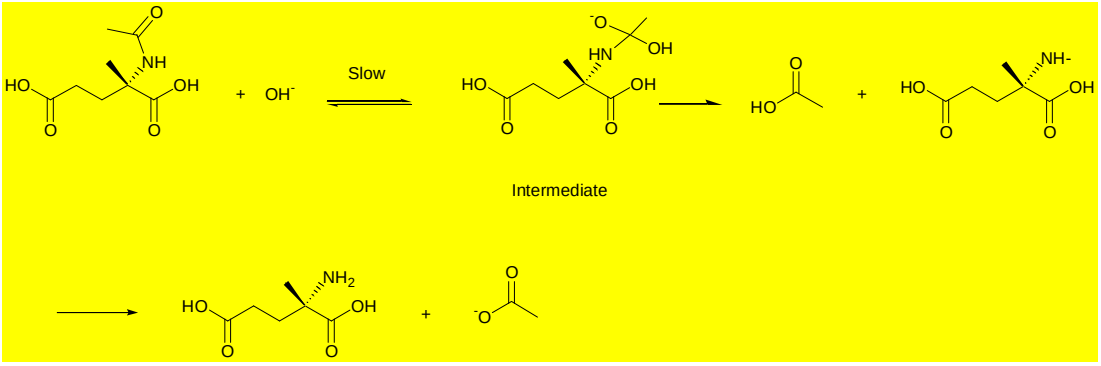
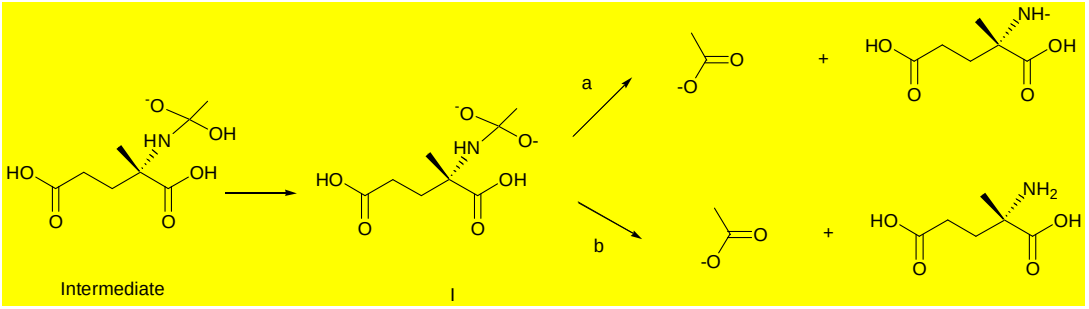


Fig. 2. Basic hydrolysis of *N*-acetylglutamate

Studies on the mechanism of amides hydrolysis based on MO have been conducted [10]. Also, kinetic studies have shown the reaction as a second order in OH-, so the intermediate is converted to structure I as follows:



Structure I can cleave through path a or path b. The rate determining step is the formation of the intermediate at high base concentration. At lower concentration of base, the cleavage of the intermediate or structure I is rate determining [11].

In this work we wish to focus on the acid and base catalyzed hydrolysis of N-acetylglutamate in gas phase with no water medium included in the quantum treatment as solvent. To the best of our knowledge only a few theoretical calculations have been devoted to the study of the amide hydrolysis mechanism. The key role played by this reaction in many biochemical processes is frequently highlighted, however its mechanism has not been completely elucidated.

2. COMPUTATIONAL METHODS

Structures of the hydrolysis reaction under acidic and basic conditions were optimized using molecular mechanics prior to calculating various molecular descriptors.

The Hartree–Fock (HF)[12,13] method was used with the 3-21G and 6-31G* basis sets. Table 1 provides the calculated electrostatic and Mulliken charges for atoms comprising NAG; the molecular structure and numbering are indicated in Fig. 3. Table 1 showed the most negative Mulliken charges for N1 and O3 (−0.89 and −0.697), respectively) calculated at the HF 3-21G level. The highest positive charge (0.889) was located on C5, the carbon atom susceptible to nucleophilic attack.

