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Original Article

Studies on the recombinant production and anticancer activity of thermostable L- asparaginase I from *Pyrococcus abyssi*

Estudos sobre a produção recombinante e atividade anticancerígena da L-asparaginase I termoestável de *Pyrococcus abyssi*

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Abstract

L-Asparaginase catalysing the breakdown of L-Asparagine to L-Aspartate and ammonia is an enzyme of therapeutic importance in the treatment of cancer, especially the lymphomas and leukaemia. The present study describes the recombinant production, properties and anticancer potential of enzyme from a hyperthermophilic archaeon *Pyrococcus abyssi*. There are two genes coding for asparaginase in the genome of this organism. A 918 bp gene encoding 305 amino acids was PCR amplified and cloned in BL21 (DE3) strain of *E. coli* using pET28a (+) plasmid. The production of recombinant enzyme was induced under 0.5mM IPTG, purified by selective heat denaturation and ion exchange chromatography. Purified enzyme was analyzed for kinetics, *in silico* structure and anticancer properties. The recombinant enzyme has shown a molecular weight of 33 kDa, specific activity of 1175 U/mg, K_M value 2.05mM, optimum temperature and pH 80°C and 8 respectively. No detectable enzyme activity found when L-Glutamine was used as the substrate. In silico studies have shown that the enzyme exists as a homodimer having Arg11, Ala87, Thr110, His112, Gln142, Leu172, and Lys232 being the putative active site residues. The free energy change calculated by molecular docking studies of enzyme and substrate was found as ΔG – 4.5 kJ/mole indicating the affinity of enzyme with the substrate. IC_{50} values of 5U/mL to 7.5U/mL were determined for FB, caco2 cells and HepG2 cells. A calculated amount of enzyme (5U/mL) exhibited 78% to 55% growth inhibition of caco2 and HepG2 cells. In conclusion, the recombinant enzyme produced and characterized in the present study offers a good candidate for the treatment of cancer. The procedures adopted in the present study can be prolonged for *in vivo* studies.

Keywords: asparaginase, cloning, kinetics, *in silico*, anticancer.

Resumo

A L-asparaginase, que catalisa a degradação da L-asparagina em L-aspartato e amônia, é uma enzima de importância terapêutica no tratamento do câncer, especialmente dos linfomas e da leucemia. O presente estudo descreve a produção recombinante, propriedades e potencial anticancerígeno da enzima de Pyrococcus abyssi, um archaeon hipertermofílico. Existem dois genes que codificam para a asparaginase no genoma desse organismo. Um gene de 918 bp, que codifica 305 aminoácidos, foi amplificado por PCR e clonado na cepa BL21 (DE3) de E. coli usando o plasmídeo pET28a (+). A produção da enzima recombinante foi induzida sob 0,5mM de IPTG, purificada por desnaturação seletiva por calor e cromatografia de troca iônica. A enzima purificada foi analisada quanto à cinética, estrutura in silico e propriedades anticancerígenas. A enzima recombinante apresentou peso molecular de 33 kDa, atividade específica de 1.175 U / mg, valor de KM 2,05 mM, temperatura ótima de 80º C e pH 8. Nenhuma atividade enzimática detectável foi encontrada quando a L-glutamina foi usada como substrato. Estudos in silico mostraram que a enzima existe como um homodímero, com Arg11, Ala87, Thr110, His112, Gln142, Leu172 e Lys232 sendo os resíduos do local ativo putativo. A mudança de energia livre calculada por estudos de docking molecular da enzima e do substrato foi encontrada como ΔG – 4,5 kJ / mol, indicando a afinidade da enzima com o substrato. Valores de IC50 de 5U / mL a 7,5U / mL foram determinados para células FB, células caco2 e células HepG2. Uma quantidade de enzima (5U / mL) apresentou inibição de crescimento de 78% a 55% das células caco2 e HepG2, respectivamente. Em conclusão, a enzima recombinante produzida e caracterizada no presente estudo é uma boa possibilidade para o tratamento do câncer. Os procedimentos adotados na presente pesquisa podem ser aplicados para estudos in vivo.

Palavras-chave: asparaginase, clonagem, cinética, in silico, anticancerígeno.

