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Apoptotic Activity of Methionine γ- Lyase on Several Cancer Cell Lines

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Abstract:

The apoptotic activity of methionine γ- lyase from Pseudomonas putida on cancer cell lines was indicated by measuring the concentration of cytochrome c in the supernatants of cell lines. The result revealed high concentration of cytochrome c in the supernatants of cancer cell lines (RD, AMGM and AMN3) respectively while the concentration of anti-apoptotic protein (Bcl-2) was very low.

Keyword: *Methionine γ- lyase, Pseudomonas putida, Cytochrome c, Bcl-2.*

Introduction:

Methionine γ- lyase is a pyridoxal 5-phosphate dependent enzyme that catalyzes the α, γ- elimination of L-methionine to α-ketobutyrate, methanethiol and ammonia (1) when the cancer cells were treated with methionine γ- lyase, the mitochondria of these cancer cells are damaged because of this treatment causing release of cytochrome c from mitochondria into the cytosol which activating the caspase cascade and subsequently apoptosis (2).

Bcl-2 is an antiapoptotic protein that mainly resides at the outer membrane of mitochondria. Bcl-2 inhibits permeability changes in the mitochondrial membrane thereby preventing release of cytochrome c from mitochondria into cytosol and subsequent apoptosis (3).

Materials and Methods:

Methionine γ- lyase (MGL)

Methionine γ- lyase was extracted from Pseudomonas putida by sonication and purified by DEAE-Sephadex ion exchange chromatography and Sephacryl S-300 gel filtration as previously described (4).

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Cell and cell culture

Rhabdomyosarcoma (RD) cell line at passage 65, Ahmed-Mohammed-Nahi-2003(AMN3) cell line at passage 180, AMGM (Ahmed – Majeed-Glioblastoma-Multiform) cell line at passage 65 and normal Rat Embryo Fibroblast (REF) cell line at passage 56, kindly provided by Iraqi center for cancer and medical genetics research (ICCMGR) were cultured in PRMI 1640 medium supplement with 10% heat inactivated fetal calf serum and incubated at 37 ºC with 5% CO2.

ELISA for detection of cytochrome c bcl-2 and

Bcl-2 ELIAS kit (Cusabio, USA) was used for detection bcl-2. Cytochrome c ELISA kit (US Biological) was used for detection cytochrome c. The procedure of each kit was carried out according to the manufacture's protocols.

Standard curve of cytochrome c

• Two hundred fifty µl of C9094-90C (Standard diluent buffer) was added to each 6 tubes labeled as 2.5, 1.25, 0.625, 0.312, 0.156 and 0.078 ng/ml cytochrome c.

• Serial dilution of standard was made as in the following table.

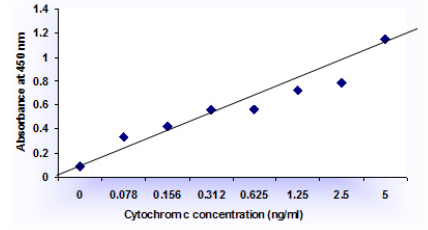
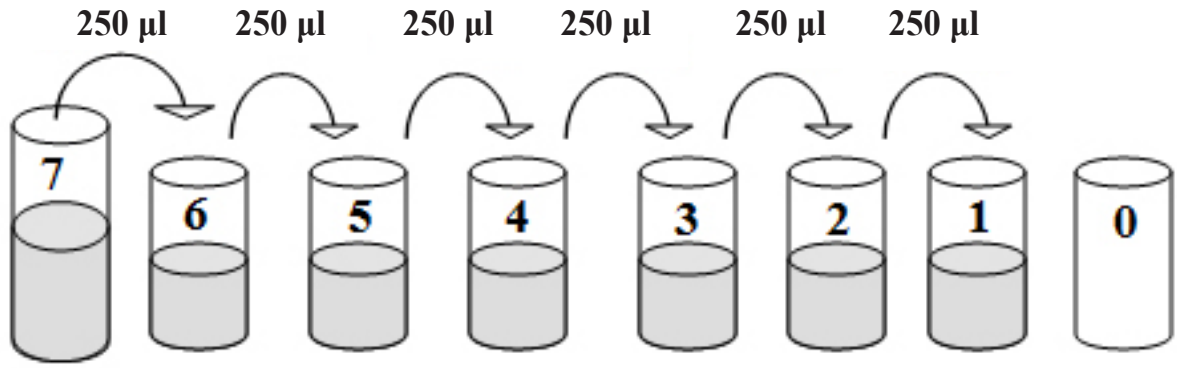


