Kinetic Study of Acetyle CoA Carboxylase-1 purified From Serum of Premenopausal Breast Cancer Women

Sabah G Mohammad 1; Susan J Ali 2; Perry H Saifullah 3

1 Tikrit University /College of Education for pure Science /Ph.D. Student sabahgazal@yahoo.com, mobile: 07816436643
2 Tikrit University /College of Education for pure Science Susan.ali@tu.edu.iq,mobile:07736518148
3 Baghdad University/ College of Science for Women afaqperryhabib@gmail.com

Abstract

Acetyle CoA Carboxylase-1 was purified from sera of premenopausal women with breast cancer( before Mastectomy or treatment ) by Gel Filtration using Sephadex G-100 and by Ion Exchange using DEAE-Cellulose A-50, also the molecular weight was estimated by the Acrylamide Electrophoresis in the absence of denaturing elements. The result showed that a single band was obtained at 220KD by Gel Filtering while Ion Exchange showed one band at 200KD. The optimum temperature of purified Acetyle CoA Carboxylase-1 was 40 °C, optimal pH at 7.5 and the optimum substrate concentration at 1.8mM. Michaelis-Menten constant (km) was 0.37Mm and Velocity Maximum (Vmax) was 25mM.min⁻¹ The Activation energy(Ea) was 28 KJ/mol.

keywords: Breast Cancer , Acetyl CoA Carboxylase, activity, purification

Introduction

Breast cancer (BC) is the most frequently diagnosed cancer and the leading cause of cancer death among females worldwide, with an estimated 2,088,849 cases and 626,679 deaths in 2018[1]. Breast cancer classification and patient stratification is crucial in terms of determining treatment strategy in clinic. Breast tumors are classified into two groups: in situ (20% of all cases) and invasive breast tumors (80% of all cases). Based on their location, in situ breast cancers are further classified into two groups: ductal carcinoma in situ (DCIS 80% of in situ breast cancers) and lobular carcinoma in situ (LCIS20% of in situ breast cancers). About 20–50% of DCIS tumors can eventually progress to an invasive carcinoma. Invasive breast cancers have the capacity to spread to other organs in the body[2]. Cancer could be regarded as a systematic dysfunction of metabolic processes. Recently, increasing research findings reveal that lipid metabolism is substantially activated during carcinogenesis and malignant tumor progression (3,4). Acetyl-CoA carboxylase (ACC) (EC 6.4.1.2) is the mediates the first step of fatty acid synthese by carboxylation of acetyl-CoA to form malonyl-CoA and functions as a rate-limiting enzyme in fatty acid synthese. Two isoforms of ACC with distinct subcellular distribution and physiological roles have been identified, of which the cytosolic isoform ACC1 is predominant in control of the fatty acid synthesis, while the mitochondrial isoform ACC2 mainly regulates the fatty acid oxidation through inhibition of carnitine palmitoyltransferase I by localized malonyl-CoA production (5,6). It also regulates oxidation of long-chain fatty acids. There are two closely related isoforms, ACC1, which predominates in the liver and adipose tissue, and ACC2, which predominates in skeletal muscle and heart. Inhibitors of ACC have been sought as potential treatments for metabolic disease and cancer[7]. Despite their diverse substrate specificities, all biotin dependent carboxylases share a common enzymatic mechanism and domain organization. Carboxylation is carried out in two half-reactions and involves three functional components. First, a biotin carboxylase (BC) catalyzes the ATP-dependent carboxylation of a biotin cofactor, which is covalently linked to a conserved lysine of the biotin carboxyl carrier protein (BCCP). Then BCCP translocates to the carboxyl transferase (CT), where the carboxyl group of the carboxybiotin intermediate is transferred to the respective substrate. In this reaction scheme, the BC and BCCP are conserved components, while the CT varies in active-site structure depending on the substrate[8]. ACC was found in human cancerogenic tumors, suggesting this enzyme as a potential target for the treatment of cancer[9].
Material and Methods

Material: All Chemical and Reagents were purchased from Sigma Aldrich / Germany, Fluka/ Switzerland, BDH / England, Pharmacia fine Chemical /Sweden.

Specimens Collection: During this study, collected were (60) blood samples from women with breast cancer and health woman(20), ages ranged between (30-45) years Patients group: collected were40 samples blood of women with breast cancer diagnosed periodically by the Medical City Hospital / Baghdad and the Iraqi Cancer Center / Baghdad for a period (December 2018-September 2019).

Methods:

Estimation of ACC-1 activity: ACC-1 Activity was estimated spectrometry according to Kroeger method \(^{(11)}\).

Estimation of Protein: protein was determined by Kite Biomaghreb Tunis.

