# Microelectronic Aspects of Hydrogen Bond Characteristics in Active Site of β-lactamase during the Acylenzyme Reaction

Rostislav Rusev, George Angelov, Elitsa Gieva, Boris Atanasov, Marin Hristov

*Abstract* – **The behavior of hydrogen bonding networks in active site of β-lactamase during the acylenzyme reaction cycle is studied. Proton transfer through the HBNs is simulated during the four intermediates of the acylenzyme reaction: free enzyme, Michaelis complex, transient state and acylenzyme. The characteristics of each hydrogen bond are calculated by Markus theory and theory of protein electrostatics. The results showed the similarity of HBN characteristics to characteristics of microelectronic devices such as: double gate MOSFET, current source, and amplifier.**

*Keywords* – **Hydrogen bonding networks, acylenzyme reaction, proton transfer, microelectronic devices.**

## I. Introduction

Integration of biomolecules in electronics is a new field known as bioelectronics. The main activities in bioelectronics include development of biosensors that convert biocatalytic processes in electronic signals [1]-[2]. Another field of research on DNA is the development of memories and biocomputers [3]. The direct use of charge transfer functions of the proteins is also field of interest for investigation. The theory of Markus [4], the superexchange charge transfer theory, and the definition of superior tunneling paths in proteins [5] allowed to study the charge transfer through proteins such as bacteriorhodopsin [6], cytochrome *c*, β-lactamase, etc.

In our previous papers [7], [8] it is shown that proton transfer takes place through the hydrogen bonds of the β-lactamase. The hydrogen bonds characteristics are compared to characteristics of various electronic elements such as transistors, amplifiers, filters, currents sources, decoders, etc. These hydrogen bonding networks (HBN) extracted from β-lactamase, consist of residues in the periphery of the protein and water molecules. B. Atanasov et. al [9] assume that proton transfer in the active site HBNs of the β-lactamase is performed during the interaction of the protein with the ligand (acylenzyme reaction). The catalyzed β-lactam nitrogen protonation is supposed to be energetically favored as the initiating event, followed by nucleophilic attack on the carbonyl carbon of the b-lactam group. Nitrogen protonation is catalyzed through a hydrogen bonding network involving the 2-carboxylate group of the substrate, S130 and K234 residues, and a water molecule. The nucleophilic attack on the carbonyl carbon is carried out by the S70 with de-protonation abstraction catalyzed by a water molecule hydrogen-bonded to the side chain of E166.

In the present paper the acylenzyme reaction cycle will be studied starting from the free enzyme (E), going through the Michaelis complex (ES), transient state (T1) and finishes with acylenzyme (EY). In particular, the formation of HBNs in the active site of β-lactamase will be studied. The change of proton transfer characteristics of each hydrogen bond depending upon the ligand-protein interaction cycles (E), (ES), (T1) and (EY) will also be examined. Hydrogen bond characteristics will be compared to characteristics of known microelectronic devices; in addition, analogy between the ligand and microelectronic device will be also specified.

## II. Materials and Methods

The x-ray crystallography of the free enzyme TEM-1 β-lactamase (1BTL) and the acylenzyme is taken from PDB [10]. The Michaelis complex (ES) and the transient state (T1) of β-lactamase are modeled on the base of iterative application of molecular mechanics/dynamics techniques at some fixed atoms in the active site. The electrostatic potentials and the pK of all protein residues are calculated depending on the pH through PHEPS server [11]. The electrostatic potentials and pK (and the free energies respectively) of donors and acceptors from HBNs in the active site including the ligand are computed as well.

For calculation of the proton transfer it is used dedicated software based on Markus theory with the parameterization given in [12]. The program code uses the following analytical relationships:

$$K = \frac{k_B T}{2\pi} \exp\left(-\frac{Eb - h\omega/2}{k_B T}\right) \qquad (1)$$

where $K$ – speed constant, $k_B$ – Boltzmann constant, $Eb$ – barrier energy, $h$ – Plank constant, $\omega$ – frequency, $T$ – temperature in Kelvins.

