Asian Journal of Chemistry Vol. 19, No. 3 (2007), 2117-2124

Approach of the Interaction Enzyme-Substrate by Molecular Modelling

S. SARI, W. MEDJAHED, J. KAJIMA MULENGI and S. GHALEM*
Laboratory of Organics Chemistry, Analyses and Naturals Substances, Cosna
Department of Chemistry, Faculty of Sciences
Aboubakr Belkaid University, Tlemcen, Algeria
Fax: 043 21 58 86; E-mail: s_ghalem2002@yahoo.fr; sari_souad@yahoo.fr

In the biological systems, the chemical reactions of the metabolism proceed seldom spontaneously, but they are generally catalysed by particular proteins which one calls enzymes. Whole of these biological functions pass by a reaction of complexation while utilizing certain atoms belonging to a given number of amino acids representing the active site. On the level of this active site, the behaviour of the atoms is relatively different as for the approach from the enzyme and the substrate. At the time of this approach, there are interactions enzyme-substrate which control the stability of a E-S complex leading to the end product according to the specificity of the enzyme. It is on this level that resides the difficulties of comprehension of the interactions between enzyme and substrate. On the basis of this idea we propose to elucidate this mechanism by molecular modelling. We are particularly interested in studying the interaction of the phospholipase A_2 (PLA_2) with aziridines structures by molecular modelling and molecular dynamics.

Key Words: Interactions enzyme-substrate, Phospholipase A_2, Aziridines, Molecular modelling.

INTRODUCTION

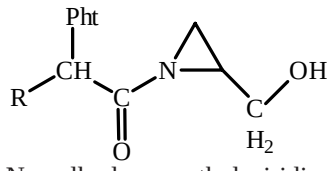
Phospholipase A_2 (PLA_2) is one of the most intensively studied membrane proteins which hydrolyze phospholipids at the sn-2 position to form fatty acid and lysophospholipid products[1]. These are small proteins and the 3-D structures are known to high resolution for several species[2]. Phospholipase A_2 proteins are of high pharmaceutical concern since they are responsible for the release of arachidonic acid from membranes and since the subsequent conversion of this fatty acid to leukotrienes and prostaglandins is part of the inflammatory response. Other functions of PLA_2 have also been documented in the litterature, such as signal transduction, host defence, blood coagulation and membrane remodelling. On the basis of their structures and enzymatic characteristics, PLA_2 secreted have been recently classified into 10 groups[3].

Aziridines are three-membered heterocycles containing nitrogen. The inherent reactivity of aziridines is due, in large part, to ring strain energy (SE) of 26.7 kcal/mol for the parent, unsubstituted aziridine[4]. Aziridines can be found in natural products such as mitomycin, porfiromycin and mitiromycin, which are potent antitumor and antibody agents[5,6].

The aziridines were the synthetic targets in the synthesis since the discovery of Gabriel[7] in year 1888, of the smallest heterocycle containing the nitrogen. The majority of the methods of preparation of the aziridines imitate significant developments of the syntheses of epoxies[8,9] or cyclopropanes[10]. The aziridines are for a long time a source of interest for the chemists, due to the multiplicity of their use in organic chemistry[11]. They are known as well to be synthons significant in organic synthesis as to compose part of the skeleton of various biologically active natural products.

EXPERIMENTAL

The construction and the optimization of all the molecules of aziridines has been carried out by program EMO (Energy of a Molecule). The conformational analysis of all structures was carried out by using the option SCAN, which allows: A sweeping of the surface of energy and to eliminate the conformers the least probably stable.



N-acylhydroxymethylaziridine

R= H (Ref. 12), R= CH_2Ph (Ref. 12), R= CH_2PhOH and R= $–CH_2COOH$

The results obtained are presented in Table-1.

TABLE-1
RESULTS OBTAINED USING PROGRAM EMO

Steric energy (KJ/mol)	Stretch.	Bending	Torsion	VDW	Electr.	Total
Az-H	10.60	568.53	32.68	40.03	-28.46	623.366
Az-CH_2Ph	13.61	573.31	7.07	74.13	-34.97	633.155
Az-CH_2COOH	13.22	581.61	36.42	65.96	-70.16	627.054
Az-CH_2PhOH	14.07	576.61	14.66	72.80	-38.30	639.825

Once the optimized structures, it is necessary to allot to each atom an electronic partial load. A calculation of load was carried out using software SPARTAN with the level of optimization B3LYP/6-31G*, NPA.

