INVESTIGATION ON THERMODYNAMIC VARIABLES OF THE ENZYME DURING THE CRYOTHERAPY

Mehrad Gavahi\textsuperscript{a}, Hong-Rong Li\textsuperscript{b}

\textsuperscript{a} School of Science, Xi'an Jiaotong University, Xi'an 710049, China.
\textsuperscript{b} School of Science, Xi'an Jiaotong University, Xi'an 710049, China.

Received: 19.12.2019 Revised: 24.01.2020 Accepted: 23.02.2020

Abstract

In this paper, we present the thermodynamic behavior of an artificial enzyme when cells are affected by the cryotherapy. In order to illustrate the Gibbs free energy and the enthalpy of the enzyme at the extreme temperature, by using the concept of the thermodynamic laws and kinetics of the enzyme, we measure the thermodynamic variables of the ground state and the transition state of the specific artificial enzyme at the different temperatures during cryotherapy. The results can be used to explain that, cells could survive at the extreme temperature below the freezing point by changing the activation enthalpy and the enthalpy.

Keywords: Classical Physics; Thermodynamics; Enzyme; Biological Physics; and Cryotherapy.

© 2019 by Advance Scientific Research. This is an open-access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/) DOI: http://dx.doi.org/10.31838/jcr.07.04.02

INTRODUCTION

The cryotherapy, or the cryosurgery, is a treatment for the cancer cells. The therapy is used for the cancer cells on the skin or inside the body by spraying or injecting the liquid nitrogen in that area. Temperature of the cancer area will be dropped below the freezing point owing to the injection of the liquid nitrogen. After the therapy, the temperature of that area returns to the normal temperature, however the normal cells on that area could survive [1,2]. Surviving of the normal cells at the extreme cold temperature motivate us to investigate the behavior of the cells at this kind of temperature.

During the recent decades, the catalysis and behavior of the enzyme have drawn more attention of many scientists. Enzyme is an organic system playing an important role in the living systems. Even the living systems have various kinds of enzyme, the function of the enzyme are the same. Enzyme accelerates the rate of all chemical reactions by catalysis, and this behavior of the enzyme is vital for life [3]. This high rate of reaction which is often studied in the branch of thermodynamics is called enzyme kinetics. This paper would like to extract thermodynamic information of the enzyme while encountering the cryotherapy.

The biological thermodynamics is a cross domain of physics and biology, which deals with the relation between energy, work, heat and temperature in the living organisms. We will focus on the Gibbs free energy for the enzyme. According to the first-law and second-law of thermodynamics, the Gibbs free energy explains the relations between the entropy and the enthalpy.

In terms of thermodynamics, cells are the isothermal system. The function of them has the essential constant temperature and the constant pressure. The energy that cells using is free energy, described by the Gibbs free- energy "G". The Gibbs free energy could announce the direction of the reactions [4].

Enzyme and operation

Enzyme is organic particle living in the cells. Enzyme is important for life to serve function of the body, such as digestion and metabolism. Enzyme accelerates the rate of all chemical reactions in the cells by decreasing the activation energy. Two theories can be used to explain how does enzyme work, the lock-and-key model and the induced-fit model. In the present article, we consider the lock-and-key model. In this model, enzyme has an active site where the substrate can bind to it. The active site of the enzyme and the substrate have the same shape, when the substrate exactly bind to the active site, the chemical reaction begins and then affects the substrate. Because of the chemical reaction, the temperature rises and the substrate turns to the product. The product finally leaves the active site, and it is used by the cells or moves to another reaction (Fig.1).

The lock-and-key model can be represented with the following equation:

\[ E + S \leftrightarrow ES \leftrightarrow [ES]^* \leftrightarrow E + P. \]  

Where E and S are the enzyme and the substrate, respectively. ES is the enzyme/substrate complex, and [ES]^* is the enzyme/substrate complex in the transition state, and P is the product.

