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Research Article

BIO-CONJUGATION OF *BACILLUS FASTIDIOSUS*-URICASE WITH METHOXY POLYETHYLENE GLYCOL DERIVATIVE AND STUDY OF PHYSIOCHEMICAL PROPERTIES

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ABSTRACT

Uricase (EC 1.7.3.3, UC) is an enzyme belonging to the class of oxidoreductases and catalyses the oxidation of uric acid to allantoin, carbon dioxide and hydrogen peroxide. In this present work, Uricase from *Bacillus fastidisous* was conjugated with methoxypolyethyleneglycol *p*-nitrophenyl carbonate (mPEG-np) a polyethylene glycol derivative, in order to improve the pharmaceutical properties of therapeutic enzyme uricase. The PEGylated conjugates (uricase-mPEG-np) were synthesized using various ratios of uricase and mPEG-np to get maximum residual activity. The PEGylated uricase showed maximum residual uricolytic activity of 90.9% compared to the unmodified uricase, which was achieved at a ratio of 1:17 of uricase to mPEG-np. PEGylated uricase was further characterized using SDS-PAGE to determine its final molecular weight and approximate number of mPEG molecules attached. The result showed that the molecular weight was increased to 79.4 KDa and the number of mPEG molecules bound per subunit of uricase was approximately 9. Stability of the PEGylated uricase at various temperature and pH was studied and found to be 32°C and pH of 9.0. Further the mechanism of binding and possible sites of binding were studied using molecular modeling and docking software tool ArgusLab 4.0.1 and the two-dimensional image of docked uricase were generated.

Keywords: Uricase, PEGylation, PEG Derivative, Docking, ArgusLab 4.0.1

INTRODUCTION

Urate oxidase or uricase (urate: oxygen oxidoreductase, EC 1.7.3.3) is a therapeutic enzyme, which catalyses the oxidative opening of the purine ring of urate to yield allantoin, carbon dioxide, and hydrogen peroxide [1]. Uric acid is present in blood plasma, as an end product of purine metabolism and also formed in the body as a result of tumor lysis syndrome, wherein the malignant cells burst and abruptly releases nucleic acids, proteins and other metabolites into the blood stream which leads to hyperuricemia [2]. Excessive concentration of uric acid in the blood can lead to Gout, painful arthritis, disfiguring urate deposits (tophi) and renal failure [3]. Gout can be cured by intravenous administration of uricase enzyme, which can convert uric acid crystals to allantoin. Allantoin being five to ten times more soluble than uric acid, gets easily eliminated through the kidneys [4]. Humans do not produce enzymatically active uricase [5]. Uricase is produced as a truncated, inactive protein fragment in humans and apes as a result of a nonsense mutation inserted into its gene during the early primate evolution [6]. Other treatments for gout can be xanthine oxidase enzyme inhibitors like Allopurinol and Febuxostat, which do not allow uric acid to form in the body, however, these treatments, can sometimes be inefficient and may lead to many complications, thus proving uricase to be the best remedy for gout treatment in comparison [7].

Uricase from microorganisms and animals is recognized as a highly antigenic foreign protein by human immune system and the chronic treatment with this enzyme frequently results in allergic reactions and anaphylactic shock. It also has a less plasma half life *in vivo*[6]. Uricase administered externally is also prone to degradation by proteolysis *in vivo.* The pharmaceutical value of an enzyme drug can be often enhanced by modification of its amino groups by its attachment to polyethylene glycol and its derivatives.[8-11] The administration of the PEGylated conjugate facilitates longer dosage intervals and reduces risk of adverse immunological reactions. The most relevant perturbations of the protein molecule following PEGylation are: size enlargement, protein surface and glycosylation function masking, charge modification, and epitope shielding[12]. Presently the PEGylated forms of uricase which are commercially available are PEGsitacase (Uricase-PEG 20) [EnzymeRx], KRYSTEXXA™ (Pegloticase), Puricase® [Savient Pharmaceuticals], PEG_{40}-Uricase [Mountain View Pharmaceuticals]. Uricase from *Candida utilis*[13-18], *Arthrobacter protoformiae*[19], *Bacillus fastidiosus*[20-21] and from mammals [22-23] have been conjugated with PEG and its derivatives and their physical, biochemical and biopharmaceutical properties have been tested.