The energy barrier is calculated by:

$$Eb = (s_A(R(DA) - t_A)^2 + v_A) + s_B E_{12} + (s_C \exp(-t_C(R(DA) - 2)) + v_C)(E_{12})^2 \qquad (2)$$

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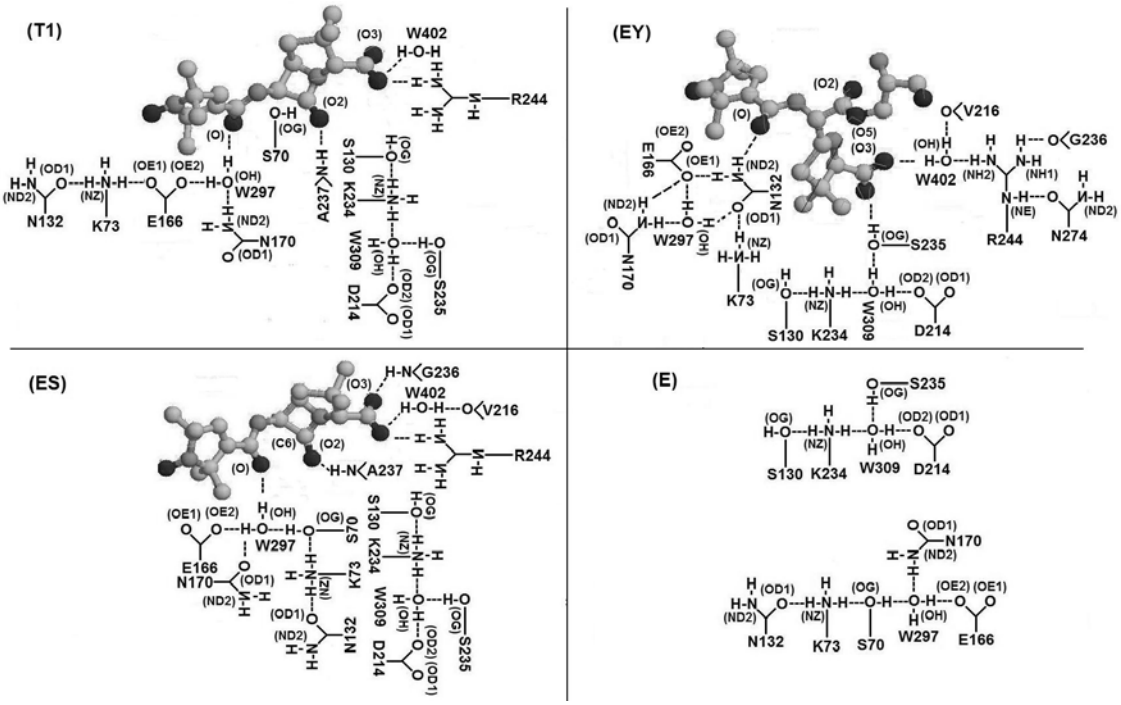


FIGURE 1. ACETYLENZYME REACTION INTERMEDIATES – HBNS IN THE ACTIVE SITE OF: (E) FREE ENZYME, (ES) MICHAELIS COMPLEX, (T1) TRANSIENT STATE, (EY) ACYLENZYME.

where *R(DA)* – distance between donor and acceptor, $E_{12}$ – the difference between the energies of donor and acceptor (cf. two-well potential), the values of the rest of the parameters are taken from [12].

## III. RESULTS AND DISCUSSION

Figure 1 shows the HBNs formed in the active site of $\beta$-lactamase: (E) the networks in the active site of the free enzyme, (ES) the formation of Michaelis complex, (T1) the transient state of reaction where the networks change due to the nucleophilic attack by S70 (the distance to C(6) is 1.53 Å), (EY) the end of the reaction when the acylenzyme is formed up and the networks of hydrogen bonds have changed due to the opening of the ligand ring and the combination of the ligand with S70.

Because many authors consider that the acyl reaction goes together with proton transfer, the proton transfer through each hydrogen bond in each reaction intermediate is simulated. This proton transfer is also compared to the current flow through known electronic elements. Besides, the ligand formation and its charge in active site, strongly affects proton transfer through the HBNs. Thus, the study of response in the HBNs will clear out the role of the ligand and it will be compared to microelectronic devices.

There are two HBNs participating in the acylenzyme reaction. The first HBN, referred to as nucleophilic, consists of residues S70, w297, N170, E166, K173, N132. The second HBN, referred to as electrophilic, consists of S130, K234, w309, D214, S235. It should be noted that there is proton transfer in parallel in both the two HBNs during the different intermediates of the reaction.

