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EXPRESSION OF MOUSE ANTICREATINE KINASE (MAK 33), F_{AB} FRAGMENT OF IgG ANTIBODY, IN THE METHYLOTROPHIC YEAST *HANSENULA POLYMORPHA*
II. Gamma chain

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ABSTRACT

The yeast *Hapsenula pdymorpha* was used as a host for expression of gamma chain peptide of F_{Ab} fragment of mouse articreatine kinase-M, IgG antibody. Two expression systems were designed and assembled based on the promoter codon of either of the preferentially highly expressed enzymes, formate dehydrogenase (FMD) and methanol oxidase (MOX) of *H. polymorpha.* Two expression plasmid vectors, PGAGU and PHAAI, with FMD and MOX promoters, representily, were contsructed and transformed into *H. pdymorpha* LR9 Expression of gamma chain gene yielded about 400 mg/L and 550 mg/L, respectively 7% and 10% of the total cell protein under the control of FMD and MOX promoters, respectively. The prepro alpha was incompletely processed and most of the gamma chain peptide accumulated within the cells, especially under the control of FMD promoter.

INTRODUCTION

The developing of monoclonal antibodies has provided general access to homogenous antibodies of predefined specificity[1]. Redesign of the antibody molecule requires the use of gene technology with convenient methods of expressing the protein[2]. Among the first expression system reported with which antibodies could be obtained in their native state were lymphoid mammalian cells[3], non-lymphoid cells[4], as well as prokaryotic cells like *Escherichia coli*[2]. A number of single-chain heterologous proteins have been reported to be secreted from yeasts, like *Saccharomyces cerevisiae*[5]. The secretion of foreign multimeric or heterodimeric proteins has been reported for the yeast *Hansenula polymorpha*[6].

The use of the methylotrophic yeast *H. polymorpha* as an expression host system brings the arsenal of yeast gene technology to antibodies production. The fast growth, efficient transformation and easy fermentation conditions of *H. polymorpha* allow rapid and sufficient production of antibodies and their fragments[6]. The antibody molecules or their fragments that contain their antigen-binding domains; F_{ab} and $F_{(ab')2}$ are particularly important for a wide variety of applications, like diagnosis and treatment of human diseases[7], as well as in affinity purification methods[8].

In a previous paper[9], we described a system for expression of kappa chain peptide of the F_{ab} fragment of MAK33 antibody in the methylotrophic yeast *Hansenula polymorpha*. This paper, describes another system for expression of gamma chain protein of the same antibodies. The cDNA coding region of gamma chain protein has been fused to glucoamylase, N-terminal coding region and prepro-α factor as leader peptide. The protein expression was controlled by either *FMD* or *MOX* promoter and *MOX* terminator in the expression plasmid.

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MATERIAL AND METHODS

Chemicals :

Restriction endonucleases, their buffers, chemilumnescent substrate, calf intestinal alkaline phosphatase klenow fragment polymerase and salmon sperm DNA were from Boehringer Mannheim, Germany, Radiolabelled nucleotides (α ^{32}P-labelled) was obtained from Hartmann Analytic, Braunschweig, Germany. Oligonucleotide DNA (primers) were from BWG-Biotec, Ebersberg, Germany. All other Chemicals were analytical grade or laboratory grade reagents.

Strains :

Hansenula polymorpha LR9 strains, *ura 3;* uracil auxotroph, was used as host strain for the integration and expression of the plasmids pGAGU (Fig. 1A), and pHAAI (Fig.1B)[10]. It was obtained from Dr. Reine Roggenkamp, Institute of Microbiology, Düsseldorf University, Germany. The *Escherichia coli* strain DH5'Fα[11] was obtained from Gibco, BRL, USA, and used for construction and amplification of the expression plasmids.

Cultures Media :

Hansenula polymorpha was grown in non-selective medium, yeast-peptone-dextrose medium (YPD) consisting of 1% w/v Bacto-yeast extract, 2% w/v Bacto-peptone, and supplemented with 2% w/v glucose. The selective medium, Yeast nitrogen base

(YNB) consisted of 0.17% w/v Difco-yeast nitrogen base w/o amino acids and 0.5% w/v ammonium sulfate (Merck) and either 2% w/v glucose in YNBG medium or 0.5% v/v methanol and 1% v/v glycerol in YNBMG (induction) medium.

Transformation :

Escherichia coli was transformed as described by Glover[12] and was grown in LB medium supplemented with 150 µg/ml ampicillin. *Hansenula polymorpha* was transformed by polyethylene glycol (PEG) as described by Klebe et al.[13] and Dohmen et al.[14]

Molecular Analytical Methods :

Southern and Northernblot analyses, as well as *in vitro* DNA manipulation were carried out according to the standard methods of molecular biology[15]. Western blot was carried out according to Towbin et al.[16].