According to Sartore *et al* [24], the monomethoxy-PEG derivatives can be used for conjugation with the amino groups or the peptide side chains of the enzymes to activate the enzyme-PEG binding, where they modified monomethoxy-PEG with *p*-nitrophenyl chloroformate and conjugated it to Superoxide Dismutase, Arginase, and Ribonuclease. The nitrophenyl carbonate group in mPEG-np reacts with the N-terminal amine in biologically active molecules like enzymes (surface accessible amino acids) to produce a urethane linkage. Urethane linkages may be formed between uricase and 4-nitrophenyl carbonate (NPC) derivative of PEG [25]. Based on this strategy, we attempted to PEGylate Uricase from *Bacillus fastidiosus* with methoxyPEG *p*-nitrophenyl carbonate. There are only a few reports of conjugation of Uricase from *Bacillus fastidiosus* to PEG molecules where it has been conjugated to linear PEG-5 kDa, branched PEG-10 kDa and Poly (N-acryloylmorpholine) -6 KDa by Schiavon *et al* in 2000[32] and with monomethoxypolyethylene glycol modified with NHS ester -5 kDa by Zhang *et al* in 2010[21]. The present work deals with the PEGylation of Uricase from *Bacillus fastidiosus* (Uc) with methoxypolyethyleneglycol-*p*-nitrophenyl-carbonate (mPEG-np), determination of molecular weight of the conjugates formed, study of stability of the bioconjugates (Uc-mPEG-np) thus formed at various pH and temperature conditions and a brief view of Uc-mPEG binding mechanism. The conjugation has been described using the molecular modeling and drug-design software ArgusLab 4.0.1[29].

MATERIALS AND METHODS

Materials

Uricase from *Bacillus fastidiosus* (average mol wt 35 kDa) [specific activity: 9 U/mg], methoxypolyethylene glycol *p*-nitrophenyl carbonate [mPEG-np] (average mol wt 5 kDa), and uric acid were obtained from the Sigma Aldrich Company, Germany. SDS PAGE Kit, reagents and the ready-to-load protein marker were obtained from Chromous Biotech, India. UV-Visible Spectrophotometer (Labomed) was used for evaluating the activity of the unmodified and PEGylated uricase.

Methods

Preparation of Uc-mPEG-np conjugates

Commercially obtained uricase from *Bacillus fastidiosus* (Uc) (1mg/mL) was allowed to react with mPEG-np at different concentration ratios of 1:13, 1:17, 1:21 and 1:25 of Uc to mPEG-np, in 100mM Sodium borate buffer solution had a pH of 9.0 as the reaction medium in a final volume of 1mL. These mixtures were allowed to

react at 30°C with slight agitation for 2 hours. The residual uricolytic activities of the conjugates formed were measured.

Enzyme assay and protein estimation

The enzymatic assay was carried out by the method described by Mahler *et al* (1955) [26]. To a 3mL of 20mM boric acid buffer of pH 9.0, 75µL of 3.57mM uric acid solution (prepared in the above mentioned boric acid buffer solution) and 20µL of uricase solution were added at 25°C. For the blank, 20µL of buffer was added, instead of the uricase solution. The blank and the test solutions were incubated at 25°C for ten minutes, after which the decrease in the uric acid concentration was measured with the aid of a UV-Visible spectrophotometer at 293nm. The difference between absorbance of the test and blank is equivalent to the decrease in uric acid concentration during the enzymatic reaction. Thus one unit of uricase activity was defined as the amount of uricase required to convert 1µmol of uric acid into allantoin per minute at 25°C and at pH 9.0, considering the milli molar extinction co-efficient of uric acid (ε) at 293nm as $12.6mM^{-1}cm^{-1}$. The decrease in activity of the enzyme before and after bio-conjugation was determined using this method.

