The Effect of Mg^{2+} and Mn^{2+} on Over-Production of Interleukin-2 in Recombinant *E.coli*

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ABSTRACT: *In order to increase the productivity of human interleukin-2 (IL-2) as a model protein in the recombinant E.coliBL21 (DE3), the effect of magnesium acetate and manganese acetate was studied based on full factorial design experiments magnesium acetate and manganese acetate and their interaction exhibited an increasing effect on IL-2 expression level upto 25.24%, 16.80% and 23.30%, respectively,in comparison to 10.76% for the standard M9 medium(as usual defined medium in high cell density culture). Magnesium acetate was the most effective salt. Other experiments were performed to investigate the effect of magnesium and acetate ions separately. Results revealed that M9 medium with 1.5 times of magnesium ion concentration resulted in the highest expression level.*

KEY WORDS: *Recombinant Escherichia coli, Human interleukin-2, Full factorial design, magnesium, manganese.*

INTRODUCTION

Human interleukin-2 has been known as a therapeutic agent in the treatment of cancer and as a promoter in proliferation of antitumoral lymphocytes [1].

The effect of inorganic compounds, especially magnesium (Mg) and manganese (Mn) salts, has been known in enzymatic reaction in different cells [2-8]. Mn improves the growth rate through enhancement of amino acid and fatty acid metabolisms [8]. In conversion of ADP to ATP and in glycolysis, Mg is a cofactor; therefore, it affects glucose uptake rate by the cells. In TCA cycle, Mg interferes in conversion of isocytrate and succynyl CoA to α-Ketoglutarate. Mg has an important role in synthesis of valine [9]. It is also necessary in DNA and RNA polymerization reaction and is a base for catalytic reactions of primase *E.coli*. This enzyme synthesizes primer of RNA that is the starter of DNA replication [10, 11]. Mg and Mn acetates, at a specific concentration, enhance the production of serine alkaline protease,. Acetate anion as a second carbon source, will enhance the growth rate and production yield [9].

Acetate plays an important role in recombinant protein production. Acetate, in concentration slower than 3g/L, can be used as a carbon source (in absence of glucose)

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and can be an inhibitor in concentrations higher than 3g/L [12-14].

To increase the expression of target gene, during the induction, it is compulsory to enhance the synthesis of related RNA molecules (messenger, ribosomal and transfer RNA) [15, 16], more necessary amino acid synthesis[6, 17, 18]consequently the increase of related enzymes activities (exp. RNA Polymerase, RNase P Ribozyme) [10, 11].

The presence of $Mg^{2+}$and Mn^{2+} may enhance the enzymatic activities, especially in M9 medium in high cell density cultures. Due to lack of a complete study on this subject in the literature, $Mg(SO_4)$ and $Mn(SO_4)$ concentrationsin M9 medium were optimized in this study, and the effect of Mg^{2+} and Mn^{2+}, besides the another important anion (acetate) and their interaction on the enhancement of IL-2 expression level, was studied.

EXPERIMENTAL SECTION

Microorganism and vector system

E.coli BL21 (Novagen, UK) was used as the host and PET21 as the inducible expression vector (Novagen). The IL-2 gene was inserted in plasmid in genetic laboratory, faculty of sciences, Tarbiat Modares University, Tehran, I.R. Iran. The host cells were transformed using the calcium chloride procedure [19].

Media and solutions

LB (Luria–Bertani) agar medium and M9 medium were used as plate and shaken-flask cultures of *E.coli* strain BL21 (DE3) [19-25]. M9 medium consisted of (g/L): glucose, 4; $Na_2HPO_4.7H_2O$, 6; KH_2PO_4, 3; $MgSO_4.7H_2O$, 0.39; NaCl, 0.5; NH_4Cl,1. Media components were sterilized separately and then were added to distilled water [19].