Plasmids :

For the construction of the vector plasmid the following plasmids were used:

1- pKK 223-3G plasmid, carrying the cDNA of gamma chain protein that could be liberated as 1.3 kb fragment following *Eco* R1-*Hind* III treatment, was a grift from Boehringer Mannheim, Germany.
2- pUC 10 GAI plasmid, carrying the cDNA gene of glucoamylase of *Schwanniomyces occidentalis* was a grift from Rhein Biotec, Dusseldorf, Germany.
3- pPFPMT 130, the source of FMD promoter and MOX terminator, was ar gift from Rhein Biotic, Dusseldorf, Germany.
4- pGRTI-16 plasmid, carrying the prepro alpha factor or *Saccharomyces cerevisiae,* was a gift from the Institute of Microbiology. Dusseldorf University, Germany.
5- pHP mox-Ae plasmid, that yield methanol oxidase promoter as 1.2 kb fragment upon *Eco* R1-*Sal I* treatment, was a grift from the Institute of Microbidgy, Dusseldorf University, Germany.

Yeast DNA analysis :

For Southern analysis, the yeast cells were grown on YPD medium to heavy growth, until the cells flocculate in the culture. After centrifugation and cell collection, the total DNA was isolated according to Davis et al.[17]. About 0.5 µg DNA was digested with the appropriate restriction enzyme (s), electrophoresed in 0.8% (w/v) agarose gel and transferred to Nylon membrane filter (Amersham) to be hybridized by radioactive probe according to Feinberg and Vogelstein[18].

RNA analysis :

Method of extraction: For Northern analysis, yeast cells were grown in YNBMG medium to O.D. of 0.8-1.0 at λ_{600nm}. Total RNA was extracted from about 20 ml culture. The cells were collected by centrifugation and lysed using glass beads (0.45 ∅ mm). Quantity of about 10 µg total RNA, per lane were loaded into RNA-formaldehyde, (1.2 w/v) agarose gel. After electrophoresis, RNA was transferred into nitrocellulose membrane filter (Schleidcher Schwell, Dassel Germany) and hydridized with glucoamylase cDNA fragment, radioactive probe.

Follow up of gamma chain mRNA: The cells were induced by growing in YNBMG medium and samples containing approximately equal number of cells were withdrawn after time intervals of 2, 4, 6, 12, and 16 hrs. The total RNA was separated and 10 µg quantities were electrophoresed and transferred to nitrocellulose membrane filter and probed as mentioned before.

Protein analysis and characterization of gamma chain peptide :

Cell-culture supernatant: Cultures were centrifuged at 10.000 rpm for 15 min., and the proteins in the supernatant were precipitated by 4% sodium deoxycholate and 10%, TCA. The precipitate was washed twice with cold acetone and dissolved in phosphate-buffered saline (PBS). About 25 µg proteins in about 20 µl, representing 10 ml culture were electrophoresed in 12% SDS-polyacrylamide gel according to Laemmli[19].

Cell-free extract: The collected cells were mechanically disintegrated as described by Ausubel et al.[20]. After centrifugation, the clear superntant was decanted and stored at -70°C in small portions until used. The proteins were analysed by electrophoresis in 12% SDS-polyacrylamide gel and either directly stained with coomassie brillient blue (CBB) or alternatively transferred into nitrocellulose membrane filter for western analysis. The expressed gamma chain was visualized by goat anti-gamma, alkaline phosphatase-conjugated antibodies (Sigma).

Quantitative analysis of protein: was carried out according to Bradford method[21].

RESULTS

Plasmids construction

To express gama chain peptide in *H. polymorpha* LR9, the plasmid pGAGU (Fig. 1A) was constructed. The cDNA of gamma chain was ligated to the PFPMT 130 plasmid[6], in between *FMD* promoter and *MOX* terminator. A fragment of 1.9kb, from the N-terminal coding region of glucoamylase, including its secretion signal sequence, was ligated after the promoter and before the cDNA of gamma chain. The prepro-alpha factor was inserted in between glucoamylase and gamma chain cDNA to function as a secretion signal sequence for gamma chain protein. Consequently, the expression cassette comprised *FMD* promoter,

glucoamylase, prepro-alpha, gamma chain and the *MOX* terminator. The expression plasmid pHAAI (Fig. 1B), was similar to pGAGU plasmid with the exception that *MOX* promoter was inserted instead of *FMD* promoter.

