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ABSTRACT

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Serpins according to their structure are proteins and those studies about fourth structural forming or folding are interesting because of their structural changes which are extremely interesting and including unstable conditions. Serpins percentage in high eukaryotes, plants and viruses are still not known in born homology against animaly and plants in prokaryotes and mycology. Al serpins are not working like a protein’s inhabit or rather some of them are known as other types proteinase like cross-class. For example crmA, virus serpins, with changing its activity restrain (1) interlokin enzymes and SccAAI or antigen-cubic cells, cysteine restrain proteinase in papain groups. Non-inhabitoryserpins show different activities which are including chaperon figures (thermal shock proteins with 47KD molecular weight) or HSP47 and proteins which are transmitted hormones like binding globulin to (CBG). Serpins vocalize around 20% from discovered proteins in blood plasma. The current study is going to investigate the purity separation of determination of the properties and optimization of the alpha-1 Antitrypsin expression increase in Saccharomyces cerevisiae. In order to separate and purify this protein, some properties such as molecular mass, the amount and capacity of inhibiting are also measured. It is worth mentioning that the materials and performing technique in the present study have been determined in the following order: strains, plasmids, cultivation environments, transformation, protein property determination, and expression increase.

INTRODUCTION

Serpins are the main parts of the protein with the different duties and turnovers which is including halter and also unhalter activities such as vocalization of lobe [15]. Serpins of clinically interesting because of the mutation which is available in their structure cause many diseases such as coagulation defects and a number of diseases also caused by structural changes and polymerized protein molecule [20, 11]. Restricting motion of human plasma proteases was determined by Fermi also Pernossi in 1984, in addition the central inhibitor clarifying for anti-proteolytic was at the beginning decomposed by Shultz in 1955, and it was named Alpha-1 Antitrypsin. Human alpha 1-antitrypsin (AAT), a serum glycoprotein, is one of the best-known models of serine protease inhibitors (serpins) superfamily [1, 17]. Alpha 1-proteinase is the main neutral serine proteinase in serum, especially Elastase, which enjoys three different additional Oligo-Saccharides, and it is 12% of its weight. Iso-electric pH of this protein varies between 4.4 and 7.4. An average amount of about 34g of this protein is synthesized by liver and secretes into serum [3, 5, 26]. In addition, AAT exists in the other types of cells, including plackets, pancreas megakaryocytes, langerhans islands [8, 14]. Its density is between 1.5 up to 3.5 gr/lit, and its plasmid half-life is between 4 to 8 days [22]. AAT forms a strong complex with proteases which is an irreversible reaction [4, 16, 23]. Ultimately, reduction of secretion results in this inhibitor shortage, and then leads to some health problems such as pulmonary emphysema [19, 28]. Giving pure AAT to these
patients decreases the disease side effects, and due to its broad treatment application, protein exploitation process could be designed from human blood [24]. Hence, its recombinant production through various sources such as Eukaryotic and prokaryotic micro-organisms is important for genetic manipulation facility and mass production [29]. In this regard, since AAT is the result of mass produced Glycosylated, consistent yeast, Eukaryotic micro-organisms is preferred. After AAT expression in Saccharomyces cerevisiae yeast, expression increased through fermentor, and the properties of this protein, including molecular mass, and inhibition are measured.

Materials and Methods

The intracellular serpins:

Serpins in high Eukaryotes are devided into zgroups:

The intra cellular serpins or ovoserpins making a complete clear group and are the ancestor of extracellular serpins. The adjustment of cell behavior is a completely understandable subject under the obscurities of serpins. Megsin is a member of serpines which is sustaining the megacaryocyte of marrow. Plasminogen has an ability to restrain tumor necrosis factor which is known as an element of apoptosis and MENT is along with agglomerating cromatine. Some of the intercellularserpins or (ovoserpins) are performing the restraining roll. For example PI-6 restrains captain G but the activities of intercellularserpins still unknowable. Any way by the exception of the ovoalbumyne which is a non-inhibitory serpins, all the intercellularserpins including parts of knee is for hibitory activities. OVO albumen which is the main part of the egg call it as an albumen , first of all use as a sauing proteins elements. Recent studies show that ovo albumen from its structural arrangement during embryonic development the extracellular serpins. The extracellular serpins; extracellular serpins are devided in 8 group called as a/b/c/d/e/f/g/h. Among them the biggest group is a, which is contouningserpins like alfa-1-antitrypsine the members of this group are in relationship with wide range of processess which the common one is inspiring the protease. For example R A S P (calistatin) alfa -1-antitrypsine, antikimotrypsine are available in this group. Some of the non-inhibitory serpins such as CBG which are transmitting the hormones and those globolines which are adjoining to (TBG-tirocsine) and peptide hormones (the carrier of angiotansionogen, vterineserpins like UTMP(uterine milk protein) and UFAP (uterina feino associated protein) are including in extracellular serpins in b/c/e groups. Uterine serpines are extremely branching and receptacle non-inhibitory knee area which their activity still mysterious. Joining ovine UTMP to the growth factor which has surveyed indicate that this serpine is completely effective to seprate the active from pregnant uterus. In group F, PEDF (pigmenteEthelium Derived Factor) serpines is available. Which is imagine that is a not o filli factor and α₂ (anti plasmine) which is known as a serpine in moray have common ancestry with mammals in group F. group d is including cofactor F (heparin). Group (g) including C₁ restrainer and in h group hsp 47 serpines are available which never have restrainer activity, and exactly they act like active pap ones in folding procelagen. Micro organisms which are used in researching for doing this research they use unidirectional bacteria and zymogenic which is indicating bellow:

1. DH5α lineage (achrichiakli) it’s a bacteria lineage which is produced by stratagene company .it is used for reproduction and keeping pelasmids. Its genetic profile as follows: (F –gyr A 96 Nlae, rec AL thi-1 hsd R 17 r-k mtk)

2. Zymogenic lineage it is used for AAT recombinant protein, as long as it has shown in genetic plans, (ura3) tubidged has been activated in this zymogenic lineage. Genetic profile (MATa pep4: his 3 prb - Δ1.6R could 1, his 3-20, ura 3-52)

3. Plasmid PYINU-at, vector indicates α1-AT human zymogenic one vector in dicate 2μ based,this image indicate that how vector Manu fractured?

Chemical materials and biologic usages in researching: subculture of bacteria LB (Luria Bertani subculture of hioquid Bacteria (LB)

- Bactopepton(Dif Co ) 10 g
- Zymogenic extract (Dif Co) 5g
- Salt (NaCl) (Merck) 10g
- Distilled water to 1litre volume
- Subculture of bacteria solid LB
- Bacto peptone (Dif Co) 10 g
- zymogenic extract (Dif Co) 5g
- Salt (Merck) (NaCl) 10g
- Aga, powder (Mir Midia) 15g
- Distilled water (to 1 liter volume)
Put these materials into the clean flasks then add distilled water up to 1 liter volume. After that all these materials dissolve in water, sterilize the culture environment by autoclave.

**Results and Discussion**

According for table 1, SDS-PAGE gel is related to Saccharomyces cerevisiae, a zymogene fermentation which is containing AAT gen with inspiration and cycle engine is depending on oxygen from left to right zero indicate a time before on inspiration and 1 to 5 hours regularly indicate after inspiration.

**Table 1**: Zymogene fermentation of Saccharomyces cerevisiae including AAT Gen along with starting of inspiration at 2 after insemination with IPTG 0.4 mM and cycle engine which is depending on oxygen

<table>
<thead>
<tr>
<th>Explanation</th>
<th>Time according to hours</th>
<th>The time after inspiration</th>
<th>OD600</th>
<th>Speed on minute</th>
<th>Temp.</th>
<th>Weather pressure according to vvm</th>
<th>pH</th>
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<td>150</td>
<td>30</td>
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</table>

**Diagram 1**: Fermentation of Saccharomyces cerevisiae yeast containing AAT gene accompanied with induction start at time 2 after insemination and oxygen-dependent revolution.