![Figure 1: The enzyme has an active site which the substrate can bind into it. The chemical reaction happens between the substrate and the enzyme while the substrate and the enzyme bind to each other, and then temperature rises because of the chemical reaction. The chemical reaction causes the substrate converts to the product and finally the product leaves the enzyme.](image1)

![Figure 2: Potential energy diagram for the lock-and-key model.](image2)
this reaction is exothermic and release heat (ΔH).

Enzyme activity is a key factor for the description of catalysis of
the enzyme. It is usually introduced by percentage over
temperature. There are two methods to measure the enzyme
activity. One is measuring the decreasing concentration of the
substrate under process. Another is to measure the increasing
concentration of the product after reaction process [5] Enzyme
activity = rate × reaction volume i.e. moles of substrate
converted per unit time or mole of product produced per unit
time. Enzyme activity is dependent on conditions and measured
by experimental.

As shown in Fig 3, the enzyme activity increases with
temperature till the enzyme begin to denature. It is means that,
when the substrate binds to the enzyme, temperature rises due
to chemical reaction until the enzyme begin to deform causing
by the high temperature. After this temperature, the enzyme
activity decreases dramatically because that the enzyme is
deformed and the substrate is separated from the active site of
the enzyme.

In this article, we used the changing concentrations of the
reactants and the products that participated in the enzyme
reaction. Changing concentrations of the reactants and the
products do not usually occur before any reactions in the living
systems, however, the living cell to follows the hyperosmotic
pressure, if the environment’s temperature and pressure
changed, the result will change concentrations of the products
and reactants inside the cells.

ATP, ADP and ATPase
Almost all essential processes in the human body work by
adenosine triphosphate, called ATP. ATP is the energy currency
of the cells. This energy is used by molecules, metabolism and
tissue. ATP could be found in the DNA or RNA and it is made up
of phosphate groups, a ribose sugar, and nitrogenous base is
called adenine.

By the series reactions, ATP hydrolysis convert to ADP
(Adenosine diphosphate) and phosphate, ADP is converted to
AMP, and AMP will be used by the molecules.

\[
\begin{align*}
\text{ATP} + \text{H}_2\text{O} & \rightarrow \text{ADP} + \text{P}, \\
\text{ADP} + \text{H}_2\text{O} & \rightarrow \text{AMP} + \text{P}.
\end{align*}
\]

Due to high rate of the activation energy of ATP, the chemical
reaction (2) happened only by presence of the enzyme. The
name of the enzyme converts ATP to ADP and phosphate is
called ATPase.

The reaction energy depends on the intracellular conditions,
like pH, ionization, concentration, etc. In order to extract
the thermodynamic information, we will consider the
concentrations of reactants and products of the reaction before
and after the reaction. The concentrations of ATP, ADP, P and
ATPase are variables which are dependent of what kind of cell
selected. Table 1 show the concentrations of ATP, P, ADP for the
different cells.

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>ATP</th>
<th>ADP</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat hepatocyte</td>
<td>3.38</td>
<td>1.32</td>
<td>4.8</td>
</tr>
<tr>
<td>Human erythrocyte</td>
<td>2.25</td>
<td>0.25</td>
<td>1.65</td>
</tr>
<tr>
<td>E. coli cell</td>
<td>7.90</td>
<td>1.04</td>
<td>7.9</td>
</tr>
</tbody>
</table>

The concentrations of ATP, ADP, P for rats, human, and E. coli
unit of concentration is presented by mM [7].

Thermodynamics and Enzyme Kinetics:
The Biological Thermodynamics is about the quantities of
energy as chemical processes occurring in the living systems
such as cells, tissues, etc. A common fundamental property of all
living systems is that thousands of the chemical reactions
happen at a low temperature with a high rate of reaction. The
high rate of reaction at a low temperature occur because of the
enzyme (enzyme-catalysis). This high rate depends on
temperature, pH, ionic strength and concentrations of reactants
[9].