Preparation of PEGylated conjugates for SDS-PAGE

For the confirmation of PEGylation of uricase, the increase in molecular weight of the conjugates relative to that of unmodified uricase was determined using SDS-PAGE. The reaction mixtures were made ready to be loaded into the SDS-PAGE kit by the following procedure: a mixture of sodium dodecyl sulphate, β-mercaptoethanol, glycerol, bromophenol blue dye were taken in an appropriate quantities to a final volume of 10µL, which was then added to 10µL of Uc-mPEG-np conjugates. The mixture was boiled for a minute and then suddenly cooled in an ice bath. The cooled solutions were then loaded into the wells of the preassembled gel matrix in the SDS-PAGE kit.

SDS-PAGE Analysis and determination of molecular weight

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the method described by Laemmli[27]. 12% and 5% acrylamide-bisacrylamide mixtures were used for the preparation of gels. Commercially obtained protein markers were in the molecular weight range of 14 kDa-110 kDa. Staining and detection of protein bands were done using coomassie blue and later destained with water. Molecular weight of the unmodified uricase and the bioconjugate were determined by the standard curve for estimation of molecular weight supplied by Chromous Biotech (India).

Effect of pH and temperature on the stability of unmodified uricase and the bioconjugate

The stability of unmodified uricase and modified uricase obtained by conjugating uricase and mPEGnp in the concentration ratio of 1:17 was studied at different pH by maintaining them in the following buffer solutions: for pH 6-7 sodium phosphate buffer, for pH 8-9, borate buffer and for pH 10 tris-HCl buffer. The stability with respect to temperature was studied by incubating unmodified uricase and modified uricase obtained by conjugating uricase and mPEGnp in the concentration ratio of 1:17, at different temperatures like 4°C, 16°C, 32°C, 80°C, 100°C in a 100mM sodium borate buffer with pH 9. The uricolytic activities of all these conjugates were determined after one hour of incubation. The pH at which the unmodified uricase showed the highest activity, was considered to possess 100% residual activity, and the activities at other pH values were evaluated with respect to the highest value of residual activity.

Molecular Modeling and Docking Studies

Due to the unavailability of 3D structure of *Bacillus fastidiosus* uricase in the Protein Data Bank (PDB) database for performing the docking studies, we performed a primary structure or protein sequence similarity test using the BLAST tool[28] from UniProt database for *Bacillus fastidiosus* uricase. Based on the availability of the 3D structure in the PDB database, we chose a structure belonging to *Bacillus sp.* (Strain TB-90) (having 57% sequence similarity to *Bacillus fastidiosus* uricase) for performing the docking studies, where the chosen 3D structure from PDB database is referred with the PDB Id: 1J2G. The 3D structure of mPEG was obtained from PubChem database. For visualizing the conjugate, molecular modeling and docking software ArgusLab 4.0.1 was used [29].

RESULTS AND DISCUSSIONS

Modification of Uricase by Bio-conjugation

Degree of modification of uricase decides its overall quality like residual activity and its bio-compatibility. Binding of the mPEG decides the degree of modification, where the binding depends on the availability of mPEG and other operating variable like temperature, degree of agitation and duration of the process. Though the effects of operating variables were insignificant, the amount of mPEG in the reaction mixture showed a significant effect on the residual activity. The aliquots of commercially purchased uricase were subjected to bio-conjugation reaction with different concentrations of mPEG-np as mentioned in the section 2.1.1., and the uricolytic activity of the conjugates were determined and compared with the unmodified uricase as shown in the **Table 1**. From the **Table 1**, it was observed that the amount of mPEG-np in the reaction mixture decides the final activity of the modified enzyme. Further, the amount of mPEG-np is not directly proportional to the residual activity of the modified enzyme; probably it could be based on the availability of mPEG to the surface accessible amino acid molecules with a free amine group at that instant for conjugation to occur.