In the first HBN (nucleophilic), participates S70; that is why it plays major role during the start and during the end of the reaction. Our simulations showed that the distance between S70 and C(6) decreases from 2.89 Å in the Michaelis complex (ES) to 1.53 Å in the transient state (T1). This attack is accompanied by proton transfer between S70(OG)....(OH)297w; the dependence of proton transfer parameter *K* from *El.Pot.* is given in Figure 2.

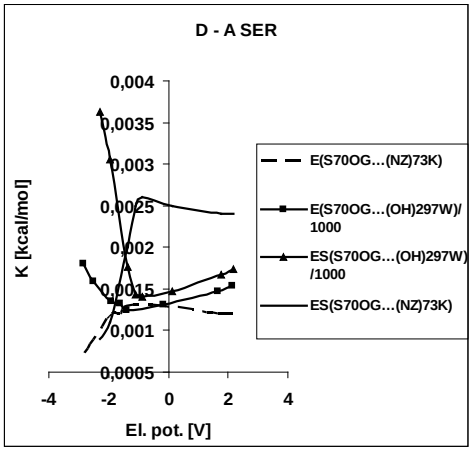


FIGURE 2. *K* VS *EL. POT.* OF O-ATOM OG OF SERINE RESIDUE S70 FOR THE FOUR INTERMEDIATES OF THE ACYLENZYME REACTION.

These characteristics are similar to a mirrored MOSFET characteristics and their level shifts during the different stages of the reaction. This shift is due to the difference in distances between donor and acceptor and the charge introduced by the ligand.

In the transient state (T1) the proton transfer should be finished and the nucleophilic attack of S70 to C(6) should be started. The proton transfer arises on condition that the water also participates in the proton-donor reaction. The water molecule donates a hydrogen and forms a hydrogen bond E166(OE2)…(OH)297w. The proton transfer characteristic of hydrogen bond E166(OE2)…(OH)297w is given in Figure 3.

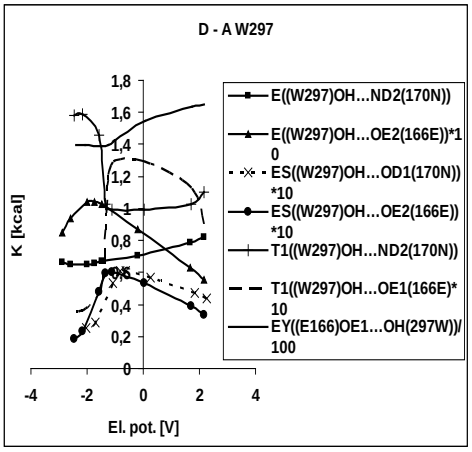


FIGURE 3. *K* VS *EL. POT.* OF O-ATOM OH OF WATER MOLECULE W297 FOR THE 4 INTERMEDIATES OF THE ACYLENZYME REACTION.

As expected, the proton transfer has increased as the reaction cycle compared to the stage of acylenzyme. This increase is determined by the correlation between the two proton transfers in the pair E166(OE2)…(OH)297w and S70(OG)....(OH)297w where the water molecule acts as both donor and acceptor. The increase is also caused by the ring break which drives the residues towards the active site. The proton transfer in E166(OE2)…(OH)297w at the acylenzyme stage is large because the distance between the donor and acceptor decreases (2.55 Å). At such distances appears the so called strong hydrogen bond, where proton is located around both heavy atoms of the hydrogen bonds.

On the other hand the proton transfer at the intermediate of the Michaelis complex is less than the transfer at the free enzyme intermediate. This phenomenon is most likely caused by the N170 residue that also forms chemical bond with w297. In the free enzyme this residue appears as proton donor in N170(NH2)...(OH)297w. After bonding of the ligand (the ES complex), N170 undergoes a conformation change (180° rotamerization around $C^{\beta} - C^{\gamma}$) and transforms itself into proton acceptor of (OH)297w, together with E166(OE2); in transient state (T1) it also transforms itself in proton donor. In EY stage there could not be realized a proton transfer between N170ND2….OH289w because the distance between the two residues is 3.23 Å.