In this section we would like to investigate the relation between
the thermodynamics and the enzyme. The enzymatic reactions
are usually exothermic i.e. the heat flows (ΔH) to the system,
and this reaction follows the transition theory.

For the chemical reactions, the Gibbs free energy can be measured by this equation:

\[
\Delta G = \Delta G_0 + RT\ln K_{eq}
\]

where \(\Delta G_0\) is the standard Gibbs free energy, R is molar gas constant, T is temperature and \(K_{eq}\) is equilibrium constant. For instance, in the process of

\[
A + B \leftrightarrow C
\]

\(K_{eq}\) is measured by this equation:

\[
K_{eq} = \frac{[C]}{[A][B]}
\]

where \([C]\), \([A]\) and \([B]\) are concentrations of the product and reactants.

\(\Delta G\) is dependent of many variables and it has definite physics
means that, \(\Delta G > 0\): the reaction is not spontaneous; \(\Delta G < 0\): the reaction is spontaneous; \(\Delta G = 0\): the reaction stays in
equilibrium. The Gibbs free energy of the enzyme with respect
to the exothermic reactions is spontaneous. The Gibbs free
energy can be decomposed to the entropy and the enthalpy:

\[
\Delta G = \Delta H - \Delta T\Delta S
\]

where \(\Delta H\) is enthalpy, and \(\Delta S\) is entropy. The enthalpy in
biology refers to the energy stored in bonds, and the entropy
means disorganization or disorder of the system.

Before introducing the rate constant of the reaction, we should
mention that the enzyme follows the transition state theory for
catalyzing the reactants. The fundamental property of the
enzyme is to reduce the activation energy, and to cause lower
activation energy, the rate of reaction increased. The high rate
of reaction is considered and studied in the enzyme kinetics.

The Ground State, the Transition State and Enzyme Kinetics
For interpretation of the exothermic reaction of enzyme and
with respect to ground state (E + S) and transition state
([ES]*), we can express:
\[ E + S \leftrightarrow [ES]^* \rightarrow P + \text{energy} \]  \hspace{1cm} (8)

\[ V = \frac{d[P]}{dt} = K[E][S] = K'[X] \]  \hspace{1cm} (9)

where the factor of \( K \) is reaction rate constant. During the reaction, two involved molecules must encounter and collide each other, and the Arrhenius law is must satisfied.

The real mechanism of the operation of enzyme is interpreted by equation (1), if we use \([X]\) to denote the transition state \([ES]^*\), then we get:

\[ V = \frac{d[P]}{dt} = K[E][S] = K'[X] \]  \hspace{1cm} (10)

Supposed that the reactants and transition state stay in the equilibrium, then the equilibrium constant is:

\[ K_{eq} = \frac{[X]}{[E][S]} \]  \hspace{1cm} (11)

We should mention if system stays in equilibrium, the activation Gibbs free energy equals:

\[ -RT\ln K_{eq} = \Delta G^* \]  \hspace{1cm} (12)

And

\[ K_{eq} = e^{-\frac{\Delta G^*}{RT}} \]  \hspace{1cm} (13)

According to equations (9) and (12), we have:

\[ V = K'e^{-\frac{\Delta G^*}{RT}}[E][S] \]  \hspace{1cm} (14)

The rate constant of the transition state is an order for transition state converting to the product. The rate constant is described by frequency. Thus, we could rewrite the rate as:

\[ K = kv \]  \hspace{1cm} (15)

where \( v \) denotes the vibration frequency and \( k \) provides a measure for generating the products versus regenerating the reactants. Here we assume that value of \( k \) is 1 without loss of generality, it means that all value of \([X]\) generates the products.