The competent cells of *H. polymorpha* LR9 were transformed with either pGAGU or pHAAI expression plasmid (3-5 µg plasmid DNA), streaked on YNBD plate and incubated for at least 3 days at 37°C. The frequencies of transformation were about 1-3, and 2-4 cells/µg plasmid DNA for pHAAI and pGAGU, respectively.

Like in kappa chain producing cells, the cells were grown to high density (10^{10} cells/ml) in YPD (non-selective) medium for about 40-50 generations followed by enrichement in YNBG (selective) medium. The cells demonstrated high stability and enrichment of uracil^{+} phenotype. Being integrating and shuttle vectors; the expression plasmids, pGAGU and pHAAI, are expected to be integrated with the yeast chromosome. This could be demonstrated by the stability of uracil^{+} phenotype and Southern analysis.

Southern analysis :

Southern analysis for the transformants of pGAGU, the plasmid exhibited one pattern of integration within the yeast chromosome, i.e. it was integrated within the yeast chromosome at the same site in all transformants, with multicopy-number plasmid integrtion. In the transformants of pHAAI plasmid, the cells showed the integration of multicopy-number of the expression plasmid into different sites within the chromosome of some transformants and low copy-number into one or more sites in another transformant.

Northern analysis of gamma mRNA :

Northern analysis for gamma chain mRNA of *H. polymorpha* LR9 transformed with either pGAGU or pHAAI expression plasmids was performed. About 10 µg total RNA in 10 µl aliquots, were electrophoresed in 1.2% agarose-formamide gel and transferred to nitrocellulose membrane by capillary technique. The membrane was hybridized with ^{32}P-labelled probe prepared from 1.3 kb gamma cDNA.

Figure (2) shows a band of 3.4kb, representing a single strand mRNA of the coding region of the expression cassette including that of gamma chain. The gamma mRNA was highly transcribed under the control of either *MOX* (lane 1) or *FMD* (lane 2) promoters. Control cells (transformed with pFPMT 130 plasmid) showed no band, of 3.4kb, of single strand mRNA (lane 3). Comparing with Southern analysis, there are no distinct differences in the transcription level between cells with multicopy-number plasmid integration and that of low copy-number integrated plasmid.

Following up gamma mRNA :

The expression of gamma chain under the control of *FMD* promoter did not show the same level of expression of kappa chain[9]. Gamma chain expression under the control of *FMD* promoter showed low level of expression and was secreted to the culture supernatant at later stage, stationary phase. For determining the maximum level and the optimum time of transcription, the level of gamma mRNA was followed up in a growing culture. The cells were induced and samples containing approximately equal number of cells were withdrawn after time intervals of 2, 4, 8, 12, and 16 hrs. The total RNA was separated, 10 µg RNA were electrophoresed, transferred to nitrocellulose membrane and probed as mentioned before. Figure (3) shows that gamma mRNA transcription starts after about 2 hrs (lane 1) and gradually increases to reach maximum level after 12 hrs (lane 4), followed by rapid degradation (lane 5). The figure also shows that mRNA transcription was going on right from the begining and mRNA has a good stability for a period of 12 hrs and the decrease in the production of protein can be attributed to other reasons rather than mRNA transcription.

Protein analysis and gamma chain peptide characterization :

The promoters *FMD* and *MOX* were induced by growing the cells of *H. polymorpha* that were transformed with pGAGU and pHAAI plasmids in YNBMG medium in parellel with pFPMT 130 transformants, as control. The cells were grown in YPD medium, subcultured in 50 ml YNBD, and incubated until cell density reached 10^{8} cells/ml and an aliquot of about 10^{4} cells was used to inoculate 50 ml YNBMG medium. The cells were incubated at 37°C with shaking to O.D. of 1.0-1.2 at λ_{600nm}. The cells were centrifuged at 13000 rpm for 15 min.

Proteins in cell culture supernatant were precipitated by deoxycholate-TCA. About 25 µg in 20 µl aliquots, representing 10 ml of the culture medium, were electrophoresed in 12% SDS-polyacrylamide gel and the protein bands were stained with CBB. Figure (4) shows the supernatant proteins of control (lane 1) and pHAAI plasmid-transformant (lane 2). The gel shows a distinct band of 67 KDa in size, representing glucoamylase peptide, another prominant band of about 35 KDa, representing the gamma and pro alpha in one peptide. The two bands are missing from the control lane. The cDNA coding region of gamma chain is about 1.3kb, however, a stop codon within an oligonuleotide linker was inserted after about 926 bps. The expected bands of gamma peptide and gamma-pro alpha peptide would be approximately 23-25 and 30-33 KDa, respectively. The gamma-pro alpha peptide, however was found to be larger than the expected and calculated one. Unfortunately, there was no band of molecular weight 23-25 KDa, representing the completely

processed gamma chain peptide in the culture supernatant. Like control, supernatant proteins of cells transformed with pGAGU expression plasmid, did not show any significant bands, except at later stage of growth (stationary phase, data not shown). Such late expression is usually accompained by secretion of many proteins and by cell death.