Table 1: Residual uricolytic activities of modified uricase

UC:m PEG-np	Residual Activity (%)	Activity (U/mg)
Unmodified Uricase	100	9.000
1:13	74.262	6.684
1:17	90.92	8.813
1:21	89.405	8.046
1:25	79.61	7.165

The PEGylation between mPEG-np and uricase is due to the reaction between nitrophenyl carbonate group in mPEG-np and free N-terminal amine containing amino acid molecules (surface accessible amino acids). Reaction between uricase and 4-nitrophenly carbonate (NPC) derivative of PEG forms a urethane linkage (-NH-(C=O)-O-) [25]. Formation of urethane linkage with mPEG-np released *p*-nitrophenol group in the reacting mixture, which produced pale yellow color in the reaction mixture, where the intensity of pale yellow color in the reaction mixture is the direct indication of the extent of conjugation [30], which could be quantified using spectroscopy technique [31]. In this present study, a yellow layer of band was observed during SDS-PAGE analysis of conjugate as shown in **Fig 1** (second layer), which was well below the conjugate (first dark layer-blue layer) due the formation of *p*-nitrophenol group as a byproduct of conjugate, which helped us to do crude confirmation of bio-conjugation [30].

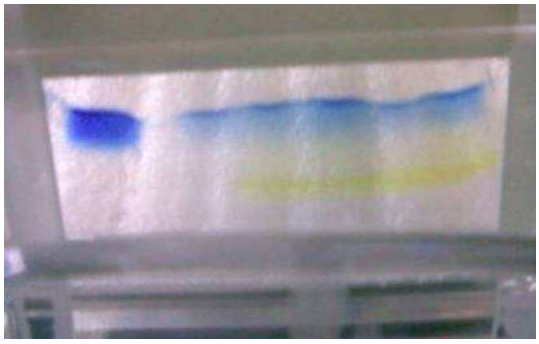

Fig. 1: SDS-PAGE Analysis

Zhang *et al* [21] in 2010 has used different ratios of modified NHS ester of mPEG of different molecular weights for the bio-conjugation of intracellular uricase from recombinant *Bacillus fastidiosus,* ATCC

29604 and have reported the maximum residual activity as 65%. Sachinova *et al*. have used different bio-conjugates like linear PEG (Mol Wt 5 kDa), PEG (Mol wt 10 kDa) and poly (N-acryloylmorpholine) (Mol Wt 6 kDa) to conjugate *Bacillus fastidiosus* uricase and found the residual activity as 40%, 76% and 24% respectively [32]. In this present work, the residual activity of bio-conjugated *Bacillus fastidiosus* uricase with mPEG-np (Mol. Wt. 5 kDa) at a ratio of 1:17 was found to be 90.92%, which is higher than the residual activity of reported literature values. Retention of such high activity even after conjugation is very significant, also a desired parameter particularly for a therapeutic enzyme for its *invivo* applications. Freitas *et al* in 2010[7], have used mPEG-np, 2-O-methoxypolyethyleneglycol-4,6-dichlorostriazine (mPEG-CN) 5 kDa to bio-conjugate *Candida utilis* uricase at a concentration ratio of 1:13. The authors have reported the residual activity as 87% for mPEG-np and 75% for mPEG-CN. A PEGylation study conducted by Bomalaski *et al* in 2002 [6], using recombinant *E. coli* uricase with succinimidyl succinimide activated 5 kDa or 20 kDa PEGs showed a residual activity as 76% and with 5 kDa mPEG as conjugate showed 50% residual activity.

Molecular weight Determination through SDS-PAGE

The change in the molecular weight of the modified uricase and the confirmation of bio-conjugation were analyzed using SDS-PAGE for crude modified enzymes. **Fig 2** shows the image of stained gel of SDS-PAGE using coomassie blue. From the **Fig 2**, the appearances of different bands were initially used to confirm the conjugation between uricase and mPEG-np.

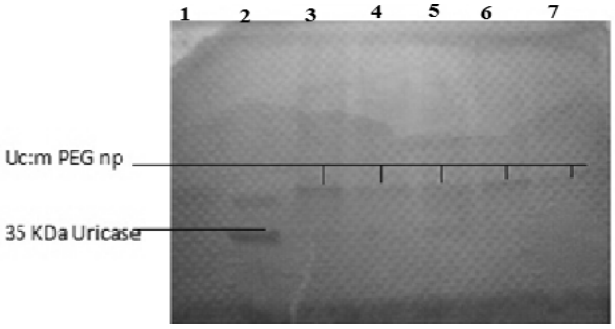

Fig. 2: SDS-PAGE Analysis (Lane 1: protein marker, Lane 2: unmodified uricase (35 KDa), Lane 3, 4, 5, 6: PEGylated complexes in various ratios)

Further, the molecular weight of the modified uricase was determined for the concentration ration of 1:17 using standard curve which is shown in **Fig 3**. The molecular weights of the unmodified and modified uricase were determined using the **Fig 3**, which were found to be 35.48 kDa and 79.4 kDa respectively, which were obtained based on the relative mobility of the proteins during electrophoresis.