The vibration bond energy with respect to Plank's law is \( E = KBT \), on another hand \( E = hv \), then we are able to write:

\[ K' = \frac{K'}{h} \]  \hspace{1cm} (16)

By combining equations (9), (13), (15), and (16), we obtain:

\[ K = -\frac{\Delta H^*}{h} e^{-\frac{\Delta G^*}{RT}} \]  \hspace{1cm} (17)

and then get:

\[ V = \frac{K'}{h} e^{-\frac{\Delta G^*}{RT}}[E][S] \]  \hspace{1cm} (18)

In this research, the activation Gibbs free energy is found by equation (17), the rate constant between reactants and transition state has name, called catalysis rate constant \( K_{cat} \). Heat capacity \( (\Delta C_p) \) is a key factor in the biological reaction, where \( \Delta C_p = \frac{2mv}{RT} \). At the modest range temperature, the heat capacity for many cases is constant. The transition state heat capacity of the cold adaption enzyme falls between negative sign and positive sing [8]. Therefore, the enthalpy can be found by:

\[ \Delta H = \Delta H_{ref} + \Delta C_p (T - T_{ref}) \]  \hspace{1cm} (19)

The entropy can be measured by:

\[ \Delta S = \Delta S_{ref} + \Delta C_p \ln \frac{T}{T_{ref}} \]  \hspace{1cm} (20)

2.3. The Arrhenius Law

Activation energy is the least possible amount of energy which is required to begin a reaction. Enzyme follows and works by transition state theory, the amount of energy needs to start a reaction (activation energy) should be more than the transition state.

The Arrhenius Law can be denoted by:

\[ K = A e^{-\frac{E_a}{RT}} \]  \hspace{1cm} (21)

where \( K \) is the rate constant, \( A \) is a pre-exponential factor (independent of temperature) and \( E_a \) denotes the activation energy.

As we mentioned, the enzyme increases the reaction rate by decreasing the activation energy. This statement is valid while the balance between the entropy and the enthalpy happens. The enzyme increases the rate of reaction by decreasing the activation enthalpy. The rate constant is depended on the temperature, while temperature changes, the rate constant of reactions changes, with supposing that all reactions are activating processes. The rate constant is exponentially dependent on the temperature by:

\[ \ln \left( \frac{K_2}{K_1} \right) = \frac{E_a}{R} \left( \frac{T_2 - T_1}{T_1 T_2} \right) \]  \hspace{1cm} (22)

The activation energy is independent of the temperature.

Cold Adaption of the Enzyme and the Cryotherapy

The enzyme is able to catalyze the reactant under the extreme temperature (freezing point), where, the relativity activation of the enzyme is as same as the enzyme is living at room temperature. According to this property, the enzyme can survive under the extreme temperature conditions. Cold-adapted enzymes reduce thermal stability. These kinds of enzyme reduce activation enthalpy and more negative activation entropy. The flexibility of the active site is the reason for reducing the entropy of the enzyme [9,10].

According to equations (16) and (19):

\[ K = K' e^{-\frac{\Delta G^*}{RT}} e^{-\frac{\Delta S^*}{hRT}} \]  \hspace{1cm} (23)

the lower \( \Delta H^* \) makes the rate less temperature dependent, and the smaller the activation enthalpy corresponding to more negative value of \( \Delta S^* \) which means flexibility of the active site [11].

Cryotherapy

The cryotherapy or cryosurgery is the treatment of the cancer cells (tumor). Such treatment depends on which part of human body is faced with this disease, but the main methods are the same. In this treatment, the temperature of the tumor decreases between −20°C to −60°C (depend on kind of cancer) around average 10 minutes, and this treatment continues until destruction of the whole tumor. The normal cells around the tumor are involved and normally all of them will survive. Metabolism and structure of cells will be affected while temperature dropping lower, the result of dropping temperature is begin to damage the cell, the cell death might happen if it is continued for a long time. During the injection of liquid nitrogen to the area of tumor, the water around the cells commence to crystalize as the temperature falls to 0°C. Crystal formation firstly occurs in the extracellular spaces, and due to hyperosmotic effect, the cell begins to dehydrate i.e. water in intracellular space leaves the cell, called cellular dehydration. This happens at temperature below the freezing point. The cellular dehydration often destroys cells in case that it continues.