According to these cases at the continuances of zymogene fermentation of Saccharomyces cerevisiae with AAT Gen and the starting of inspiration during 3/4/5/6/7 hours after insemination with IPTG 0.4 mM with cycle engine which is related to oxygen had done.

Tables 2 to 6 with graphs indicate those explanations.

**Table 2**: Zymogene fermentation of Saccharomyces cerevisiae including AAT Gen along with starting of inspiration at 3 after insemination with IPTG 0.4 mM and cycle engine which is depending on oxygen

<table>
<thead>
<tr>
<th>Explanation</th>
<th>Time according to hours</th>
<th>The time after inspiration</th>
<th>OD600</th>
<th>Speed on minute</th>
<th>Temp.</th>
<th>Weather pressure according to vvm</th>
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<td>0.3</td>
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<tr>
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</table>
**Diagram. 2:** Fermentation of Saccharomyces cerevisiae yeast containing AAT gene accompanied with induction start at time 3 after insemination and oxygen-dependent revolution

**Table 3:** Zymogene fermentation of Saccharomyces cerevisiae including AAT Gen along with starting of inspiration at 4 after insemination with IPTG0.4m M and cycle enginge which is depending on oxygen.
Diagram. 3: Fermentation of Saccharomyces cerevisiae yeast containing AAT gene accompanied with induction start at time 4 after insemination and oxygen-dependent revolution

Table 4: Zymogene fermentation of Saccharomyces cerevisiae including AAT Gen along with starting of inspiration at 5 after insemination with IPTG 0.4m M and cycle engine which is depending on oxygen.

<table>
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<tr>
<th>exlanation</th>
<th>Time according to hours</th>
<th>The time after inspiration</th>
<th>OD 600</th>
<th>Speed on minute</th>
<th>Temp.</th>
<th>Weather pressure according to vvm</th>
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<td>215</td>
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<td>7</td>
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<td>2.3</td>
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</table>
Diagram. 4: Fermentation of Saccharomyces cerevisiae yeast containing AAT gene accompanied with induction start at time 5 after insemination and oxygen-dependent revolution

Table 5: Zymogene fermentation of saccharomyces cerevisiae including AAT Gen along with starting of inspiration at 6 after insemination with IPTG0.4m M and cycle enginge which is depending on oxygen

<table>
<thead>
<tr>
<th>Explanation</th>
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<th>The time after inspiration</th>
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<th>Speed on minute</th>
<th>Temp.</th>
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<td>2.41</td>
<td>390</td>
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<td>1</td>
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</table>
Diagram. 5: Fermentation of Saccharomyces cerevisiae yeast containing AAT gene accompanied with induction start at time 6 after insemination and oxygen-dependent revolution

Table 6: Zymogene fermentation of saccharomyces cerevisiae including AAT Gen along with starting of inspiration at 7 after insemination with IPTG 0.4m M and cycle enginge which is depending on oxygen