Figure 1: Standard curve of cytochrome c

Table 1: Standard curve of cytochrome c

Standard concentration (ng/ml)	Add	Into
5ng/ml	Prepare in 2.2.13.2	0
2.5 ng/ml	0.25 ml of 5 ng/ml standard	0.25 ml of C9094-90C
1.25ng/ml	0.25 ml of 2.5 ng/ml standard	0.25 ml of C9094-90C
0.625ng/ml	0.25 ml of 1.25 ng/ml standard	0.25 ml of C9094-90C
0.312 ng/ml	0.25 ml of 0.625 ng/ml standard.	0.25 ml of C9094-90C
0.156 ng/ml	0.25 ml of 0.312 ng /ml standard	0.25 ml of C9094-90C
0.078 ng/ml	0.25 ml of 0.156ng/ml standard	0.25 ml of C9094-90C
0 ng/ml	0.25 ml of C9094-90C	0.25 ml of C9094-90C

Standard curve of B-cell leukemia /lymphoma 2 (Bcl-2)
The standard was centrifuged at 6000-10000rpm for 30s.
The standard was reconstituted with 1 ml of sample diluent (stock solution 60ng/ml); the standard was mixed to ensure complete reconstitution and allowed to sit for 15 min with gentle agitation.
Two hundred fifty µl of sample diluent was added into each tube (S0-S6). The stock solution (60ng/ml) was used to produce a 2-fold dilution series (as below).



Tube	S7	S6	S5	S4	S3	S2	S1	S0
ng/ml	60	30	15	7.5	3.75	1.8	0.9	0

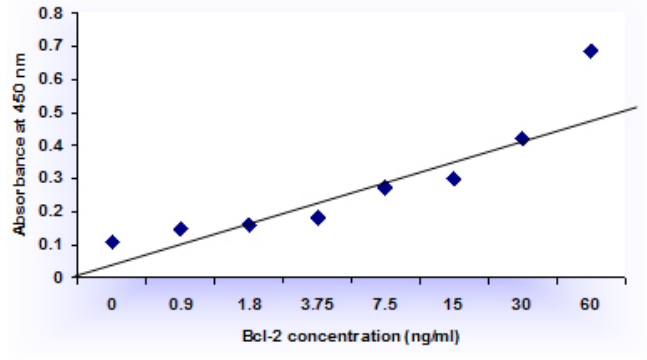


Figure 3: Standard curve of Bcl-2

Results:

Detection of cytochrome c

The concentrations of cytochrome c in the supernatant of cell lines (REF, RD, AMGM and AMN3) were estimated according to the standard curve of cytochrome c figure (1). These values were expressed in ng/ml and illustrate in figure (2), in which cytochrome c concentrations were variable from one cell lines to another. The results were indicated that RD express high concentration of cytochrome c then AMGM and AMN3 respectively in comparison with control (cancer cell lines without treatment with enzyme) thus results were demonstrated that methionine γ- lyase have apoptotic effect against cell lines according to the concentration of cytochrome c released from this cell lines. Measurement of cytochrome c is a tool to detect the first early steps for initiating apoptosis in cells.

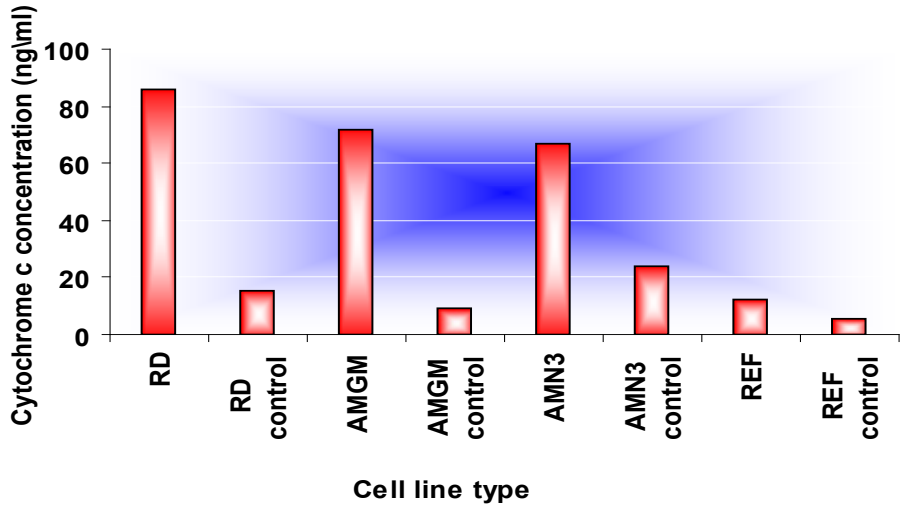


Figure 2: concentrations of cytochrome c release from cancer and normal cell lines (1x105 cell/ml) treated with methionine γ- lyase.