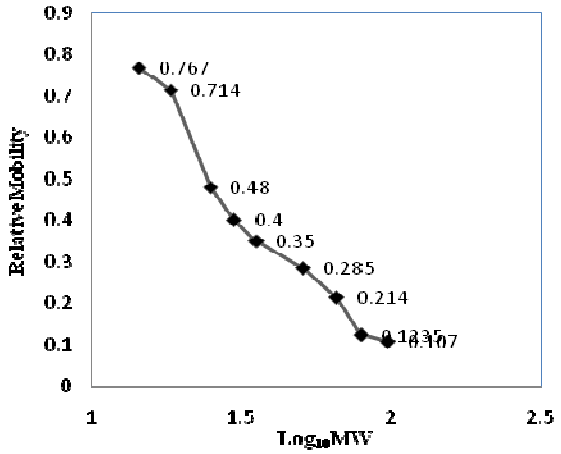

Fig. 3: Standard Curve for the estimation of Molecular Weight using SDS-PAGE

The similarity between the molecular weight of the commercially purchased uricase and its SDS-PAGE result confirmed the accuracy of the present molecular weight detection through SDS-PAGE. Similar results were reported by Freitas *et al* in 2010[7] for bioconjugation of *Candida utills* uricase with mPEG-np and mPEG-CN, where the reported molecular weights were 50 kDa and 65 kDa respectively. Further, the number of mPEG attached to uricase was approximately calculated using the difference between the unmodified uricase molecular weight and modified uricase molecular weight. The probable number mPEG molecules attached with single sub unit of uricase would be approximately 9 molecules, where the molecular weight of mPEG was considered as 4.8kDa. Literature studies showed that the degree of modification of uricase is related to its molecular structure, and also the number of surface accessible amino acids with a free n-terminal amino group. When these surface accessible amino acid residues are in close proximity, attachment of mPEG to a single lysine would produce steric hindrance for the neighboring n terminal amino acids [18, 23]. Although the primary structure of each subunit of *Bacillus fastidiosus* uricase composed of many amino acid residues with free N-terminal group that would be available for PEGylation, only a few residues of each subunit would be accessible on the surface of the uricase tetramer for binding.

Stability of native and modified uricase at various pH and temperature conditions

The effects of pH and temperature on the stability of the modified enzyme were determined at various pH and temperature. **Fig 4** shows the stability of modified uricase at different pH values. From **Fig 4,** it was observed that the modified uricase (1:17 concentration ratio of uricase to mPEG-np) was more stable at a pH value of 9, which is near to the physiological pH**,** thus making these conjugates more suitable for possessing maximum uricolytic activity *invivo*. *Bacillus fastidiosus* uricase exhibits its maximum uricolytic activity at a pH of 9.0-9.5 [33], since it is localized in peroxisomes. Similarly Zhang *et al* [21] observed that the unmodified *Bacillus fastidiosus* ATCC 29604 uricase had an optimum pH slightly below 9.2, which was not altered by modification with NHS ester of mPEG 5K or mPEG 350. Further the author has found that, at pH 7.4 the residual activity of uricases modified with NHS ester of mPEG 5K or mPEG 350 was still about 16% that of at pH 9.2. Schiavon *et al* [32] have reported that the stability tests were conducted using 0.05 M sodium acetate (pH 4–6), 0.05 M phosphate (pH 7), and 0.05 M sodium borate (pH 8–11) and the residual activity was evaluated after 24 hours.