From microelectronics point of view, the characteristics of ES(W297)OH…OE2(166E), ES(W297)OH…OD1(170N), T1(W297)OH…OE1(166E) are similar to a tunnel diode; the characteristics of T1(W297)OH…ND2(170N) are similar to a reverse diode, the characteristics of EY(W297)OH…OE1(166E) are similar to transfer characteristic of double-gate FET. The residues theirselves can functionally operate as: signal adders E166, the water molecule w297 acts as both adder and splitter of signals since it is both donor and acceptor.

On the other hand S70(OG) forms hydrogen bond with K73(NZ). Many authors note that this is an alternative way for deprotonization of S70(OG) at the beginning of the nucleophilic attack. In this reaction K73(NZ) should be acceptor of S70(OG) and both donor of N132(OD1).

The parameter calculations of proton transfers (*K*) as a function of *El.Pot.* for S70(OG)...K73(NZ) and K73(NZ).... N132(OD1) are given in Figures 4. It is observed that during the process of proton transfer between S70(OG)...K73(NZ) there is no proton transfer between K73(NZ).... N132(OD1) : *K* (*El.pot.*) = 0.0001. For intermediate EY when the reaction is over the b-lactam the ring is opened and this causes rearrangement of the hydrogen bonding network. We can conclude that probability of K73 to become a proton acceptor of S70(OG) and to participate in the nucleophilic attack is little since this could have affected the proton transfer parameter characteristics of K73(NZ)....N132(OD1).

From microelectronics point of view, the *K*(*El.Pot.*) characteristic of K73(NZ).... N132(OD1) hydrogen bond is similar to current source for intermediates E, ES, T1; *K*(*El.Pot.*) characteristic is similar to FET for intermediate EY.

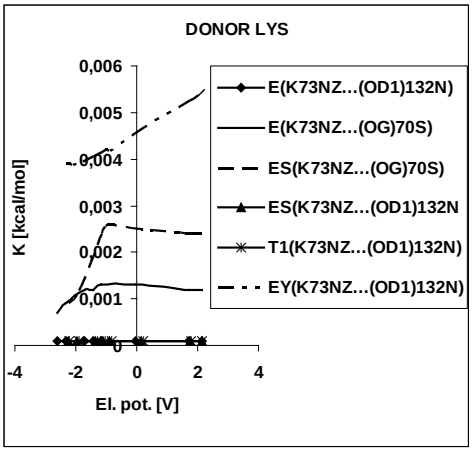


FIGURE 4. *K* VS *EL. POT.* OF N-ATOM NZ OF LYSINE RESIDUE K73 FOR THE FOUR INTERMEDIATES OF THE ACYLENZYME REACTION.

The second HBN in the active site which is influenced by the enzyme-substrate reaction is noted above as electrophilic chain. The results show that proton transfer takes place not only in the nucelophilic HBN but also in the electrophilic HBN. It is supposed that S130OG attacks electrophilic N(4) from the substrate forming a hydrogen bond with it. The simulations show that the distance between S130OG….N(4) is 3. 30 Å in the Michaelis complex and 3.08 Å in T1 state. Probably S130OG begins to exert electrostatic influence on the acylenzyme reaction at intermediate ES rearranging the charges in the ring. Its influence increases in transient state (T1) by forming hydrogen bond with N(4) allowing for the start of the nucelophilic attack by S70OG.

This electrophilic reaction affects the proton transfer between the other members of the electrophilic HBN. In Figure 5 it is shown that the parameter of the proton transfer between the pair K234NZ…(OG)130S increases during the reaction course E→ ES →T1: the form of the curves is the same but the curves are shifted to each other. This phenomenon is due to the electrostatic influence of the ligand on the electrophilic HBN in the active site. In the intermediate EY of the reaction the characteristic of K234NZ…(OG)130S differs from the previous characteristics for intermediates E, ES, T1. This is due to the mechanical influence of the ligand on the electrophilic HBN in the active site.

In the same Figure 5 it is shown the proton transfer between the pair K234(NZ)…(OH)309w. It can be seen that the curves are similar to each other and the proton

transfer parameter decreases at stages E→ ES →T1 to EY.