Some useful parameters for analyzing the reactions are proton affinity, Gibbs free energy, and equilibrium constants. Computational chemical models were constructed to examine differences between hydrolyses under acidic and basic conditions and its favorability.

Fig. 3. Molecular structure of N-acetylglutamate with atomic numbering

Table 1. **Calculated electrostatic and Mulliken charges for *N*-acetylglutamate**

Atom	Electrostatic		Mulliken	
	HF 3-21G	HF 6-31G*	HF 3-21G	HF 6-31G*
N1	−0.746	−0.715	−0.890	−0.827
O3	−0.718	−0.566	−0.697	−0.583
C4	−0.031	0.171	−0.146	−0.071
C5	1.058	0.965	0.889	0.802
C6	−0.917	−0.848	−0.676	−0.579
H9	0.400	0.392	0.402	0.437

3.GEOMETRIC PARAMETERS

The geometry of the main structures involved in the acid- and base-catalysed hydrolysis of NAG are shown in Table 2. The highest difference in the C–N bond distances at the carbonyl site in NAG where hydrolysis occurs under acidic conditions was calculated as C4–N1 = 1.469 Å, while the acid intermediary showed a value of 1.517 Å and amine (product) 1.504 Å (Figs.4 and 5). However, the molecular angles generally tended to reduce, with angles of 108.24°, 106.66°, and 104.08° for H7–C4–N1 of NAG, intermediary, and amin e product, respectively.

Bond distances and angles in the basic hydrolysis reaction exhibited smaller differences between the intermediates and the product in comparison with acidic hydrolysis. By taking into account the prior example (C4–N1 and H7–C4–N1), it was found that the basic intermediate and the product (glutamic acid) had a C4–N1 distance of 1.483 Å and 1.459 Å, respectively. The angles were decreased; the intermediary and glutamic acid had H7–C4–N1 angles of 113.46° and 109.98°, respectively. The glutamate product is neutral, adopting the more stable configuration compared with the acid hydrolysis product. The bond lengths and angles vary, depending on the bonds broken and formed in each structure, i.e.

based on the differences that develop because of the different attack modes on NAG.

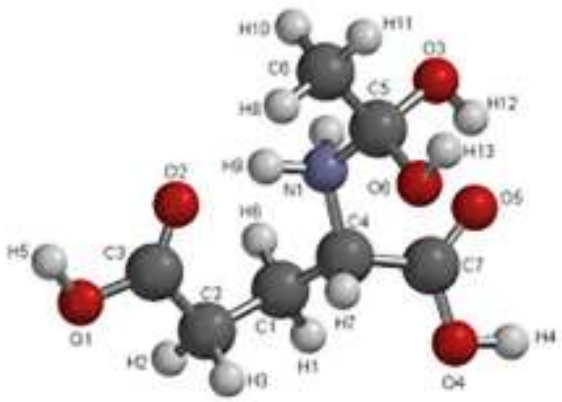


Fig. 4. Acid intermediary of *N*-acetylglutamate hydrolysis

Fig. 5. Glutamic acid originated from *N*-acetylglutamate hydrolysis

The attacks on NAG under each hydrolysis condition generate two intermediates, where only one present H12 Fig. 4. which create an attraction in O3 at the same time it moves away from C5; therefore, it reduces the angle O3–C5–C6. Regarding the final products, the glutamic acid (Fig. 5) and protonated glutamic acid Fig. 6. differ in H10, which increases the distances of N1–H8 and reduces the angle H9–C4– N1.