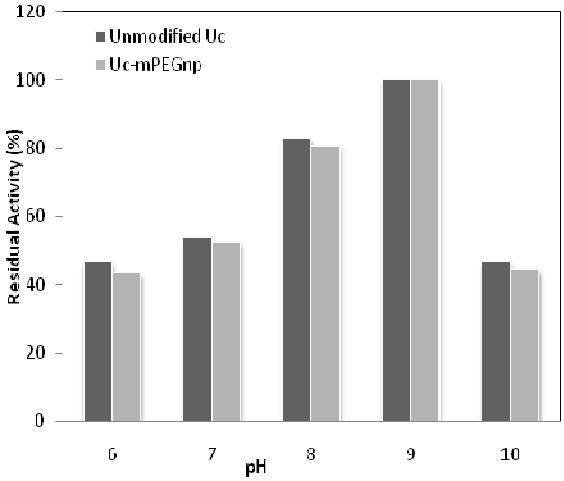

Fig. 4: Stability of modified and unmodified uricase at different pH.

The effect of temperature on the stability of the modified enzyme was analyzed at the following different temperatures 4°C, 16°C, 32°C, 80°C, 100°C. **Fig 5** shows the stability of modified enzyme at different temperatures. From **Fig 5**, it was observed that both unmodified and modified uricase were stable at 32°C with a

maximum residual activity of 100%. Zhang *et al* [21] had reported similar result that the unmodified uricase had an optimum reaction temperature below 25°C, and modification with the NHS esters of mPEG 5K and mPEG 350 with uricase, did not bring about any change in the optimum reaction temperature. Bio-conjugated *Candida utilis* uricase with mPEG-np showed maximum stability at a pH of 7.5 and 37°C.

Molecular Modeling and docking studies

In this present study, docking studies of uricase-mPEG binding were performed using ArgusLab 4.0.1. As an initially step, the 3D structure of *Bacillus fastidiosus* uricase was searched for in the protein data bank (PDB Database), due to unavailability of the *Bacillus fastidiosus* uricase 3D structure, sequence similarity tool BLAST was used to find an alternate 3D structure of uricase form other species. BLAST sequence similarity test was conducted using *Bacillus fastidiosus* uricase as a query sequence. In the sequence similarity test, uricase from *Bacillus sp.* (Strain TB-90) showed 57% similarity with the uricase from *Bacillus fastidious* which is shown in **Fig 6**. Further the 3D structure of uricase from *Bacillus sp.* (Strain TB-90) from PDB was chosen to perform the docking studies.