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1. Introduction

L- asparaginase (EC 3.5.1.1) is the enzyme that catalysis the hydrolytic deamination of L-asparagine to produce L-aspartate and ammonia (Pritsa and Kyriakidis, 2001; Borek et al., 2004). The enzyme found in a wide range of prokaryotic and eukaryotic sources (Moharib, 2018; Aisha et al., 2020). Physiologically, L-asparaginase has a vital role in the amino acid catabolism. The aspartate produced in the catalyzed reaction can enter the Krebs cycle or it can be used for protein synthesis (Purwaha et al., 2014), the enzyme is also involved in the synthesis of methionine, threonine, and lysine (Guo et al., 2017). Enzyme isolated from different sources has shown variable biological activity e.g. L-asparaginase from *Streptomyces rochei* has 119.5 U/mg of proteins (El-Naggar and El-Shweihy, 2020), from *Anoxybacillus flavithermus* 165U/mg (Maqsood et al., 2020), and enzyme isolated from *Bacillus altitudinis* exhibited 800 U/mg (Prakash et al., 2020). L-asparaginase from different species has been intensively used for the treatment of leukaemia (Verma et al., 2007; Hourani et al., 2008; Lanvers-Kaminsky et al., 2020). The enzyme has also shown growth suppression effects in many other cancer cells including HEPG2 and HCT-116, HELLA (Moharib, 2018), MCF-7 cells (Baskar et al., 2015), K-562, Jurkat clone E6-1, HL-60 (Abakumova et al., 2012; Mahajan et al., 2014). The anticancer activity of L-asparaginase is based on the hydrolytic deamination of L-asparagine, the normal cells can produce L-asparagine. However, it becomes an essential amino acid for cancer cells due to absence of asparagine synthase activity in these cancer cells (Kotzia and Labrou, 2007; Dhankhar et al., 2020). In addition to its anticancer activity the enzyme also has an ability to prohibit or reduce the production of toxic compounds such as acrylamide in the baked or fried foods. Free L-asparagine present in the raw vegetable and meat based food materials can react with reducing sugars at high temperatures. The treatment of food materials with L-asparaginase before baking has found to significantly reduce the production of acrylamide (Paul and Tiwary, 2020; Koszucka et al., 2020; Muneer et al., 2020). Owning a great pharmaceutical and industrial importance L-asparaginase has an ever increasing demand in the international market that cannot be fulfilled by natural resources. Recombinant DNA technology has made it possible to produce bulk quantities of enzymes and proteins of pharmaceutical importance in *E. coli* and *Bacillus* species. Genetically modified bacterial strains with disrupted native protease and RNase enzymes, preferred tRNA genes and genome having lysogenically introduced bacteriophage T7 RNA polymerase gene are often used as a tool for recombinant protein production (Gopal and Kumar, 2013; Correddu et al., 2020; Duarte et al., 2020; Doozandeh-Juibari et al., 2020). *Pyrococcus abyssi* is an archaeon that grows under stringent conditions with optimum growth temperature 96°C, under anaerobic high pressure conditions (Erauso et al., 1993), its genome has been analysed (Cohen et al., 2003). To our knowledge, the species has not been explored for the characteristics of its proteins and enzymes. Therefore, the present study was aimed to characterize the recombinant L-asparaginase I from *Pyrococcus abyssi* and its effectiveness as anticancer molecule.

2. Materials and Methods

2.1. Materials

Gene specific primers for PCR with restriction sites for *Nco*I and *Bam*HI were commercially synthesized from Macrogen (South Korea), PCR amplification was carried out using ABL (Applied Bio systems) thermocycler. *E. coli* BL21 (DE3) strain (Catalogue No. CMC0024) were obtained from MERK, pET-28a (+) plasmid was obtained from Novagen. Other chemicals including kanamycin, ampicillin, IPTG, NaCl, acrylamide solution, LB-medium, trisma base, glacial acetic acid, Nessler's reagent, trichloroacetic acid, L-glutamine, L-asparagine, were obtained from Millipore Sigma. DEAE-Sephadex was obtained from GE Healthcare Bio-Sciences. All of these and other chemicals and reagents used in the present study were of molecular biology grade.