Rapid cooling treatment does not allow time for water to leave the cell dramatically. Because the water stays inside cells, which will continue the metabolism and survive. Many cells have water crystal formation in intracellular space during the temperature drops below the freezing point [12,13].

This article would like to illustrate how the cells are able to survive at the extreme cold temperature and continue metabolism. Metabolism inside the cells happens by the
In the next section we will define a physical model, which present the assumptions of our work, measure the thermodynamic variables and consider the enzyme kinetics during the process in which the artificial cell encountered the cryotherapy.

Physical Model and Measurement:
The rapid cooling treatment is the reason for cells survival while the extracellular temperature drops below the freezing point. This situation gives us motivations for investigating the behavior of the enzyme during the cell is affected by the rapid treatment. As we have explained before, enzyme and substrate can bind by lock-and-key model. We here assume that, on one hand, while the enzyme and the substrate are binding to each other, there are exactly locked to each other and there is no way for separation i.e. $k = 1$. On the other hand, the concentrations of the reactants and products will be changed during the time the cells are affected by Cryotherapy. The cell has certain concentrations of the products and reactants at the human body temperature. While temperature decreases, due to the hyperosmotic pressure, the cell begins to dehydrate. The dehydration of the cell causes changing volume of the cell, and concentrations of the substrates and products in the intracellular space increase.

The most energy required for the reactions in the intracellular space is transported from ATP hydrolysis. In the presence of ATPase, ATP reacts with the water molecule and breaks bonds. Then the new bonds formation, ADP, and P release. We would like to probe the effects of changing concentrations and temperatures on enzymatic thermodynamic while the cell affected by cryotherapy.

On the one hand, according to property of the cold adaption enzyme, the cold adaption enzyme could be existed by decreasing the activation enthalpy and activation entropy, and on the other hand, the cold adaption of the enzyme just focuses on the activation state (transition state). Because temperature and pressure on the living cell are usually constant, we can use $\Delta G_p$ for achieving the activation enthalpy and activation entropy. However, in this special case, when the cells encounter the cryotherapy, the temperature and pressure of the cells dramatically change and we suppose that these variables affect the ground state (i.e. changing temperature affects volume of the cell, because of changing volume, the concentration of the products and reactants changes. In this research, for simplicity, we assume that first the temperature and concentration of the cells change, then the enzyme gets active and begins to catalyze. This assumption is like the steady state. It means for the ground state, the thermodynamic variable depends on the concentration and temperature, and for the transition state, we can use the heat capacity for the constant pressure for achieving thermodynamic variable for the activation state).

We suppose that the cells involved by the cryotherapy have different temperature as shown at Fig. 4. By injecting the nitrogen liquid to the tumor area, the temperature of that area decreases to 253K. The nearest cells around the tumor area should have the same temperature with tumor area. As far from that area, the temperature should increase until the cell's temperature is as same as the human body temperature. In this case, we would like to show the thermodynamic variable for the specific temperature as shown in (Fig. 4).

The concentration of the substrates and products changes by the temperature. These concentrations can be achieved by experimental. In this article, we use the literatures for finding the concentrations of ATP, ADP and thermodynamic variables for concluding and solving our problem for the artificial cell. The concentration of ATP, ADP, P is shown in Table 2.

![Figure 4: The nitrogen liquid injected to the tumor area is shown in black. The temperature of the tumor area drops at 253K and the nearest cells around the tumor have the same temperature as the tumor area. As far from that area, the effect of the nitrogen liquid on the temperature decreases. The furthest cells from the tumor area have the body temperature.](image)

As mentioned, there is two state can explain the enzymatic function, the ground state where introduce the Gibbs free energy, enthalpy and entropy, and the transition state where introduce activation the Gibbs free energy, activation entropy and activation entropy.