Purification of ACC-1: Performed through protein precipitation by 40% Ammonium sulfate, was dissolved in phosphate buffer at (0.1 M,PH 7.8) and proceeded into a further purification by applying into a Sephadex G-100 Column (2.5 x 40 cm) using phosphate buffer (PH 7.8, 0.1 M) as elution buffer. The resultant was extra purified by Ion Exchange Chromatography using DEAE-Cellulose (2.5 x 25 cm) and a washing solution consisting of phosphate buffer Solution (20mM, PH7.8) which contains (10 mM of sodium citrate) and the dilution solution that consists of phosphate buffer (0.13M,0.75M ,pH7.8) which contains (10mM) of sodium citrate\(^{(12,13,14)}\).

Electrophoresis: Used Garfine method for electrophoresis Polyacrylamide under non denatured condition\(^{(15)}\).

The molecular weight of the purified enzyme ACC-1 was measured compared to standard proteins standard proteins solution: Prepare to dissolve standard proteins Bovine albumin, albumin Eggs, Chymotrypsinogen, Lysozyme, Glucose Oxidas, Alcohol dehydrogenase and Immunoglobulins In the sample solution at a concentration of 5 mg / ml.

Temperature Effect: The effect of temperature on the purified enzyme(ACC) from sera at different temperatures (20,25,30,37,40,45,50\(^{\circ}\)C) and the temperature was adjusted by thermostat and the activity was measured used Kroeger method\(^{(11)}\).

The activation energy (Ea) is calculated by drawing the relationship between 1 / T (K-1) against log Vmax as shown in Figure (6) represented by the following Arenos equation:

\[
\log A = \frac{-Ea}{2.3R} \times \frac{1}{T} + \log k, \quad \text{Slop} = \frac{-Ea}{2.3R}
\]

pH Effect : The studied effect of the acid function on the purified serum solution the reaction mixture solutions were prepared in different acid function (4.5,5,5.5,6,6.5,7,7.5,8,8.5,9) and the pH of the prepared solutions was adjusted by Hydrochloric acid 1M and sodium hydroxide 1M and the activity was measured used Kroeger method at optimum temperature.

Substrate Concentration Effect: The studied effect of the substrate (Acetyl Co-A) on the purified serum solution prepared concentrations different of substrate (0.1,0.2,0.3,0.4,0.6,0.8,1.2,1.4,1.8,2 mM) and the activity was measured Used Kroeger method At optimum temperature and optimum pH\(^{(11)}\). The value(km)of the Substrate (Acetyl Co-A) of the ACC-1 purified enzyme from the blood Serum of women with breast cancer was calculated using the Lineweaver- Burk equation .

Results and Discussion

The result of ACC-1purification protocol could be illustrated in table1 , Fig1 and Fig 2

| Step                  | Vol (ml) | Activity (IU/ml) | Total activity (IU) | Protein Conc(g) | Specific activity IU/g | fold | % yield |
|-----------------------|----------|------------------|---------------------|-----------------|------------------------|------|---------|
| Grude Serum           | 6        | 25.03            | 150.18              | 1.57            | 15.94                  | 1    | 100     |
| Ammonium Sulphates    | 5        | 21.18            | 105.9               | 0.18            | 117.66                 | 7.38 | 70.51   |
| Dialysis              | 4        | 15.08            | 60.32               | 0.09            | 167.55                 | 11.17| 40.21   |
| Gel Filtration        | 3        | 10.9             | 31.8                | 0.04            | 272.5                  | 17.09| 21.11   |
| Ion exchange          | 3        | 7.33             | 21.99               | 0.02            | 366.5                  | 22.99| 14.64   |
Figure 1: Gel filtration of ACC1 from breast cancer serums blood in premenopausal group before treatment or Mastectomy.

Figure 2: Ion exchange of ACC1 from serum with premenopausal breast cancer before treatment or Mastectomy.
These results are agree with the results of Roessler, whe obtained one single peak of the purified ACC enzyme from the filtered Cyclotella Cryptica by gel filtering method \(^{(14)}\). It also agreed with Dehaye and his group where he obtained one single peak of the purified ACC enzyme from the seeds of the gel filtration method \(^{(16)}\). Electrophoresis on acryl amide, in the absence of denatured agents, the above results are proven, as it was observed that a single package appeared in the exudate part of the gel filtration as shown in Fig (3) molecular weight (220 kd) and ion exchange and molecular weights 210 Kd) as shown in Fig (3) of the serum of the Ab group.

These results were in agreement with the findings of Roessler, where he obtained a band 200KD molecular weight using gel filter method \(^{(16)}\) as well as with the chenge and his group, where the ACC enzyme was purified from humans and Rat, where a band was found at 200KD \(^{(17)}\). It also agreed with Dehaye and his group that he found the protein band at 210KD \(^{(18)}\).