2.2. Manipulation of l-asparaginase gene

A complete open reading frame (ORF) of the L-asparaginase I gene from *Pyrococcus abyssi* comprising 918 bp was PCR amplified using the forward and reverse primers with nucleotide sequences 5'-ccatggtggctattatcgttcatg-3' and 5'- ttaaggatccctaaacaccagcct-3' respectively. The sites for restriction enzymes *Nco*I and *BamH*I were introduced at the both ends of ORF. The PCR product was initially cloned by pJET1.2 plasmid vector using the procedure provided with the CloneJET PCR Cloning Kit (Catalogue No. K1232 - Thermo Scientific TM). The successfully transformed bacterial colonies were cultured in LB broth supplemented with 50 µg of kanamycin per millilitre of medium. The recombinant plasmid was isolated from the confirmed colonies, gene was restricted out and subcloned in pET28a (+). Successful transformation was screened on LB agar plates containing 50 µg of kanamycin per millilitre of medium. The recombinant plasmid was confirmed by colony PCR and restriction analysis.

2.3. Induction of l-asparaginase production

The confirmed *E. coli* cells transformed with pET28a (+) plasmid ligated with L-asparaginase gene (pET28- Asn+) were cultured in LB broth containing 50µg/mL kanamycin. The conical glass flasks of 250 mL capacity containing 25mL medium were inoculated with 1% overnight culture of transformed cells. The 2nd culture was incubated in a shaking incubator adjusted at 200 rpm and 37°C, expression of L-asparaginase gene was persuaded by the addition of 0.5mM IPTG (isopropylβ-D-thiogalactopyranoside) when the bacterial culture attained an absorbance of 0.5 to 0.6 at 600 nm. *E. coli* cells transformed with pET28a (+) without L-asparaginase gene (pET28-Asn-) was also used in a parallel negative control experiment. Induction of gene expression was carried out for 3 h followed by centrifugation of bacterial cells at 2,000×g for 5 min at 4°C. The harvested cell precipitate was suspended in ice chilled 20 mM phosphate buffer pH 7.5 and the suspension was sonicated (Model MSK-USP3N by MTI corporation) for 5 to 7 min using 30 seconds moderate pulse exposure followed by1 minute incubation on ice. The cell debris was

removed by centrifugation at 15000 x g / 10min / 4°C and supernatant was used for further investigations.

2.4. Measurement of enzyme activity and protein content

Total protein component of supernatant was estimated by using Bradford method (Bradford, 1976), aqueous solution of bovine serum albumin was used for the preparation of standard curve. The activity of enzyme was measured by a modified method adopted from literature (Chohan and Rashid, 2013). In brief, the standard curve of ammonia was prepared using known molar concentrations of ammonium chloride. The reaction mixture comprising 100 mM L-asparagine prepared in phosphate buffer pH 7.5, adjusted at 80°C was mixed with 50µL enzyme solution and incubated at the same temperature in a water bath for 5 min, the amount of urea released was estimated by Nessler's reagent method measuring absorbance at 480 nm. One unit was defined as the amount of enzyme that can release 1 µmole of ammonia per minute under above assay conditions. Glutaminase activity of enzyme was determined by the procedure adopted from the recent literature (Shah et al., 2019).

2.5. Purification of enzyme and SDS-PAGE analysis

The supernatant I was subjected to 80°C in a water bath for 15 min and heat denatured *E. coli* proteins were precipitated and discarded by centrifugation at 12000 x g / 10 min / 4°C. The supernatant II was dialyzed overnight in 10 volumes of buffer A (20 mM phosphate buffer pH 7.5) at 4°C and loaded onto a manual chromatography colum (2.5 x 30 cm) packed with 30 to 35mL of DEAE-Sephadex and equilibrated with buffer A. After washing out the unbound proteins, the bound proteins were eluted by 0 to 0.5M linear NaCl gradient at a flow rate of 2mL per minute. The specific activity, total protein content, number of units were noticed at each purification step. The fractions with maximum protein content were subjected to SDS-PAGE (Laemmli and Favre, 1970).