The optimization of the geometry of the PLA_2 was carried out using the force field Amber99 (Assisted Model Building with Energy Refinement), the principal chain (backbone) was maintained rigid, while the side chains remain flexible. This approximation allows the side chains proteins to more easily find the position in which the interactions are most favourable. AMBER99 was developed by Case *et al.*[13]. The equation used can be simplified by the following equation:

$$E_{tot} = \sum_{liaisons} K_r (r - r_{eq})^2 + \sum_{valence} K_\theta (\theta - \theta_{eq})^2 +$$

$$\sum_{dièdres} \frac{V_n}{2}[1 + \cos(n\theta - \gamma)] + \sum_{i<j} \left[\frac{A_{ij}}{R_{ij}^{12}} - \frac{B_{ij}}{R_{ij}^{6}} + \frac{q_i q_j}{\varepsilon R_{ij}} \right]$$

The torsion, stretching and bending energies are represented by a simple diagonal harmonic expression. The energy of interaction of van der Waals is represented by a potential 6-12 and electrostatic energy is modelled by Coulombiennes interactions of loads centered on the atoms.

The following step is the positioning of the ligands in the active site of the PLA_2. using the program Dock in ChemOffice.Once that the receiving complex ligand- is formed, this one will adapt the most stable conformation, *i.e.* with the lowest energy level.

In present studies, a minimization of the geometry and a molecular dynamics calculation were carried out on the PLA_2 alone, the substrates and complexes PLA_2-aziridines.

RESULTS AND DISCUSSION

The first step is downloading of the PLA_2 from the Bookhaven Protein Data Bank (access code is 1 kvo). It crystallizes in the hexamer forms; each monomer consists of 124 amino acids, 2 calcium atoms and the inhibitor OAP which was used for the co-crystallization (Fig. 1).

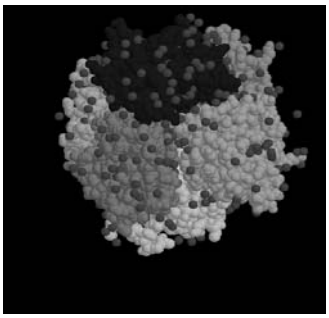


Fig. 1. Hexamer form of the PLA_2

The molecular modelling have a reducing effect, thus in present model that first 70 amino acids of the monomeric form as well as only one calcium atom present by eliminating all the molecules from water and the OAP (Fig. 2).

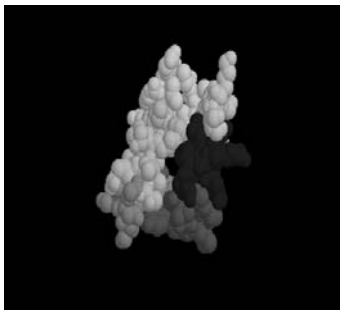


Fig. 2. Monomer chain, 70 amino acids and 1 atom of calcium

This step is followed by the optimization of the geometry and a calculation of molecular dynamics of all the starting structures.

Molecular dynamics of the PLA_2: Constraints on the level of the principal chain (backbone) were applied to the PLA_2 alone like with the various studied complexes.These constraints maintains the skeleton of rigid polypeptide, allowing the distances coordination between calcium and the complex to remain stable (Figs. 3 and 4).

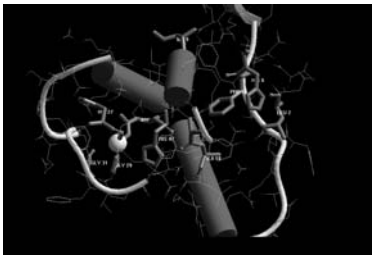


Fig. 3. Residues of PLA2 constituting the active site

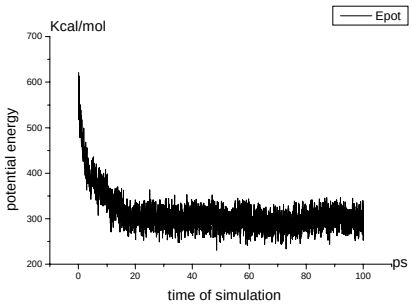


Fig. 4. Variation of the potential energy of the PLA2 according to time

Molecular dynamics calculations of complexes PLA_2-aziridines are given in Figs. 5 to 12.