**Effect of Acidic function**

The Effected the acidic on speed of the enzymatic reaction through its effect on the ionic groups associated with the surface of the enzyme, where the ionic residues are taken in the active site of the enzymes and the creation of an enzymatic reaction that maintains the catalytic activity of the enzyme and Substrate, this pH agree with Manning and his group particularly the ACC-1 multiple enzyme that contains ionized residues in the centers reaction, the activity of charting the relationship between different degrees of the acidic function and reaction speed of the ACC-1 enzyme. The reaction velocity is observed with an increase in pH to the limit of reaching the maximum speed at an acidic function of 7.5. \(^{(19)}\) as shown in Fig (4).
Temperature Effect

Fig (5) shown the effect of temperature on the reaction speed of the ACC-1 enzyme where a temperature rise is observed to reach the maximum reaction speed at a temperature of 40 °C, then the reaction speed begins to decrease when the temperature increases above 40 °C. It is agree with Manning and his group. Where the temperature affects in the case of ionization of the active groups present on the surface of the enzyme and the substrate which leads to an increase in the enzyme activity with increasing temperature. Because the enzymes are complex protein molecules, their catalytic activity affects the three-dimensional and even four-dimensional structure as the number of collisions between the enzyme and the substrate increases with increasing temperature. At the same time, the enzyme cannot perform its catalytic role when the temperature rises to the extent that leads to a change in protein synthesis and the occurrence of denaturation processes, and then the enzyme loses its catalytic activity. The activation energy (Ea) is calculated by drawing the relationship between $1/T$ (K-1) against log $V_{max}$ as shown in Fig (6).
Figure (6) : The relationship between the inverse of the absolute temperature \(1/T(k^{-1})\) and against Log \(V_{\text{max}}\) for the ACC-1

Where show Fig (6) that the reaction speed of the ACC-1 enzyme purified is subject to the relationship of Arenos to the dependence of the reaction on temperature and Energy activation \(E_a\) of the reaction enzyme. Calculated according to the relationship of Arenos is equal 28 kJ and that the value of activation energy is positive and this indicates that the enzymatic reaction depends on the temperature.

Table (2): the activation energy calculated according to the Aarhus relationship

| Substrate ACC-1 | \(T_{\text{optimum}}\) | \(E_a\) (KJ/mol) |
|-----------------|-----------------|-----------------|
| Acetyl Co-A     | 40°C            | 28              |

Effect of Substrate Concentration (Acetyl Co-A)

The effect of the Substrate concentrations (Acetyl Co-A) on the speed of the enzymatic reaction was studied and the optimum concentration of this substance was determined, which gives the maximum reaction speed. Fig (7) shows the effect of the concentration of (Acetyl Co-A) on the speed of the ACC-1 reaction. An increase in the reaction velocity is observed with an increase in the concentration of the Substrate until the maximum reaction velocity is reached at a concentration of (1.8mM.) Then the reaction speed is established as a result of saturation of sites activity of the enzyme ACC (ACC-1).

Figure (7): The effect of different concentrations of Acetyl Co-A on the Activity of the purified ACC-1

The Michaelis-Menten constant is one of the important physical constants in the kinetic studies of life compounds as it reflects the enzyme affinity of the Substrate and it can be known that Substrate concentration when the velocity of the enzymatic reaction is half of its maximum velocity \(V_{\text{max}}\). The value \(k_m\) of the Substrate (Acetyl Co-A) of the ACC-1 purified enzyme from the blood Serum of women
with breast cancer was the $K_m$ (0.37mM) and $V_{\text{max}}$ (25mM min$^{-1}$) calculated using the Lineweaver- Burk equation and the Hanes-Woolf equation.

Figure (8): Lineweaver- Burk method for calculating the ($K_m$)

Figure (9): Hans-Wolf's method for calculating the ($K_m$)

Table (3) : Values of Michaelis-Menten constant ($K_m$) $V_{\text{max}}$ for ACC-1

| equation       | Substrate | Con optimum | $K_m$(mM) | $V_{\text{max}}$ |
|----------------|-----------|-------------|-----------|------------------|
| Lineweaver- Burk | Acetyl CoA | 1.8mM       | 0.37      | 25               |
| Hans-Wolf's     |           |             |           | 25               |

Where shows table (3) the value of $K_m$ and $V_{\text{max}}$ calculated according to the Lineweaver- Burk and Hans-Wolf's equations for Acety CoA the Substrate for the enzyme ACC( ACC-1) purified from sera with breast cancer at optimal conditions for the reaction the $K_m$ reflects a high affinity of the enzyme for substrate because a low conc of substrate is needed to half saturate the enzyme- that is reach a velocity of $\frac{1}{2} V_{\text{max}}$.
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