2.6. Enzyme stability, specificity and kinetic properties

To determine the temperature stability, the purified enzyme solution (3-5µg/µL) prepared in buffer A was incubated at different temperatures (40°C to 100°C) for 5 minutes, immediately transferred to ice box and its activity was measured. To determine the enzyme specificity, activity was measured using L-asparagine and L-glutamine solutions in parallel reactions. The temperature for optimum enzyme activity was determined by adjusting the temperature of reaction mixture at different temperatures from 40°C to 100°C. The pH value for the optimum enzyme activity was determined by calculating the activity of enzyme in the reaction mixtures prepared in buffer solutions adjusted at different pH (6 to 10). To determine the K_M and Vmax values, a linearly increasing concentration of L-asparagine was used with all constant assay parameters. The inverse of enzyme activity was plotted against the inverse of substrate concentration. The values of K_M and Vmax were calculated by Lineweaver-Burk plot.

2.7. In silico studies

3D structure of asparaginase from *Pyrococcus abyssi* (Asn-PA) was generated by Swiss-Model (Biasini et al., 2014) and validated by RAMPAGE which demonstrated the structure of proteins in terms of cBeta, psi and phi deviations in terms of Ramachandran plot. The polypeptide comprising of 305 residues was aligned structurally using information available in the data basis. L-asparagine ligand was obtained from PubChem (CID_6267), and energy minimized and prepared for docking using Molecular Operating Environment (MOE) software (Version 2018) and ConSurf. Protein structure firstly energy minimization, partially charged, and protonated using MOE. Ligand protein interaction analyzed using PLIP webserver (Celniker et al., 2013; Salentin et al., 2015).

2.8. Anticancer effect of l-asparaginase

The cancerous cell lines including human HepG2 cells (hepatocellular carcinoma cells) and caco2 cells (colorectal adenocarcinoma cells) and non-cancerous FB cells (human fibroblast cells were used to determine the cytotoxic and anticancer effects of purified L-asparaginase on these cells (cancerous and non-cancerous). In brief, 100 µL well calculated dilutions with known enzyme activity were prepared in DMEM medium and incubated with approximately $6x10^4$ cells per millilitre of medium. This treatment was given to all three types of cells in parallel experiments. A modified neutral red assay procedure (Weyermann et al., 2005), was used to determine the 48 h post treatment cytotoxic effects of enzyme. IC_{50} is the amount of enzyme required to kill 50% of cells in a culture. The degree of anticancer selectivity SI was determined by IC_{50} of enzyme in a normal non-cancerous cell line divided by the IC_{50} enzyme against cancer cells.

3. Results

3.1. Production and purification of recombinant l-asparaginase

The complete 918 bp L-asparaginase gene consisting was amplified by polymerase chain reaction, ligated into pJET1.2 blunt end cloning vector and subcloned in pET28a (+) plasmid. Production of recombinant enzyme was induced under 0.5 mM IPTG for 3 h. The clear supernatant obtained from disrupted *E. coli* cells exhibited a 33kDa protein band on SDS-PAGE indicating an overexpression of L-asparaginase gene (Figure 1). Recombinant enzyme was found in soluble and active form. It was subsequently purified by selective heat denaturation followed by DEAE-Sephadex based colum chromatography. The finally purified fractions gave a single band on SDS-PAGE (Figure 1). Enzyme specific activity, protein content, total number of units and level of purity were determined at each purification step. The enzyme was purified up to 44.57 folds and its specific activity was found as 1175U/mg of protein (Table 1).

3.2. Stability, substrate specificity and kinetics

The purified enzyme solution was found stable to a wide range of temperature levels. After incubation at 40°C

to 70°C there was no detectable change in the enzyme activity. However, the post temperature treatment enzyme activity was decreased from 80°C to 100°C (Figure 2). The activity of enzyme was only about 45% in the reaction mixture adjusted at 40°C, it increased with the increase in temperature and optimum activity was found at 80°C (Figure 2). There was no detectable activity of enzyme at low acidic pH, it exhibited activity from pH 5 to 10 with maximum activity at pH 8 (Figure 3). The KM and Vmax values for purified recombinant L-asparaginase was found at 2.051mM L-asparagine and 256.4 µmoles of ammonia detected per minute (Figure 4).