Fig. 6 Protonated Glutamic acid

Table 2 Geometric parameters of N-acetylglutamate, acid and basic intermediates, amine and glutamic acid

Distance and angle	*N*-acetylglutamate	Acid Intermediary	Basic Intermediary	Amine	Glutamic Acid
C4-N1	1.469 Å	1.517 Å	1.483 Å	1.504 Å	1.459 Å
N1-H9	1.018 Å	1.034 Å	1.014 Å	1.018 Å	1.006 Å
N1-C5	1.369 Å	1.457 Å	1.430 Å	-	-
C5-O3	1.229 Å	1.397 Å	1.299 Å	-	-
C5-C6	1.504 Å	1.529 Å	1.526 Å	-	-
N1-H8	-	-	-	1.040 Å	1.004 Å
C4-H7	-	-	-	1.096 Å	1.078 Å
C4-C1	-	-	-	1.537 Å	1.545 Å
C7-C4-N1	109.16°	111.53°	108.05°	111.31°	111.34°
H7-C4-N1	108.24°	106.66°	113.46°	104.08°	109.98°
C4-N1-H9	114.82°	108.43°	110.80°	108.95°	112.31°
N1-C5-O3	122.75°	110.93°	108.97°	-	-
O3-C5-C6	122.36°	107.46°	110.79°	-	-
N1-C4-C1	-	-	-	110.95°	110.77°
C4-H7	-	-	-	1.096 Å	1.078 Å
C4-C1	-	-	-	1.537 Å	1.545 Å
C7-C4-N1	109.16°	111.53°	108.05°	111.31°	111.34°
H7-C4-N1	108.24°	106.66°	113.46°	104.08°	109.98°
C4-N1-H9	114.82°	108.43°	110.80°	108.95°	112.31°
N1-C5-O3	122.75°	110.93°	108.97°	-	-
O3-C5-C6	122.36°	107.46°	110.79°	-	-
N1-C4-C1	-	-	-	110.95°	110.77°

Table 3 Proton affinity for acidic hydrolysis of *N*-acetylglutamate

Method	Protonation (Kcal/mol)	N1	Protonation (Kcal/mol)	O3
HF 3-21	24524.7		24543.2	
HF 6-31G*	24660.9		24678.9	

The proton affinity was calculated for acid hydrolysis; the initial protonation occurs on O3 rather than on N1. The highest proton affinity values (24543.2 and 24678.9 kcal/mol) calculated by HF methods corresponded to that of O3 Table 3.

4.THERMODYNAMIC PROPERTIES

The Gibbs free energy was calculated as shown in Table 4. The lowest Gibbs free energy was found to be −278.04 kJ/mol by the HF 3-21G method, suggesting that hydrolysis under basic conditions is more favourable.

Table 4 Gibbs free energy of acid and basic hydrolysis of *N*-acetylglutamate

Method	Acidic hydrolysis (kJ/mol)	Basic hydrolysis (kJ/mol)
HF 3-21G	−225.96	−278.04
HF 6-31G*	−267.25	−268.25