Aliquots (20 µl) of cell-free extract containing approximately 50 µg proteins were electrophoresed in 12% SDS-polyacrylamide gel and stained with GBB for band detection. Figure (5) shows proteins in the cell-free extract of pGAGU (lane 2) and pHAAI (lane 3) transformants, concurrently with that of control (lane 4). In comparison with control, the two transformants show the same two bands as those is in the supernatant; the glucoamylase band (at about 67 KD) a and gamma-pro alpha peptide band (at about 34 KDa). There was an extra band (at about 49 KDa), which could be that of the gamma chain peptide providing that the inserted stop codon was missed.

The total expressed proteins were estimated by Braford method[21]. It was found that pGAGU-transformant produced about 20 mg/L and 380 mg/L gamma protein in the cell culture supernatant and cell-free extract, respectively, representing about 7% of the total cell proteins. pHAAI-transformant produced about 50 mg/L and 500 gamma protein in the supernatant and within the cells, respectively, representing about 10% of the total cell proteins.

Western analysis :

Western analysis of cell-free extract and culture supernatant of *H. polymorpha* LR9 transformed with pGAGI and pHAA 1 plasmids were carried out. After transfer of proteins to nitrocellulose membrane, the bands were detected and visulized by goat alkaline phosphatase-connjugated anti-mouse Ig, gamma-specific anibodies (Sigma). Lane I, in Fig. 6, represent-ed culture supernatant of pHAAI-transformant, while lanes 2 and 3 represented the cell-free extract of pHAAI and pGAGU transformants in respective manner. The bands at about 35KDa were detected by the antibodies and these results confim the eariler results of CBB-stained gel and the right calculation of the expected molecular weight of gamma-pro alpha peptide. It was noticed that the band at about 49 KDa (lane 4) was also identified by the antibodies. Moreover, there was another band of about 106 KDa in both pHAAI and pGAGU-transformants which could represent glucoa-mylase-prepro alpha-gamma peptide as one peptide that remained unprocessed within the cells. However, many bands of different sizes could be identified by the used antibodies. This could be attributed to either unspecificity of the used antibodies, the unstability of the expressed gamma peptide to degradation or the acquisition of the peptide conformations or forms that would consequently affect the rate of migration in the polyacrylamide gel.

DISCUSSION

Hansenula polymorpha has been reported to be an efficient expression host for expression of heterologous proteins that offers several advantages like *Saccharomyces cerevisiae*[22]. The transformation of *H. polymopha* with polyethylene glycol (PEG) was found to provide a satisfactory rate of transformation. The cells were treated with DNA under hypertonic conditions in presence of PEG. The addition of sheared, freshly, heat-denatured fish-DNA and histamine was found to enhance and improve the transfer of the foreign DNA from outside of inside the cell[23]. The low rate of transformation (1-3 cells/µg DNA) by PEG-mediated transformation seems to be a specific feature for *H. polymorpha*.

Multiple integration of foreign DNA have been observed in the yeast *H. polymorpha*[23]. The explanation for multiple integration, can be attributed to the presence of two or more of foreign DNA fragments ligated to each other in the yeast cells. This large fragment, a concatamer, can undergo integration with the yeast chromosome[24]. It is also possible that the occurance of recombination, integration and replication in mitosis at the same time leads to a multiplication of the integrated sequence.

Scorer et al.[25], found that the mRNA level of foreign genes under the control of *AOX1* promoter increased progressively with the copy-number of the expression plasmid in *Pichia pastoris* transformants. Thus, the dependence of expression levels on the vector numbers seems to be general phenomenon, independent of foreign gene. Melefors and Hentze[26], reported that the expression of certain eukaryotic gene is, at least, partially controlled at the level of mRNA translation. and the step of initiation of translation represents the primary target for regulation. Northern analysis, (Figure 4) showed the abundance of mRNA of gamma chain in the transformed cells under the induction conditions. The relation betwen mRNA level and plasmid copy-number in the transformants is not linear, and this could be attributed to the low stability of gamma mRNA under the stated conditions.