3.3. In silico analysis

3D model of L-asparaginase appeared as a homodimer when built in the Swiss-Model software and QSQE server and visualized by PyMOL. According to RAMPAGE results, the generated model showed 93.2% (~98.0% expected) of residues were in their favoured region, 5.4% (~2.0% expected) were in their allowed region, and only 1.4% at outlier region. The interaction of L-asparaginase enzyme with L-asparagine exhibited a free energy change (ΔG value) slightly negative (ΔG - 4.5 kJ/mole) (Figure 5). The interaction of our enzyme with L-asparagine resulted in formation of hydrogen bonds in the ES-complex (Table 2). Five residues that forming hydrogen bonds were located at highly conserved region of the enzymes have been described in the present study. These include Ala87, Thr110, His112, Gln142, and Leu172 (Figure 6).

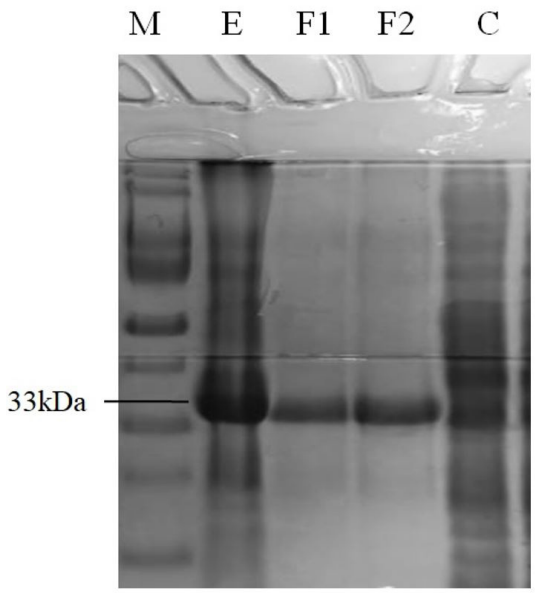

Figure 1. SDS-PAGE photograph. M, Protein marker; E, cellular extract from experimental culture; F1 and F2, purified enzyme fractions collected from chromatography colum; C, negative control experiment (extract of cells transformed with plasmid without gene of interest).

3.4. Anticancer activity of enzyme

The safety pattern of purified L-Asparaginase was determined by using human fibroblast cells. The IC_{50} values ranging from 5 to 7.5U/mL were determined. The anticancer activity of enzyme was determined against caco2 cells and HepG2 cell lines. The selected amount of enzyme (5U/mL) has shown 78% to 55% growth inhibition of caco2 and HepG2 cells when subjected to 48h incubation time.

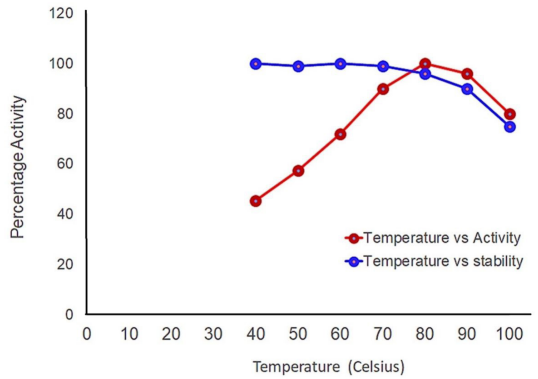

Figure 2. Effect of temperature on the stability and activity of enzyme. The optimum activity of purified enzyme was calculated at 80°C. The enzyme was considerably stable at temperatures up to 100°C.

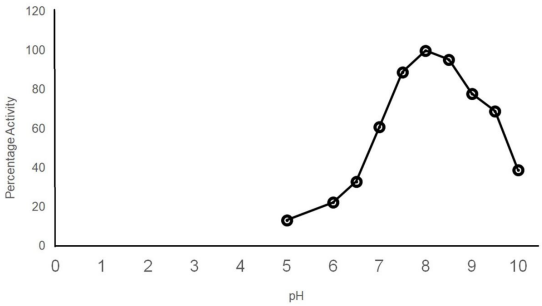

Figure 3. Effect of pH on the enzyme activity. The pH value for optimum enzyme activity was found 8.