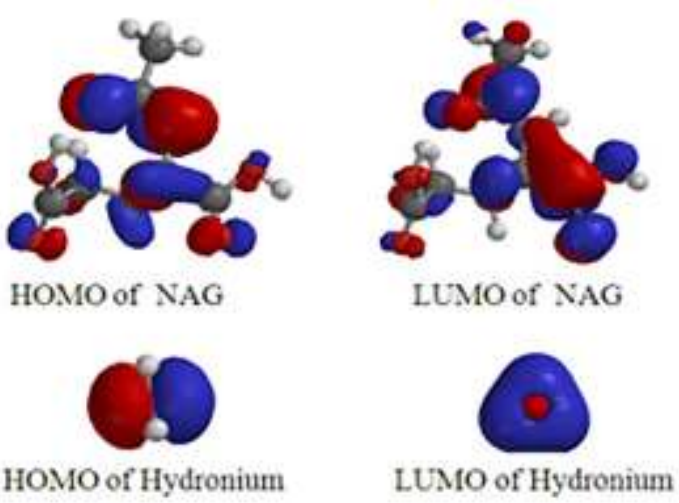


Fig. 7. HOMO and LUMO orbitals acid hydrolysis reaction

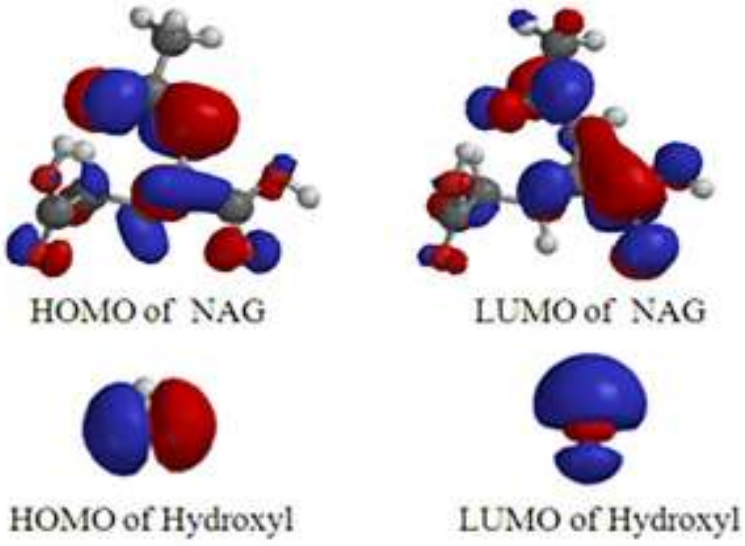


Fig. 8. HOMO and LUMO orbitals in basic hydrolysis reaction

The equilibrium constant was calculated Table 5 from the Gibbs free energy, using the following equation:

$$Keq = e^{-\frac{\Delta G}{RT}}$$

The obtained results confirm the favorability of basic hydrolysis, which shows higher equilibrium constant values.

Table 5 Equilibrium constants calculated for acidic/basic hydrolysis of *N*-acetylglutamate.

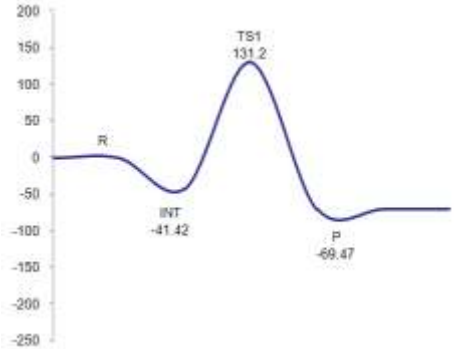
Method	Acid hydrolysis	Basic hydrolysis
HF 3-21G*	$4.048159071 \times 10^{39}$	$5.384308964 \times 10^{43}$
HF 6-31G*	$7.017182065 \times 10^{46}$	$1.038150503 \times 10^{47}$

Within the molecular descriptors, the HOMO and LUMO Fig. 7 and Fig. 8 variation energies were obtained to calculate Δ (Frontier Molecular Orbital Theory), which provides an indication where the reaction is taking place under acidic and basic conditions Table 6.

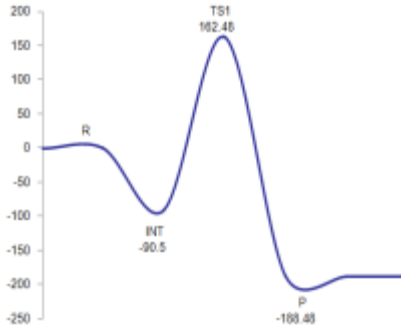
An energy profile is a tool to analyze and compare the relation between the reactions and their favourabilities. Graphics 1 and 2 show the energy profiles of the acidic and basic hydrolysis reactions, respectively, which include the reagents, intermediates, transition states, and products under acidic and basic hydrolyses.

Table 6 Values of Δ between LUMO and HOMO energies for acidic and basic hydrolyses

	Method	Molecule	HOMO (eV)	LUMO (eV)	Δ (eV)
ACID	HF 3-21G	NAG	H1: −10.95	L2: 3.85	Δ1: 8.61
		Hydronium	H2: −25.12	L1: −2.34	Δ2: 28.97
	HF 6-31G*	NAG	H1: −11.33	L2: 3.84	Δ1: 8.12
		Hydronium	H2: −25.18	L1: −3.21	Δ2: 29.02
BASIC	HF 3-21G	NAG	H1: −10.95	L2: 3.85	Δ1: 29.01
		Hydroxyl	H2: 0.52	L1: 18.06	Δ2: 3.33
	HF 6-31G*	NAG	H1: −11.33	L2: 3.84	Δ1: 26.65
		Hydroxyl	H2: −1.03	L1: 15.32	Δ2: 4.87



Graphic 1. Energy profile of acidic hydrolysis of *N*-acetylglutamate.