The abundance of mRNA is not only dependent on its synthesis but also on its degradation which is an essential pre-requisite for maintenance of gene expression flexibility with accuracy. The relation between the specific protein production and the abundance of its specific mRNA is usually linear, which means that the translation rate is a function of the transcription rate. This seems not to occure with gamma protein under the control of *FMD* promoter (Figure 5). Although mRNA was quite abundant, the produced protein was low. This could be due to the instability of either gamma mRNA or gamma peptide. The second possibility was ruled out and results in Figure (6), showed that the mRNA of gamma was being stable over

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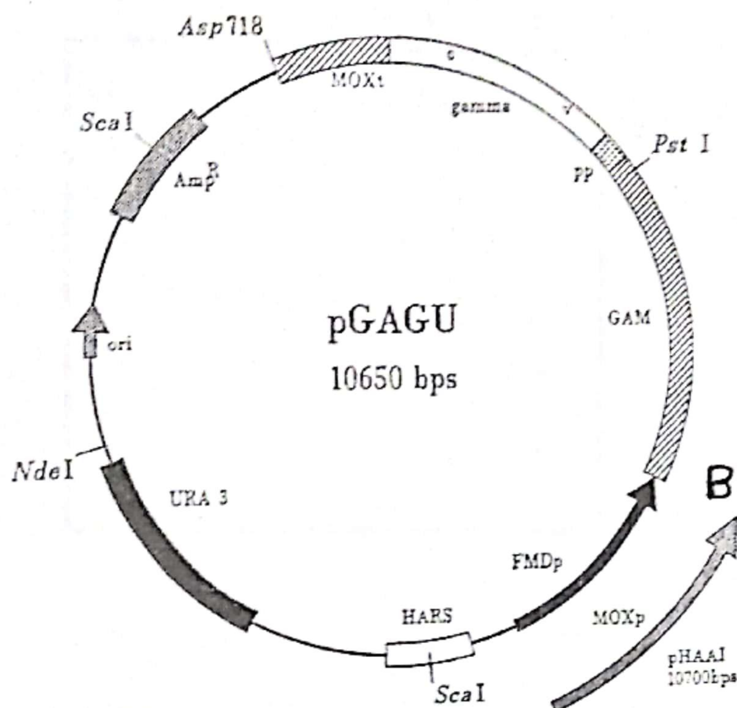

Figure (1): Diagram of the expression vectors, pGAGU (A) and pHAAI (B) plasmids, containing a gamma coding region. The coding region cDNA of gamma gene was inserted in between either *FMD* promoter in pGAGU plasmid of *MOX* promoter in pHAAI plasmid and *MOX* terminator. A 1.9kb fragmentt from the N-terminal of the coding region of glucoamylase gene *(Schwanniomyces occidentalis, GAM1)* with its secretion signal sequence was ligated in between *FMD* or *MOX* promoter and gamma gene. Prepro-α was inserted in between gamma and glucoamylase genes to be a secretion signals sequence for gamma chain peptide.

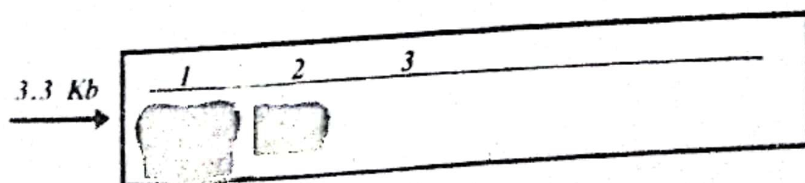

Figure (2): Northern analysis of gamma mRNA of the induced cells of *H. polymorpha* LR9 transformed with either pHAAI (lane 1), pGAGU (lane 2) or pFPMT 130 (lane 3, control). A band of 3.3kb representing the expected mRNA of gamma, pre-pro-α- factor, and glucoamylase coding regions that are missing in the control.