Table 1. Illustration of different parameters of enzyme during each purification step.

Sr. No.	Enzyme purification steps	Activity (U/mL)	Protein content (mg/mL)	Specific activity (U/mg)	Total units	Percentage recovery	Fold purification
1	Crude extract	145	5.5	26.36	45700	100%	1
2	Selective heat denaturation	133	4.0	33.25	41580	91%	1.26
3	Chromatography	940	0.8	1175	23000	50.3%	44.57

At our experimental conditions, 95% enzyme activity was retained at 37°C for 72h. The selectivity index was 3.0 and 0.98 against caco2 and HepG2 respectively indicating a better anticancer impact of enzyme against caco2 cells.

4. Discussion

L-asparaginase is an enzyme found in plants, fungi and microbes. It has been widely used as an anticancer therapeutic agent. The enzyme from many sources has L-glutaminase activity which has cytotoxic and hazardous effects (Parmentier et al., 2015; Amadasun, 2020). These findings emphasize to continue the search for highly substrate specific enzyme that can be more stable and useful in anticancer therapy. *Pyrococcus abyssi* is a hyperthermophilic archaeon that grows at about 96°C under anaerobic and high pressure conditions (Erauso et al., 1993). According to the genomic studies, *P. abyssi* has two L-asparaginase genes. In the present study we have described the short chain L-asparaginase I for its characteristics and anticancer effects. Genetic engineering tools and techniques have been widely used for the production of enzyme from various resources (Einsfeldt et al., 2016; Saeed et al., 2018). In the present study we have produced a recombinant of L-asparaginase from *P. abyssi* in *E. coli*, purified and characterized the enzyme and evaluated its anticancer potential. Genomic DNA of *P. abyssi* was obtained from DSMZ (InhoffenstraBe7B 38124, Braunschweig, Germany). The 918 bp gene coding for asparaginase was cloned in pJET1.2 blunt end cloning vector and subcloned in pET28a (+) plasmid. The later plasmid has T7 promoter that is highly specific for T7 RNA polymerase coded by the modified bacterial genomic DNA. The enzyme production was induced under 0.5mM IPTG. Normally the concentration of IPTG from 0.2 to 1mM is used for the induction of gene expression (Silaban et al., 2019). The crude extract obtained after sonication of bacterial culture was purified by selective heat denaturation and ion-exchange chromatography. The chromatography conditions were optimized using the information from our previous studies. The purified enzyme gave a 33 kDa band on SDS-PAGE (Figure 1). The enzyme with different molecular weight has been reported in the literature, as for example enzyme isolated from corn cob has molecular weight of 11.2 kDa (Makky et al., 2013) and from *Penicillium cyclopium* 55 kDa (Shafei et al., 2015). Our subject L- Asparaginase has exhibited stability at high temperatures and maximum activity at 80°C (Figure 2). The enzyme from various sources has shown an optimum activity at different temperatures starting from 37°C (Shafei et al., 2015), to 85°C (Muneer et al., 2020).

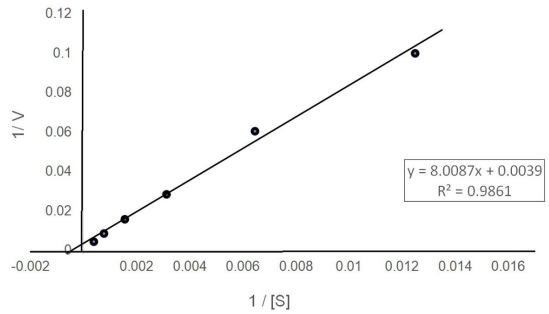

Figure 4. Lineweaver-Burk plot indicating used for the calculation of KM and Vmax of recombinant L-asparaginase.

Figure 5. 3D structure of L-asparaginase, α-helices are shown in red, β-pleated sheets in yellow. The interaction of enzyme and L-asparagine is shown, ligand atoms are shown in balls at active site.

Table 2. Hydrogen bonds formed from interaction of L-Asparaginase enzyme and L-Asparagine.