Energy of interaction: With the object of establishing correlations between energies of interaction and the activities and to consider by which type of forces the nature of the interactions PLA_2-ligands is controlled, one often refers to a decomposition of the total potential energy of the molecule in several terms (electrostatic energy, VDW energy...) (Table-6). Energies of interaction between the various studied substrates and the PLA_2 is obtained using the following relation:

$$E_{interaction} = (E_{total\ potential\ energy\ of\ the\ complex}) - (E_{total\ potential\ energy\ of\ PLA_2} + E_{total\ potential\ energy\ of\ substrate})$$

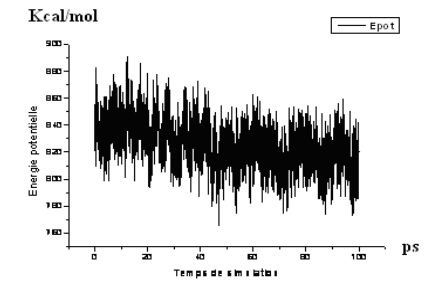


Fig. 5. Variation of the potential energy of the complexe PLA_2-(Az-H) according to time

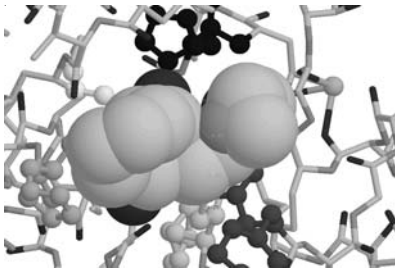


Fig. 6. Complex PLA_2-(AZ-H)

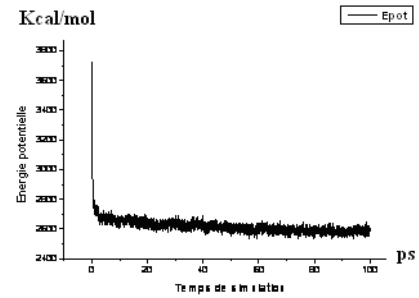


Fig. 7. Variation of the potential energy of the complexe PLA_2-(Az-CH_2Ph) according to time

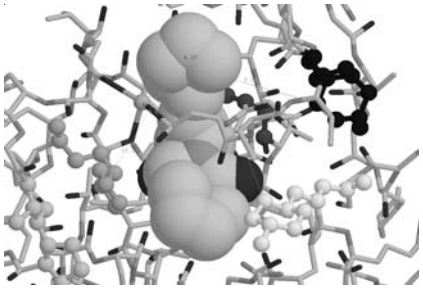


Fig. 8. Complex PLA_2-(AZ-CH_2Ph)

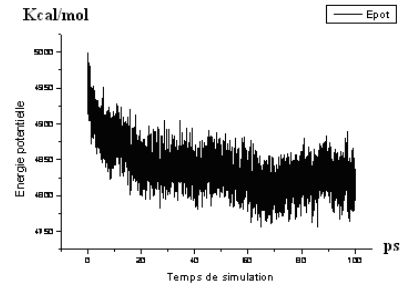


Fig. 9. Variation of the potential energy of the complex PLA_2-(Az-CH_2PhOH) according to time

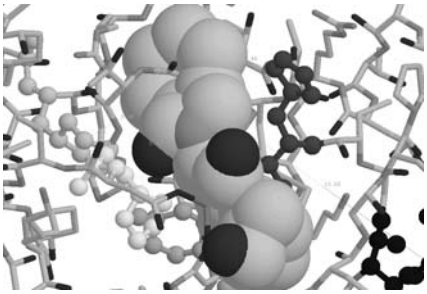


Fig. 10 Complex PLA_2-(AZ-CH_2PhOH)