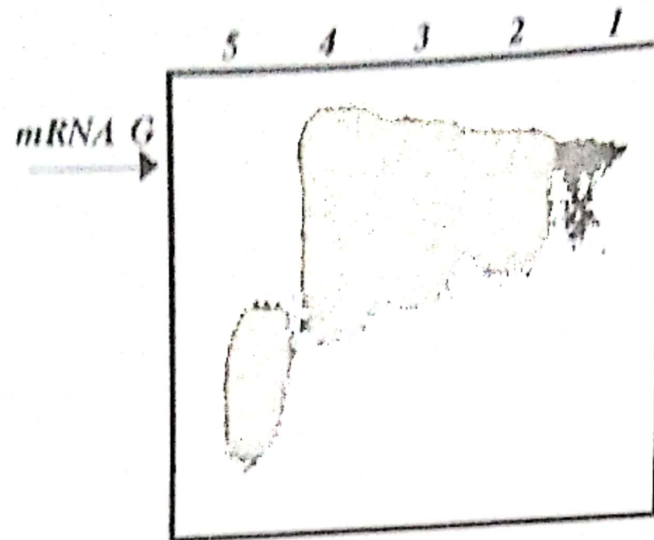

Figure (3): Following up the gamma mRNA level of the induced cells of *H. polymorpha* LR9 transformed with pGAGU after 2, 4, 8, 12, and 16 hrs in lanes 1, 2, 3, 4, and 5 in respective order. The Arrow indicates a band of 3.3. kb representing the single strand mRAN of the coding regions of glucoamylase, prepro-α and gamma genes under the control of *FMD* promoter ad *MOX* terminator. The single strand mRNA was transcribed after 2 hrs (lane 1) and reached maximum after 12 hrs (lane 4) and rapidly degraded after 16 hrs (lane 5).

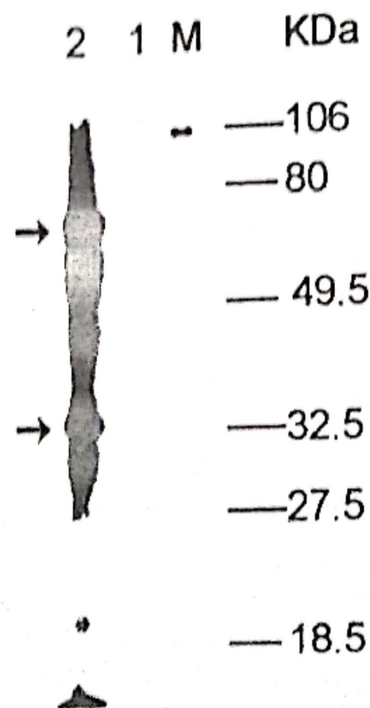

Figure (4): 12% SDS polyacrylamide gel stained with coomassie brillient blue (CBB) of the cell culture supernatant of the induced cells of *H. polymorpha* LR9 transformed with either pHAAI or pFPMT 130 (control). Plasmid. Lane M, molecular weight marker (in right side); lane 1, control; lane 2, pHAAI-transformants. The culture supernatant of pH AI-transformant showed two distinct bands that are missing in control and indicated by arrows in left side, the first band is glucoamylase band (67 KDa) and the second band is the pro-α with gamma chain in one peptide (35 KDa).

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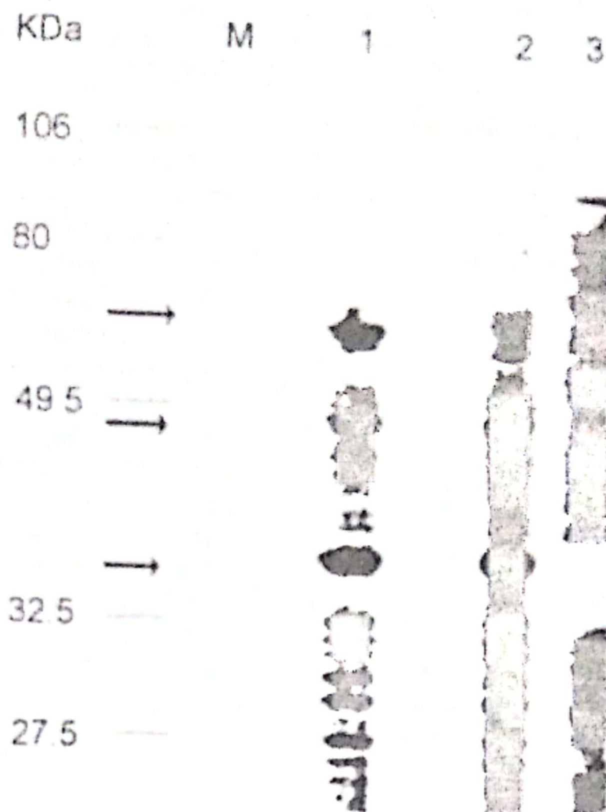

Figure (5): 12% SDS-polyacrylamide gel stained with coomassie brillient blue (CBB) of the cell -free extract of the induced cells of *H. polymorpha* LR9. Lane M, molecular weight marker (in left side), lane 1, pHAAI-transformant, Lane 2, pGAGU- transformant; lane 3, pFPMT 130-transformant (control). There are three bands (indicated by arrows) that are missing in control, a band of 67 KDa representing glucoamylase peptide, a band of 35KDa representing pro-segment with gamma chain in one peptide and a band of about 49KDa representing gamma chain peptide with that missed the inserted stop codon.