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Sidechain	Donor Atom	Acceptor Atom
1	11A	GLY	2.28	3.24	157.37	X	X	4482 [N3]	70 [O2]
2	87A	ALA	2.09	3.05	163.88	✓	X	641 [Nam]	4461 [O.co2]
3	87A	ALA	2	2.93	164.05	X	X	4461 [O.co2]	644 [O2]
4	110A	THR	3.24	4.05	138	X	X	4464 [N3]	810 [O2]
5	112A	HIS	3.12	4.08	158.83	X	X	4465 [Nam]	825 [O2]
6	142A	GLN	3.41	4.08	127.22	✓	✓	1072 [Nam]	4483 [Nam]
7	172A	LEU	2.21	3.17	163.78	✓	X	1326 [Nam]	4480 [O.co2]
7	172A	LEU	2.21	3.17	163.78	✓	X	1326 [Nam]	4480 [O.co2]
8	232A	LYS	2.54	3.41	142.36	✓	✓	3973 [N3+]	4461 [O.co2]

Abbreviations: AA= amino acid, H-A= distance between hydrogen and acceptor atom, D-A= distance between donor and acceptor atom.

Figure 6. ConSurf generated conserved sequences of L-asparaginase gene, the active site residues associated with interaction of ligand are shown in boxes.

The recombinant enzyme has shown maximum activity at pH8 (Figure 3). L-asparaginase from different species with optimum activity at pH values ranging from pH 6 (Kishore et al., 2015) to pH 10 (Kumar et al., 2011) have been reported. The enzyme has shown 2.05 K_M value for L-asparagine (Figure 4) and it has shown no activity with L-glutamine. The enzymes without L-glutaminase activity have been reported (Kumar et al., 2011; Meena et al., 2016). The KM value of our investigated enzyme is lower than 5.5mM and 10mM as calculated for the enzyme isolated from *Thermococcus kodakaraensis* and *Streptomyces fradiae* respectively (Chohan and Rashid, 2013). However, the L-asparaginase with K_M values 0.81mM have also been reported from *Aspergillus niger* (Vala et al., 2018) that represents a better affinity with the substrate. In silico studies have shown that the enzyme if found as a homodimer (Figure 5). The molecular docking studies have shown free energy change of – 4.5 k Joule/ mole for the binding of enzyme with the substrate. The asparaginases from *E. coli* and *Pyrococcus horikoshii* have been reported to exist as dimers (Yun et al., 2007; Vimal and Kumar, 2017). The molecular docking studies with substrate have shown Arg11, Ala87, Thr110, His112, Gln142, and Leu172 as the putative active site amino acids (Figure 6, Table 2). However, according to literature, the calculations made by molecular docking studies have limited probability of confirmation in the wet labs (Ramírez and Caballero, 2016). The enzyme cytotoxicity and anticancer efficacy has been

evaluated in the present study. According to our findings the IC_{50} values of 5U/ mL to 7.5U/mL were determined for FB, caco2 cells and HepG2 cells. The growth of caco2 cells was inhibited by 78% and that of HepG2 cells was inhibited up to 55% when subjected to 5 U of enzyme for 48h. Our subject enzyme is less effective than asparaginase from *E. coli* against U 937 cells (Aljewari et al., 2010), the less efficacy can be due to higher optimum temperature for subject enzyme activity. The activity against caco2 cells and HepG2 cells has shown the potential anticancer activity of our subject enzyme against the precursor tumour types i.e. colorectal cancer and hepatocellular carenoma (Taheri et al., 2020; Hajiasgharzadeh et al., 2020). It has shown better anticancer activity as compared to enzyme isolated from *Rhodospirillum rubrum* (Zhdanov et al., 2017). More than 90% enzyme activity was retained in the cell culture after 72h that indicated the long term stability of the enzyme in cell culture.

5. Conclusions

Absence of L- glutaminase activity, ability to remain active at a wide range of pH and temperature, long term stability at cell culture conditions, better binding affinity with the substrate, advocate the subject enzyme a good therapeutic candidate for the treatment of colorectal cancer and hepatocellular carcinoma.

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