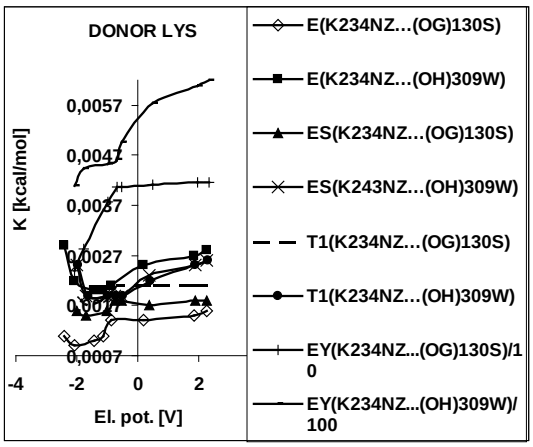


FIGURE 5. *K* VS *EL. POT.* OF N-ATOM NZ OF LYSINE RESIDUE K234 FOR THE 4 INTERMEDIATES OF THE ACYLENZYME REACTION.

From microelectronics point of view, the bonds K234(NZ)…(OH)309w and S130OG….(NZ)234K can be compared to the characteristics of double gate MOS transistor for the stage E → ES → T1 (but for the intermediate EY the characteristics are different). The reason again is the rearrangement of the HBN at the end of the acylenzyme reaction.

Generally, at each intermediate of the reaction the charge distribution in the substrate changes; it also affects the electrophilic HBN and the proton transfer in it. This influence decreases after W309. In Figure 6 there are shown proton transfers parameters between K234(NZ)…(OH)309w и W309(OH)….(OG)235S. It is clear that they do not change at stages E→ ES →T1 which means that the electrostatic influence of the ligand decreases through the HBN.

An exception is the proton transfers at stage EY when acetylenzyme form strongly rearranges the network and changes the distances between donors and acceptors. These characteristics are similar to current source for the hydrogen bond w309(OH)….(OG)235S and the characteristics of the w309(OH)…(OD2)214D bond can be compared to amplifier characteristics.

It is also observed that the ligand itself rearranges the HBNs and with its charge it changes and controls the characteristics of the individual hydrogen bonds. This is similar to function of control gate voltage in the MOS transistor device. Finally, we may conclude that the ligand acts like a control switch in relation to the HBNs in active site by rearranging them by means of mechanical and electrostatic interaction.

## IV. CONCLUSION

Results proved that hydrogen bonds in active site of $\beta$-lactamase during the acylenzyme reaction cycle are functionally similar to microelectronic devices. The obtained set of characteristics of some hydrogen bonds depend on the charge of the ligand (which is an external charge to the protein electrophilic HBN) similarly like the output set of characteristics of MOS transistor depend on the external controlling gate voltage, i.e. the ligand acts like a control terminal. Other hydrogen bonds behave like current source, amplifier.

Finally, it can be concluded that the electrophilic and nucleophilic networks of hydrogen bonds in the active site can operate like an integrated circuit consisting of individual devices in the form of hydrogen bonds.

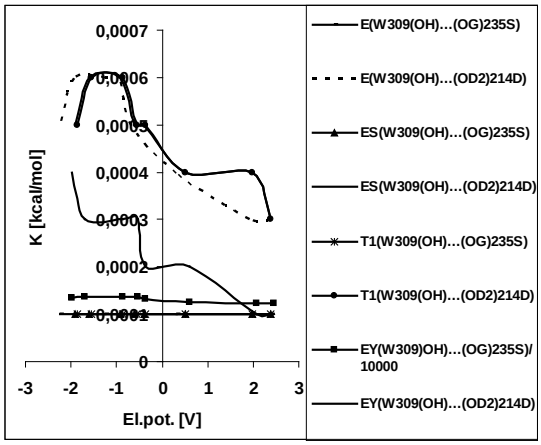


FIGURE 6. *K* VS *EL. POT.* OF O-ATOM OH OF WATER MOLECULE W309 FOR THE 4 INTERMEDIATES OF THE ACYLENZYME REACTION.

## ACKNOWLEDGEMENT

The research is related to the project No BG051PO001-3.3.06-0046 “Development support of PhD students, postdoctoral researchers and young scientists in the field of virtual engineering and industrial technologies”. The project is implemented with the financial support of the Operational Programme Human Resources Development, co-financed by the European Union through the European Social Fund.”

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