Cultivation methods

Shaken-flasks were incubated at 37°C and 200rpm. Overnight-incubated seed culture (15 mL, OD_{600} = 0.7–1) was added to the flasks containing 150 mL of modified M9 medium. Harvested culture was induced at OD_{600} =1 with 1.5mL of 1molar MIPTG solution. Culture pH was maintained at 7 by addition of 3 Molar NaOH or 3 Molar H_3PO_4 solutions.

Analytical procedures

Cell growth was monitored by optical density (600 nm)

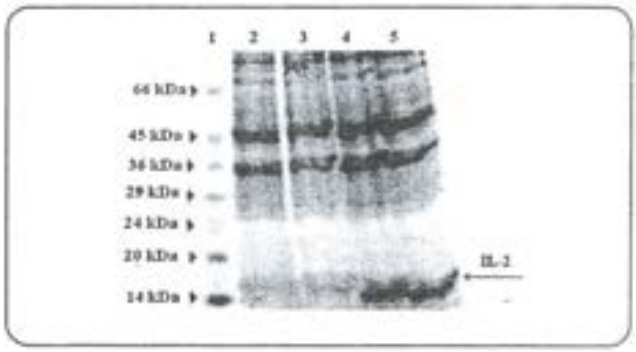

Fig. 1: SDS-PAGE of $MgSO_4$ optimization experiments (4 hours after induction).1- molecular weight marker, 2- IL-2 bond for expression in M9 medium (standard culture), 3,4and 5 are the IL-2 bonds for expression in M9 medium containing 3, 2 and 1.5 times of $MgSO_4$ concentration, respectively.

measurement of cultures. The expression levels of IL-2 were determined by SDS-PAGE using 15% (w/v) poly-acrylamide gels. The gels were stained with Coomassie Brilliant Blue R250 and then gel quantifications were done by a gel densitometer. The biological activity of IL-2 was measured by the Lymphocyte Transformation Test (LTT) [20].

RESULTS AND DISCUSSIONS

Optimization of $MgSO_4$ in M9 medium

At first,sulphate salt of $Mg^{2+}$was used to verify the sole effect of Mg^{2+}, not the acetate anion, on the IL-2 expression level. $MgSO_4$ concentration was changed in 4 levels as 1, 1.5, 2 and 3 times of the original concentration in M9 medium. Fig. 1 shows the results of IL-2 expression.Gel densitometry analysis on the gel showed that IL-2 expression percents in lanes 2 to 5 were 10.75%, 12.56%, 17.67%and 19.61%, respectively. Then, optimum concentration of $MgSO_4$ was found to be 1.5 times of its concentration in M9 medium.

These experiments revealed that $MgSO_4$ concentration in M9 medium plays a crucial role in recombinant protein production, which is not optimum for maximal recombinant protein production [19-25]. Optimum concentration of Mg^{2+} affects enzymes' activity especially in RNA polymerase activity and consequently changes expression level to maximum attainable expression level in culture conditions.

Effects of $Mn(AC)_2$ and $Mg(AC)_2$ on IL-2 expression level

M9 medium does not have any of Mn salts, but

in many of high cell density cultures 1mL of a trace element solution containing Mn was added to cultures [19-25]. The trace element stock solution contained 2g/L of $MnCl_2.4H_2O$ [19-25], which is equivalent to1.73 g/L $Mn(AC)_2$ or 0.973 g/L $MnSO_4$ on molar basis.

In order to investigate the effect of acetate salts of Mg^{2+} and Mn^{2+}, by maintaining these cations molar concentrations in the medium, $Mg(AC)_2$ and $Mn(AC)_2$ were used instead of $MgSO_4$ and $MnSO_4$ salts, respectively. A full factorial experimental design was used for 2 variables in 2 levels (with or without each a cetate salt).

Fig. 2 shows the effect of $Mg(AC)_2$ and $Mn(AC)_2$ on IL-2 expression level in full factorial design experiments in comparison with standard culture(M9).