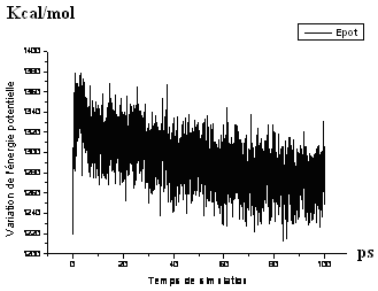


Fig. 11. Variation of the potential energy of the complex PLA_2-(Az-CH_2COOH) according to time

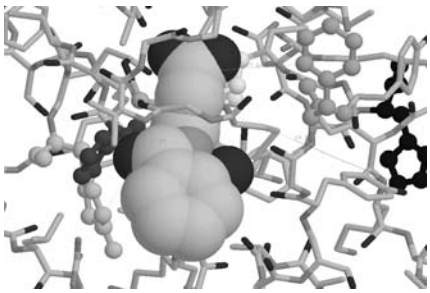


Fig. 12. Complex PLA_2-(AZ-CH_2COOH)

In the light of the results obtained, it arises that the introduction of the cumbersome groupings generates a conformational rearrangement within the cavity of the active site, which probably will increase complimentary activity.

The case of R=H presents a characteristic because of perfect linearity, not presenting any steric obstruction; its energy of van der Waals is equal to -92.29 Kcal/mol.

It could be discuss that increasing or decreasing the interval of dimensions of the cavity of the active site having in present case a width of 10 Å and a depth of 14 Å which narrows by reaching a value of 8 Å.

The distances between the groupings R of the aziridine and those of the side chains of the constituent amino acids were measured. The active site and possibly of other groupings of the principal chain of the enzyme responsible of the interactions which can generate a conformation favourable significant activity. The results obtained are reported in Tables 2-5. The measured distances vary between 3.46 and 19.95 Å for the whole of the studied complexes.

The interactions ranging between 2.5 and 3.1 Å are regarded as strong and ranging between 3.1 and 3.55 Å are supposed to be average. This situation was observed in the case of Concanavaline A with a monosaccharide[14].

TABLE-2
DISTANCES MEASURED BETWEEN THE GROUP OF THE AZIRIDINE (R=H) AND THE GROUPS OF THE SIDE CHAINS OF AMINO ACIDS RESPONSIBLE FOR INTERACTIONS

Distances (Å)	Phe23	His6	Phe5	His47	Tyr21
R = H	4.25	6.20	6.45	8.75	11.00

TABLE-3
DISTANCES MEASURED BETWEEN THE GROUP OF THE AZIRIDINE (R=CH_2Ph) AND THE GROUPS OF THE SIDE CHAINS OF AMINO ACIDS RESPONSIBLE FOR INTERACTIONS

Distances (Å)	His27	Pro36	His47	Tyr51	Tyr66
R = CH_2Ph	9.67	12.69	4.37	9.30	8.80

TABLE-4
DISTANCES MEASURED BETWEEN THE GROUP OF THE AZIRIDINE (R=CH_2PhOH) AND THE GROUPS OF THE SIDE CHAINS OF AMINO ACIDS RESPONSIBLE FOR INTERACTIONS

Distances (Å)	Phe5	Tyr21	His27	His47	Tyr24
R = CH_2PhOH	5.08	6.94	3.46	5.18	13.65

TABLE-5
DISTANCES MEASURED BETWEEN THE GROUP OF THE AZIRIDINE (R=CH_2COOH) AND THE GROUPS OF THE SIDE CHAINS OF AMINO ACIDS RESPONSIBLE FOR INTERACTIONS

Distances (Å)	His6	Tyr21	Phe23	His27	His47
R = CH_2COOH	19.97	5.71	7.69	7.38	12.08

TABLE-6
VALUES OBTAINED OF INTERACTIONS ENERGY IN Kcal/mol

R	$E_{total\ pot}$ complex E-S	$E_{total.\ pot}$ S	VDW Energy Complex E-S	VDW Energy S	VDW Inter. Energy	Total inter. Energy
H	785.2017	236.4357	-273.860	-0.3260	-92.2908	305.2199
CH_2Ph	2552.6130	438.2930	-194.122	5.9238	-18.8028	1828.5954
CH_2PhOH	4775.0330	247.4404	-163.452	3.0965	14.6945	4284.0465
CH_2COOH	1248.8980	224.5801	-126.280	4.6933	50.2697	759.2937