Graphic 2. Energy profile of basic hydrolysis of *N*-acetylglutamate.

In general, these profiles demonstrated that the two reactions are favorable; however, Graphic 2 is in agreement with our preliminary equilibrium constant findings, showing a greater favorability for basic hydrolysis.

5. CONCLUSIONS

From the Mulliken charges calculated for the hydrolysis of NAG under acidic conditions, it is clear that N1 and O3 are the most negatively charged atoms, and therefore, more susceptible to electrophilic attack. Under basic conditions, the highest positively charged atom is C5, rendering this site susceptible to nucleophilic attack.

Based on the difference between the HOMO and LUMO energies, the lowest Δ value indicates which orbitals are involved in the reaction. Under acidic conditions, the HOMO of NAG and LUMO of hydronium are the reacting orbitals. Under basic conditions, the hydroxyl HOMO and NAG LUMO are the reacting orbitals. Table 6.

Furthermore, by analyzing the calculated values for the Gibbs free energy, it was observed that the NAG hydrolysis under basic conditions is more favorable. The calculated equilibrium constants are in agreement with the favorability of hydrolysis under basic conditions, which is consistent with the biochemical process. Proton affinity reveals that under acidic conditions, the more susceptible atom for protonation is O3.

REFERENCES

[1] Thoden J, Miran S, Philips J, Howard A, Raushel M, Holden H. Biochemistry; 1998.

[2] Shi D, Sagar V, Jin Z, Yu X, Caldovic L, Morizono H, Allewell N, Tuchman M;The crystal structure of N-acetyl-L-glutamate Synthase from Neisseria gonorrhoeae Provides Insights into Mechanisms of catalysis and regulation. J. Biol. Chem. 2008 ;283(11):7176-7184

[3] Antonczak S, Ruiz M, Louis R.The hydrolysis mechanism of formamide revisited; comparison between ab initio semiempirical and DFT results. J. Mol Model; 1997;3: 434.)

[4] Bednárová L, Malon P, Bour P. Spectroscopy properties of the nonpolar amide group A computational study. Chirality; 2007.,19:775

[5] Zahn D. Car-Parrinello molecular dynamics simulations of base-catalyzed amide hydrolysis in aqueous solution. Chemical Physics Letters; 2004; (383):134-137

[6] Zahn D. On the role of water in amide hydrolysis. Eur. J. Org. Chem. 2004; 4020-4023.

[7] Stanton R. V, Peräkylä, Bakowies D, Kollman P.A. Combined ab initio and Free Energy Calculations To Study Reactions in Enzymes and Solution: Amide Hydrolysis in Trypsin and Aqueous Solution. J. Am. Chem.Soc. 1998; (120);3448-3457

[8] Bakowies D, Kollman P.A. Theoretical Study of Base-Catalyzed Amide Hydrolysis: Gas- and Aqueous-Phase Hydrolysis of Formamide. J. Am. Chem.Soc. 1999, (121) 5712-5726.

[9] Yates K, Stevens J. B. The ionization behavior of amides in concentrated sulfuric acids: ii. Applications of the ha function to rates and equilibria. Canadian Journal of Chemistry, 1965, 43(3): 529-537

[10] Hori K, Kamimura A, Ando K, Mizumura M, Ihara Y. Ab initio molecular orbital study on the mechanism of amide hydrolysis dependent on leaving groups. Tetrahedron, 1997, 53, 4317-4330

[11] Smith M.B, March J. March´s Advanced organic Chemistry. Seventh edition. 2013

[12], Beltran Juan A. J. Química Teórica y Computacional.;. 2000.

[13]Spartan '08 for Windows. Tutorial and User's Guide