Gel densitometry analysis on the above gel showed that IL-2 expression percents in lanes 1 to 4 were 23.30%, 16.80%, 16.08%, and 25.24%, respectively, in comparison to the standard culture in the 10.75% (lane 6). These results revealed that the presence of $MnSO_4$ in culture scan rise expression level to 16.08% and $Mn(AC)_2$ to 16.80%. By maintaining equal Mn molar concentration in these two cultures, difference between expression levels can be related to the effect of acetate in comparison with sulphate anion. Culture containing $Mg(AC)_2$ had the best IL-2 expression level(lane 4), however presence of $Mg(AC)_2$ in addition with $Mn(AC)_2$ decreased the IL-2 %(lane 1). It means that interaction of these two salts lowered the IL-2 expression level or had inhibition on $Mg(AC)_2$ efficiency. Then, $Mn(AC)_2$ increased expression level, but $Mg(AC)_2$ was the most effective salt in enhancement of IL-2 expression.

The function of acetate in the IL-2 % enhancement

By maintaining equal Mg molar concentrations, $Mg(AC)_2$ enriched culture had better IL-2 %(25.24%) than $MgSO_4$ enriched culture (19.61%). Therefore, AC has positive effect on IL-2 expression level, probably due to providing an extra carbon source.To have a better insight on the role of acetate ion, with maintaining Mg molar concentration in optimum amount, different amounts of $Mg(AC)_2$ and $MgSO_4$ were added to flasks 1 to 5 (optimum concentration of $MgSO_4$ was 1.5 times of its concentration in M9 culture). These flasks contained M9 medium and 0.5 times of M9's Mg molar concentration in different mixtures as below:

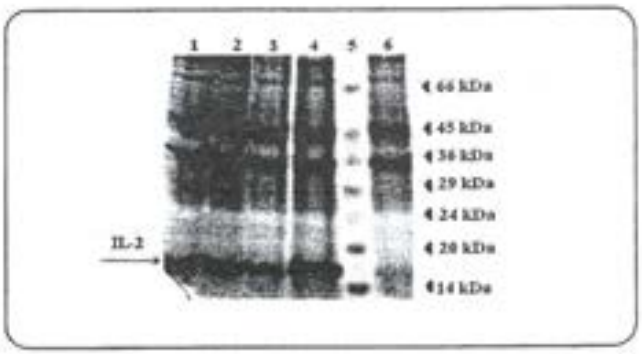

Fig. 2: SDS-PAGE of full factorial design experiments (4 hours after induction). IL-2 bond from culture containing 1- $Mg(AC)_2$ and $Mn(AC)_2$, 2- $Mn(AC)_2$, 3- $MnSO_4$, 4- $Mg(AC)_2$, 5- Molecular weight marker, 6- $MgSO_4$(standard culture).

Table 1: The IL-2 expression level in acetate role experiment.

Flask number	1	2	3	4	5
IL-2%	20.72	28.99	28.35	19.16	10.75

Flask1: a half $MgSO_4$ and the other $Mg(AC)_2$. Flask2: $Mg(AC)_2$. Flask3: $MgSO_4$ and additional glucose (0.014g) equal to acetate carbon-mole in flask 2. Flask 4: $MgSO_4$. Flask 5: Standard culture (contained just M9).

IL-2 expression level, in all of the flasks was investigated. Table 1 presents the IL-2 expression level. In Table 1, IL-2% is 19.16%, 28.35%, 28.99% and 20.72% in samples of flasks 4 to 1, respectively. By addition of acetate concentration in flasks1 to 2, IL-2% increased. The equality of IL-2% in samples of flasks 3 and 2 means that cells can use acetate as well as glucose as a carbon source.

CONCLUSIONS

Presence of $MnSO_4$ in M9 defined medium raised IL-2 expression level from 10.76% to 16.08% in comparison with standard culture. The requirement of recombinant *E.coli* to more Mg is not dependent on IL-2 as a model protein, since large similar of ities exist in recombinant protein expression in *E.coli*. Therefore, Mg optimization can be useful in enhancement of expression level of all recombinant proteins in *E.coli* hosts.

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