Detection of B-cell leukemia /lymphoma 2 (Bcl-2)

The concentration of Bcl-2 in cell lysates of cell lines was calculated according to the standard curve of Bcl-2 represented in figure (3).The concentration was expressed in ng/ml and demonstrated in figure (4).The results were indicated that cell lines (RD, AMGM and AMN3) expressed low concentration of Bcl-2 because of the Bcl-2 have the ability to block the release of cytochrome c from mitochondria

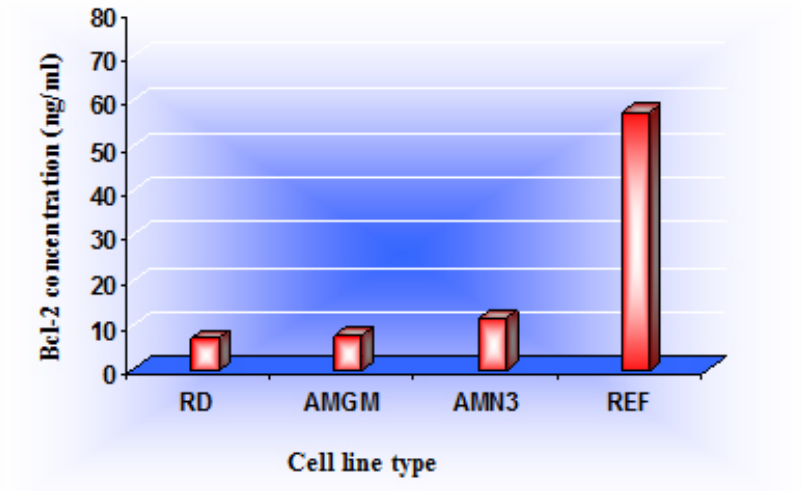


Figure 4: Concentration of Bcl-2 from cancer and normal cell lines (1x105 cell/ml) treated with methionine γ- lyase.

Discussions:

Cytochrome c was a component required for the crucial steps in apoptosis, caspase-3 activation and DNA fragmentation (5). Cytochrome c was shown to distribute from mitochondria to cytosol during apoptosis in cells. Mitochondrial cytochrome c is a water soluble protein of 15kDa with a positive charge, found in the mitochondrial intermembrane space and is associated with the inner membrane (6). The molecular mechanisms responsible for the translocation of cytochrome c from mitochondria to cytosol during apoptosis are unknown (7). Reduction in mitochondrial transmembrane potential has been reported to accompany early apoptosis (6); the release of cytochrome c into cytosol leads to activation of caspase dependent pathway, cytochrome c a chives this activation by interaction other cytosolic factors forming a complex apoptosome which composed of cytochrome c, Apaf-1, dATP and Apaf-3/caspase 9 (8).

Bcl-2 possessed channel-forming activity and the properties of the Bcl-2 channel differ between proapoptotic proteins, the Bcl-2 form channel in lipid membranes of mitochondria only at low pH and these channels are cation-selective whereas proapoptotic protein form channels at natural pH that are anion –selective(9)

proapoptotic proteins such as Bax are a cytosolic monomer but during apoptosis changes its conformation ,integrates into the outer mitochondrial membrane and oligomerizes, thus Bax and Bak oligomers are believed to contribute to the permeabilization of the outer mitochondrial membrane (PT) either by forming channels by themselves or by interacting with components of the PT pore such as voltage –dependent anion channel (VDAC) ,thereby inducing hyperpolarization and permeability transition , these events cause entry of water and solutes, matrix swelling and rupture of the outer mitochondrial membrane which allows the release of cytochrome c (10) .

In contrast antiapoptotic Bcl-2 members sequester proapoptotic Bcl-2 members by binding to their BH3 domains and thereby prevent Bax or Bak activation/ oligomerization and consequently inhibit mitochondrial proapoptotic events , overexpression of Bcl-2 prevent cells from undergo apoptosis by suppressing the generation of ROS , stabilizing mitochondrial inner membrane potential , preventing PT and consequently blocking the release of cytochrome c (11) ,thus Bcl-2 can regulate apoptosis by controlling permeability of outer mitochondrial membrane to ions and proteins such as cytochrome c (6,12).

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فعاليه الموت المبرمج للأنزيم methionine γ-lyase في بعض خطوط الخلايا السرطانية

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الخلاصة:

درست فعالية الموت المبرمج للأنزيم methionine γ-lyase المنتج من بكتريا Pseudomonas putida بواسطة قياس تركيز سايتوكروم سي في رائق الخلايا السرطانية , آذ أظهرت النتائج تراكيز عالية للسايتوكروم سي في رائق الخلايا السرطانية RD,AMGM and AMN3 بالتتابع بينما كانت تراكيز البروتين المثبط للموت المبرمج(Bcl-2) قليله جدا.