For the ground state, the Gibbs free energy is calculated by equation (4), because enthalpy of this model depends on the temperature and concentration. It could be calculated by:

$$
\Delta H = -2.303 R \frac{\Delta \log [ATP]}{\Delta \log [ADP]/[P]} \tag{24}
$$

The entropy can be found by equation (7). For the transition state, the activation Gibbs free energy is found by equation (17) and the activation enthalpy is followed by equation (19). Also, the Gibbs free energy for [ES] could be measured by $\Delta G_{ES} = RT \ln K_{m}$. The initial concentrations of [ATP], [ADP] and [P] for the artificial cell are selected 2.25μM, 0.25 μM, 16.5 μM respectively, and $\Delta G_{m} = -35 \frac{\mu \text{M}}{\text{mol}}$ and $\Delta H_{m} = -24 \frac{\mu \text{M}}{\text{mol}}$. According to the changed concentration of the reactants and products with different temperature, the equilibrium constant will be changed by temperature. The results of this measurement are shown in the next section.

<table>
<thead>
<tr>
<th>Table 2:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>T= 298K</td>
</tr>
<tr>
<td>T= 283K</td>
</tr>
<tr>
<td>T= 273K</td>
</tr>
<tr>
<td>T= 263K</td>
</tr>
<tr>
<td>T= 253K</td>
</tr>
</tbody>
</table>

The concentrations of ATP, ADP, and P for the artificial cells with different temperature, while the cell follows the hyperosmotic pressure. Unit of concentration is presented by nM [14].

Measurement and Discussion:
We have measured the theoretical variables of the thermodynamics and enzyme kinetics while the artificial cells encounter the cryotherapy. We suppose that the cells follow the hyperosmotic pressure. The concentration of the products and the reactants increase when temperature decreases. The maximum concentrations of ATP, ADP and P are examined at 273 K, while the water crystal is presented inside the cell. Because much more heat is released from the enzyme and flowed to the cell, this crystal water is converted to the water and the concentration of reactants and products decrease.
As mentioned, by decreasing temperature, the activation Gibbs free energy changed. The balance between the activation enthalpy and entropy allowed our enzyme to work at the extreme temperature. The significant property as shown in this table is the rate of the constant for catalysis (\( k_{cat} \)). As shown, this variable was dramatically changed by decreasing the temperature and we expect after 253K, \( k_{cat} \) changes the sign i.e. the reactants don't intend to change to the product and the equilibrium constant stays in the reactant side.

As we expected, according to the cold adaption enzyme, the activation enthalpy (\( \Delta H^\ddagger \)) decreased while the temperature dropped. This property of the enzyme lets it work at the cold temperature. However due to the hyperosmotic pressure and the change of the concentration on the cells, the enthalpy of reaction breaks, and the result is a dead cell. With respect to the experimental result, the dead cell occurs at -40°C.

**Figure 5:** The activation enthalpy of ATP hydrolysis at 298K is longer than that at 253K. This property allows the enzyme to work at the cold temperature. However, the heat released for this reaction at 253K is more than 298K and this is a reason that the cell does not die at cold temperature.

Decrease of the temperature in the human cell affects the enzyme kinetics as shown in table 4. \( k_{cat} \) dramatically drops. Because of dropping \( k_{cat} \), the maximum velocity of the enzyme decreases. \( V_{max} \) reflects how fast the enzyme can catalyze the reaction. A low value of \( V_{max} \) means that the enzyme does not convert much substrate to the product per unit time. However, the heat input to the cell is more over at the low temperature.

**Figure 6:** Michaelis-Menten equation for temperature 263K (blue) and 253K (orange). The maximum velocity for the above temperatures dramatically differs.

The human cell has several enzymatic reactions that work at different temperatures and different concentrations. Because several enzymes exist in a cell and they can input heat in the environment (cell) at a below temperature, it can be a reason for the cells to not die when encountering the cryotherapy.