Vdw energy of PLA_2 = -181.243 Kcal/mol, Pot. Energy of PLA_2 = 243.5461 Kcal/mol, E-S: complex Enzyme-substrate, S: substrate

Tyrosin having a pK_a side chain (phenol) of 10.07 Å is strongly deprotoned, possibly being able to have interactions cation-π, *e.g.* the distances separating Ca^{2+} and the groups of the aziridines are:

R = H (13.23 Å), R = CH_2Ph (5.25 Å), R = CH_2PhOH (4.57 Å) and R = CH_2COOH (3.25 Å)

Another approach is that of exploitation of the hydrophobic interactions which remain among the principal interactions which are established between the residues preparing the hydrophobic cavity of the enzyme and the hydrophobic part of the ligands during the introduction inside the active site, in order to avoid the exposure to the solvent.

Conclusions

This contribution which consists with the elucidation of the mechanisms of interaction between the enzyme and of the inhibitors in order to establish a correlation between the activity and the energy of interaction of the complexes advances the following assumptions while waiting for the biological tests of these aziridines to validate present model.

In present work, because of the presence of the phthalimido grouping the interaction of van der Waals and cation-π, distances separating the groups from the aziridines and those of the principal amino acids of the side chain of the enzyme, the classification by increasing activity is according to:

$$R = H > CH_2Ph > CH_2PhOH > CH_2COOH$$

while being based on the energy of total interaction, the order is slightly disturbed and as follows:

$$R = H > CH_2COOH > CH_2Ph > CH_2PhOH$$

REFERENCES

1. D.L. Scott, S.P. White, Z. Otwinowski, W. Yuan, M.H. Gelb and P.B. Sliger, *Science*, **250**, 1541 (1990).
2. B.W. Dijkstra, K.H. Kalk, W.G.J. Hol and J. Drenth, *J. Mol. Bio.*, **147**, 93 (1981).
3. E.A. Dennis, *TIBS*, **22**, 1 (1997).
4. R.D. Bach and O. Dmitrenko, *J. Org. Chem.*, **67**, 3884 (2002).
5. H.M.I. Osborn, *J. Tetrahedron Asym.*, **8**, 1693 (1997).
6. Y.Wang and S. Kohn, *J. Am. Chem. Soc.*, **124**, 4666 (2002).
7. S. Gabriel, *Chem. Ber*, **21**, 1049 (1888).
8. a) W.H. Pearson, B.N. Lian and S.C. Bergmeier, in eds.: A.R. Katritsky, C.W. Reese and E.F. Scriven, Comprehensive Heterocyclic Chemistry II, Vol. 1A, p. 1 (1996); b) D. Tanner, *Angew. Chem. Int. Ed.*, **33**, 599 (1994).
9. I. Erden, in eds.: A.R. Katritsky, C.W. Reese and E.F. Scriven, Comprehensive Heterocyclic Chemistry II, Vol. 1A, p. 97 (1996).
10. T. Aratani, E.N. Jacobsen, A. Pfaltz and H. Yamamoto, *Springer*, **3**, 1451 (1999).
11. R.H. Garrett and M.C. Grisham, Biochemistry, Brooks & Cole, edn. 3 (2004).
12. W. Medjahed, A.T. Zatla, J.K. Mulengi, F.Z.B. Ahmed and H. Merzouk, *Tetrahedron Lett.*, **45**, 1211(2004).
13. D.A. Case, D.A. Pearlman, J.W. Caldwell, T.E. Cheatham III, W.S. Ross, C.L. Simmerling, T.A. Darden, K.M. Merz, R.V. Stanton, A.L. Cheng, J.J. Vincent, M. Crowley, V. Tsui, R.J. Radmer, Y. Duan, J. Pitera, I. Massova, G.L. Seibel, U.C. Singh, P.K. Weiner and P.A. Kollman, Programme Amber 6, University of California, San Francisco (1999).
14. A. Imberty, K.D.Hardman, J.P. Carver and S. Pérez, *Glycobiology*, **1**, 631(1991).

(*Received*: 1 May 2006; *Accepted*: 26 October 2006) AJC-5225