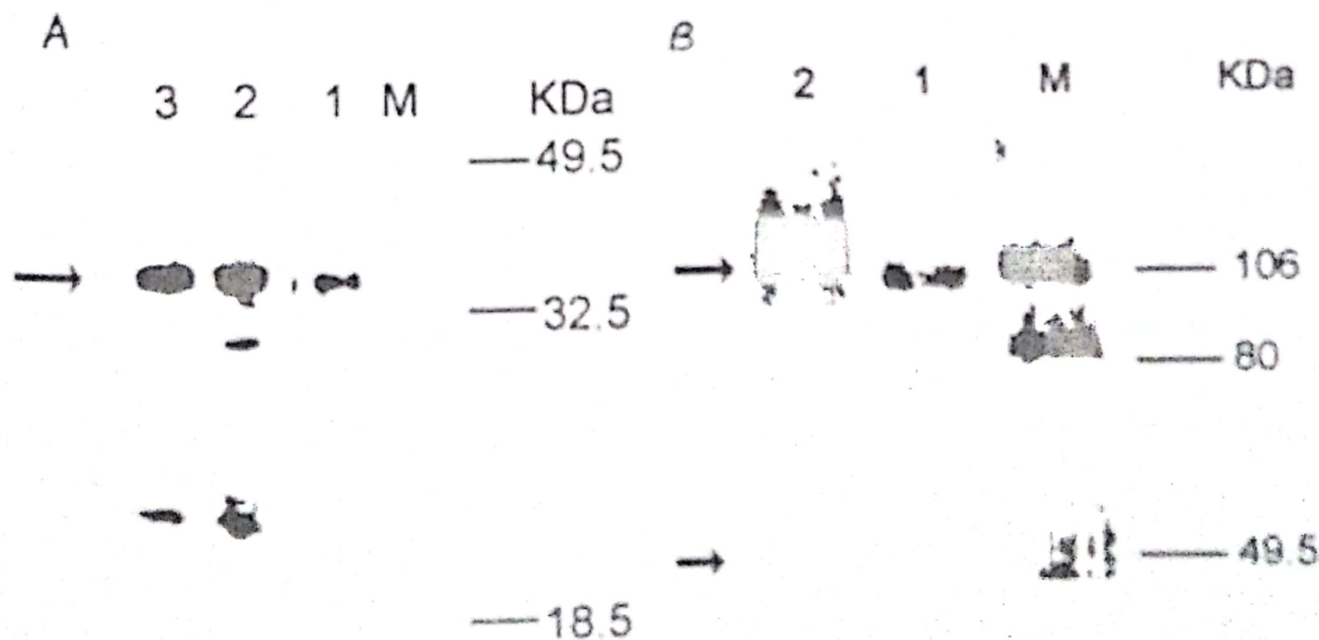

Figure (6): Western analysis of the cell culture supernatant and cell-free extract of the induced cells of *H. polymorpha* LR9.

A. Lane M, molecular weight marker (in right side), land 1, supernatant of pHAAI-transformant; lane 2, cell-free extract of pHAAI transformant; lane 3, pGAGU-transformant. The bands were detected and visualized by anti-mouse, gamma-specific, alkaline phosphatase conjugated antibodies (Sigma). The band of 35KDa (indicated by arrow in left side) was detected by the antibodies in both cell culture supernatant and cell-free extract.

B. lane M, molecular weight marker (in right side), lane 1, cell-free extract of pGAGU-transformant; lane 2, pHAAI-transformant. There are two bands (indicated by arrows in left side) detected by the anti-gamma antibodies, a band of 106KDa representing the native translated peptide and a bands of 49 KDa representing gamma peptide [illegible] that the inserted stop codon was missed by the ribosome.

period of 2-12 hrs. The little degradation of mRNA, demonstrated in the figure may be due to the preparation technique or it may represent the degradation of mRNA that had been translated.