**CONCLUSION**

The important side effect of the cryotherapy is involving the normal cells around the tumor area. Because the cell's membrane has the hyperosmotic property, the dehydration of water in the intracellular area is the reason for the cells to start destruction. For this reason, the rapid treatment is presented by the scientists. However, decreasing temperature below the freezing point makes crystal water inside the cells. In this article, we tried to show that the function of enzymes in intracellular area is a reason for the cells to not die. By decreasing the activation enthalpy, enzyme can work at the extreme temperature and it releases the more heat flow to the environment (cell) to raise the temperature for living. In this situation, the rate of reaction of the enzyme decreases. Although, the human cells made up of several enzymes could work at different temperature and different concentrations. Also, we measured the thermodynamic variables for the ground state and transition state of ATPase for the artificial cell. We supposed that as we go farther away from the tumor area, the temperature of the involving cells increases, and this different temperature affects the cells concentrations. Different concentration and temperature are the reasons for changing thermodynamic variables of the enzyme. At low temperature, the enzyme can work by fixing the activation Gibbs free energy. For this reason, the activation enthalpy and the activation entropy will change. High activation enthalpy and entropy are unfavorable and decrease the rate of reaction. Enzyme decreases \( \Delta H \) to increase the reaction rate. Changing concentration and temperature affect enthalpy of the ground state. For this reason, the enzyme releases more heat into the environment. This heat can warm the cell at low temperature and resist for cell destruction. As we mentioned, this research does not probe the ionization and electrostatic of the enzyme. Electron distribution could affect \( k_{cat} \).

**REFERENCE**


**Table 3:**

<table>
<thead>
<tr>
<th>T (K)</th>
<th>( \Delta G ) (( \text{mol}^2 ))</th>
<th>( \Delta H ) (( \text{mol}^2 ))</th>
<th>( \Delta S ) (( \text{K} \times \text{mol}^{-1} ))</th>
<th>( \ln K_{eq} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>298K</td>
<td>-5.550</td>
<td>-2.450</td>
<td>0.102</td>
<td>-6.60</td>
</tr>
<tr>
<td>283K</td>
<td>-4.244</td>
<td>-2.340</td>
<td>0.089</td>
<td>-8.50</td>
</tr>
<tr>
<td>273K</td>
<td>-3.660</td>
<td>-4.384</td>
<td>0.035</td>
<td>-8.20</td>
</tr>
<tr>
<td>253K</td>
<td>-2.554</td>
<td>-3.914</td>
<td>0.050</td>
<td>-8.34</td>
</tr>
</tbody>
</table>

The ground state thermodynamic variables for ATP hydrolysis at different temperatures.

**Table 4:**

<table>
<thead>
<tr>
<th>T (K)</th>
<th>( V_0 )</th>
<th>( k_{cat} )</th>
<th>( k_a )</th>
<th>( k_c )</th>
<th>( K_m )</th>
<th>( K_a )</th>
<th>( K_c )</th>
</tr>
</thead>
<tbody>
<tr>
<td>298K</td>
<td>8.88</td>
<td>1.33</td>
<td>9.1</td>
<td>2.21</td>
<td>1.86</td>
<td>1.36</td>
<td>1.36</td>
</tr>
<tr>
<td>283K</td>
<td>5.91</td>
<td>0.035</td>
<td>0.022</td>
<td>0.015</td>
<td>0.015</td>
<td>0.015</td>
<td>0.015</td>
</tr>
<tr>
<td>298K</td>
<td>5.91</td>
<td>0.035</td>
<td>0.022</td>
<td>0.015</td>
<td>0.015</td>
<td>0.015</td>
<td>0.015</td>
</tr>
</tbody>
</table>
13. Andrew A. Gage, M.D.1,§,* John G. Baust, Ph.D.2 , Technology in Cancer Research & Treatment. ISSN 1533-0346 Volume 3, Number 2, April (2004)