<table>
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<th>explanation</th>
<th>Time according to hours</th>
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<th>OD 600</th>
<th>Speed on minute</th>
<th>Temp.</th>
<th>Weather pressure according to vvm</th>
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<td>1.80</td>
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<td>100μg/ml ampicilin</td>
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<td>1.85</td>
<td>290</td>
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Serpin A1, also known as Alpha-1 antitrypsin (AAT), is a prototype member of the serpin super family of the serine protease inhibitors [12]. This serine protease inhibitor blocks the protease, neutrophil elastase. Alpha-1 antitrypsin is mainly produced in the liver and acts as an antiprotease [18, 13]. Its principal function is to inactivate neutrophil elastases, preventing tissue damage. Alpha1-antitrypsin, an acute phase protein and the classical neutrophil elastase inhibitor, is localized within lipid rafts in primary human monocytes in vitro [27]. It association with monocytes is inhibited by cholesterol depleting/efflux-stimulating agents (nystatin, filipin, MbetaCD (methyl-beta-cyclodextrin) and oxidized low-density lipoprotein (oxLDL) and conversely, enhanced by free cholesterol. Furthermore, SerpinA1/monocyte association per se depletes lipid raft cholesterol as characterized by the activation of extracellular signal-regulated kinase 2, formation of cytosolic lipid droplets, and a complete inhibition of oxLDL uptake by monocytes [7, 25]. Previous population studies have suggested that heterozygote status for the AAT gene (SerpinA1) is a risk factor for chronic rhinosinusitis with nasal polyposis (CRSwNP). Alpha-1 antitrypsin deficiency is a recently identified genetic disease that occurs almost as frequently as cystic fibrosis. It is caused by various mutations in the SerpinA1 gene, and has numerous clinical implications [2]. Alpha-1 antitrypsin deficiency is an inherited disease affecting the lung and liver. In the liver, alpha-1 antitrypsin deficiency may manifest as benign neonatal hepatitis syndrome; a small percentage of adults develop liver fibrosis, with progression to cirrhosis and hepatocellular carcinoma [6, 10]. It’s most important physiologic functions are the protection of pulmonary tissue from aggressive proteolytic enzymes and regulation of pulmonary immune processes [21]. It is apparent that Zymogen IA is similar to hog pepsinogen in several respects. It is known that hog pepsinogen is stable in a mild alkaline medium in which hog pepsin is unstable. The observation that gastricsin and human pepsin are produced from the activation of the Zymogen IA fraction suggests two possibilities. (a) Zymogen IA is a common precursor for gastricsin and human pepsin, and (b) Zymogen IA consists of twozymogens, pepsinogen and a zymogen for gastricsin. The evidences in favor of the “common precursor” are (i) Zymogen IA appeared as a single boundary in ultracentrifugation studies and as a single band in starch gel electrophoresis; (ii) the ratio of pepsin to gastricsin varied as a function of the activating pH whereas the total amount of enzyme produced from Zymogene. Gastricsin and human pepsin were both found to be present as a zymogen in human gastric mucosa. In the attempts to purify the zymogen, a fraction (IA) has been obtained from the alkaline extract of human gastric mucosa after ammonium sulfate
precipitation and diethylamino ethyl cellulose chromatography. Zymogen IA behaved as a single component in ultracentrifugation and starch gel electrophoresis. It was stable in a solution of pH 8.5. However, once acidified to pH 5 or below, it was no longer stable at pH 8.5. When acid-activated Zymogen IA was fractionated in an Amberlite IRC-50 column or by starch gel electrophoresis, gastric sin and human pepsin were obtained. The physicochemical and enzymatic properties of the enzymes obtained from the activation of Zymogen IA were identical to gastric sin and human pepsin isolated from human gastric juice. It appeared that both enzymes might be derived from this same zymogen. Single-chain pro-urokinase (pro-uPA) is present both in the medium and lysate of the A431 epidermoid carcinoma cell line. Most of the cell-associated pro-uPA is on the cell surface, as shown by indirect immuno-fluorescence and by surface lodination. Pro-uPA is not an integral membrane protein but is bound to a specific surface receptor that is completely saturated [9]. A mild acid treatment uncovers the surface receptors by dissociating pro-uPA. Resaturation of uncovered receptors has been studied by reincubating cells in normal medium; within 40 min, 50% of the free sites are reoccupied. Excess uPA-specific antibodies prevent rebinding of ligand to the receptors. Thus, A431 cells first secrete uPA, which then binds to the surface receptor. We propose that the synthesis of uPA and uPA receptor by the same cell may provide a pathway for the activation of the metastatic potential of malignant cells.

REFERENCES