The rate of degradation of gamma mRNA transcribed under the control of *FMD* promoter was higher than that under *MOX* promoter. This could be attributed to the difference in the 5'-untranslated region (5'-UTR) of gamma mRNA under the control of *FMD* promoter than that of MOX promoter. In the 5'-UTR of gamma mRNA produced under *MOX* promoter, it was noticed that there is an additional ATG, translation start codon, in the nucleotide sequence of *MOX* promoter. This additional start codon was near to the first TATA box in the promoter sequence, which in turn would increase the efficiency of transcription and translation. Wakiyama et al.[27], were able to increase the efficiency of translation by 1.6 times of pre-α-lactalbumin by insertion of additional AUG codon in the mRNA, *in vitro*. Ernst[28] had. Also, similar results when he used *S. cerevisiae* transformants for secretion of human granulocyte macrophage-colony stimulating factor (hGM-CSF) under the control of *MF-α, CYC1* and *ACT* promoters. Although the mRNA of hGM-CSF was abundant under the control of the promoters, yet there was still a difference in the protein level produced. The author referred that difference in the secreted protein to the difference in the strength of promoters in the construction procedure and consequently to their 5'-UTR. In the present study both *FMD* and *MOX* promoters are not equal in their strength, as the upstream repression sequence (URS) region of the *MOX* promoter was deleted (Pierera and Hollenberg, personal communication).

The improvement of the production of heterologus proteins in recombinant organisms, is achieved by the development of a secretion system. However, the secretion efficiency is a measurement of the percentage of the product secreted to either the periplasmic space or the culture broth[29]. There are several factors that affect protein secretion. In addition to the expressed protein characters, there are genetic factors including host strain characters, signal sequence, promoter strength and chaperone availability[28,30]. Furthermore, some yeast secretion signal sequences are well-identified in the same or another yeast species, while others are not identified and therefore unprocessed. For example, the pre-pro-alpha factor of *S. cerevisiae* was completely processed and interleukin was efficiently secreted to the culture medium when expressed in *S. cerevisiae* transformants[31]. On the other hand, interferon was neither completely processed nor secreted to the medium although the same leader peptide and the same expression host cells were used[32]. In the present investigation, gamma peptide was not completely processed and pro-alpha was usually accompained with gamma peptide. This could be explained on the basis that pro-alpha was not processed as foreign leader peptide. In contrast, the glucoamylase signal peptide was completely processed and glucoamylase protein was secreted to the culture medium as reported before by Gellissen et al.[6].

KEX2 gene could be cloned within the expression plasmid for efficient processing and secretion of foreign proteins in the case of prepro-α as leader peptide in the yeast *H. polymorpha*. Comparing with the results of kappa chain[9], it was found that kappa chain was more efficiently secreted than gamma chain. Gamma chain was not completely processed and was found as single peptide with the alpha pro-segment. This delay in the pro-segment removal may be due to either missfolding and missassembly of gamma peptide or insufficiency of KEX2 enzyme. Immunoglobulins in the polymorphic structure is composed of heavy and light chains and was efficiently secreted into the ER lumen in the lymphoid cells. In the absence of light chain, heavy chains are retained in the ER lumen, bound to the heavy chain-binding protein (BiP) (Hendershot et al., 1988)[33]. It is not known so far whether the yeast, *H. polymorpha* has similar protein or not.

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استخدام خميرة الهنسنيولا بوليمورفا فى تخليق جزىء اميونو جلوبيولين جاما (IgG) الفئرانى، الجسم المناعى المضاد لإنزيم كيرياتين كيناز (MAK 33)

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فى بحث سابق وصف إستخدام خلايا خميرة الهنسنيولا بوليمورفا فى انتاج السلسلة الحقيقة (كابا) من مقطع(F_{ab})من جزىء بروتين الاميونوجلوبيولين جاما (IgG) . وفى هذا البحث أمكن استخدام خلايا خميرة الهنسنيولا بوليمورفا كأداة لترجمه الشفرة الوراثية الخاصة ببروتين السللة الثقيلة (جاما) الى البروتين المطلوب.

فقد تم تصميم وتركيب بلازميدات تحمل الشفرة الوراثية لتركيب بروتين السللة جاما بالإضافة إلى مورثات خاصة بالتحكيم بإنتاج إنزيمات خاصة بالخميرة وأخرى خاصة بإفراز البروتينات الى خارج الخلية فى خلايا خميرة السكاروميسيس وخميرة الهنسنيولا.

وأمكن نقل هذه البلازميدات الى خميرة الهنسنيولا للحصول على هجائن تنتج بروتين السلسلة جاما بنسب ٧، ١٠٪ من الإنتاج الكلى للبروتين فى الخليــــة وبما يعادل ما يقــرب من ٤٠٠ مجم، ٥٥٠ مجم للتر من مزرعة الخميرة وذلك تحت نظامى تحكم FMD و MOX (الخاصة بإنتاج إنزيمات فى الخلية) بالترتيب، ولكن آلية افراز هذا البروتين الى خارج الخلايا لم تكن مكتملة بما أدى الى تراكم معظمه فى داخل الخلايا.