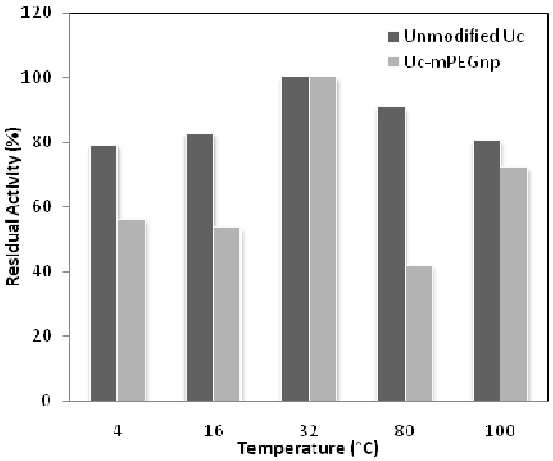

Fig. 5: Stability of unmodified uricase and Uc-mPEG-np at various temperatures

www.uniprot.org/blast/uniprot/2011120750XL5NZ39Q?sort=score&desc=true&page=1

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Alignments	Entry	Entry name	Status	Protein names	Organism	Length	Identity	Score	E-value	Gene names
	C5HDG5	C5HDG5_9BACI	☆	**Uricase**	Bacillus fastidiosus	320	100.0%	1,650	0.0	
	F5SCJ4	F5SCJ4_9BACL	☆	**Uricase**	Desmospora sp. 8437	530	63.0%	1,027	1.0×10^{-109}	HMPREF9374_0825
	D5WQV0	D5WQV0_BACT2	☆	**Uricase**	Bacillus tusciae (strain DSM 2912 / NBRC 15312 / T2)	326	58.0%	943	1.0×10^{-99}	Btus_2019
	O32141	PUCL_BACSU	★	**Uric acid degradation bifunctional protein Pu...**	Bacillus subtilis	494	56.0%	928	6.0×10^{-98}	**pucL** yunL BSU32450
	Q45697	PUCL_BACSB	★	**Uric acid degradation bifunctional protein**	Bacillus sp. (strain TB-90)	502	57.0%	926	1.0×10^{-97}	**uao**
	E0U0Q6	E0U0Q6_BACPZ	☆	**Uricase**	Bacillus subtilis subsp. spizizenii (strain ATCC 23059 / NRRL B-14472 / W23)	494	56.0%	926	1.0×10^{-97}	**pucL** BSUW23_15825
	D5N1J6	D5N1J6_BACPN	☆	**Uricase**	Bacillus subtilis subsp. spizizenii ATCC 6633	494	56.0%	926	1.0×10^{-97}	BSU6633_11715
	F8FF76	F8FF76_PAEMK	☆	**Uricase**	Paenibacillus mucilaginosus (strain KNP414)	344	57.0%	919	7.0×10^{-97}	**uao** KNP414_03236
	E8V930	E8V930_BACST	☆	**Uricase**	Bacillus subtilis (strain BSn5)	494	55.0%	917	1.0×10^{-96}	BSn5_07115
	D4G104	D4G104_BACNA	☆	**Uricase**	Bacillus subtilis subsp. natto BEST195	494	56.0%	917	1.0×10^{-96}	**pucL** BSNT_04802
	Q5WBJ3	Q5WBJ3_BACSK	☆	**Uricase**	Bacillus clausii (strain KSM-K16)	341	54.0%	901	9.0×10^{-95}	ABC3735
	D6XY45	D6XY45_BACIE	☆	**Uricase**	Bacillus selenitireducens (strain ATCC 700615 / DSM 15326 / MLS10)	318	55.0%	892	1.0×10^{-93}	Bsel_0582
	Q9KEU0	Q9KEU0_BACHD	☆	**Uricase**	Bacillus halodurans	329	56.0%	891	1.0×10^{-93}	BH0759
	E0IDR4	E0IDR4_9BACL	☆	**Uricase**	Paenibacillus curdlanolyticus YK9	329	54.0%	886	5.0×10^{-93}	PaecuDRAFT_3805
	C6D261	C6D261_PAESJ	☆	**Uricase**	Paenibacillus sp. (strain JDR-2)	319	52.0%	839	1.0×10^{-87}	Pjdr2_3315
	D4GPU7	URIC_HALVD	★	**Uricase**	Haloferax volcanii (strain ATCC 29605 / DSM 3757 / JCM 8879 / NBRC 14742 / NCIMB 2012 / VKM B-1768 / DS2) (Halobacterium volcanii)	308	50.0%	776	3.0×10^{-80}	HVO_B0300
	D8JBB7	URIC_HALJB	★	**Uricase**	Halalkalicoccus jeotgali (strain DSM 18796 / CECT 7217 / JCM 14584 / KCTC 4019 / B3)	306	50.0%	773	6.0×10^{-80}	HacjB3_16061
	A5YRZ8	A5YRZ8_9EURY	☆	**Uricase**	Uncultured haloarchaeon	322	47.0%	718	1.0×10^{-73}	
	G0LH78	G0LH78_HALWC	☆	**Uricase**	Haloquadratum walsbyi (strain DSM 16854 / JCM 12705 / C23)	309	47.0%	706	4.0×10^{-72}	**pucL1** Hqrw_2223
	F7Q5N1	F7Q5N1_9GAMM	☆	**Uricase**	Salinisphaera shabanensis E1L3A	309	40.0%	604	2.0×10^{-60}	SSPSH_05217
	D6TYV6	D6TYV6_9CHLR	☆	**Uricase**	Ktedonobacter racemifer DSM 44963	313	41.0%	570	2.0×10^{-56}	Krac_6350

Fig. 6: BLAST results for sequence similarity test for Uricase from *Bacillus fastidiosus.*

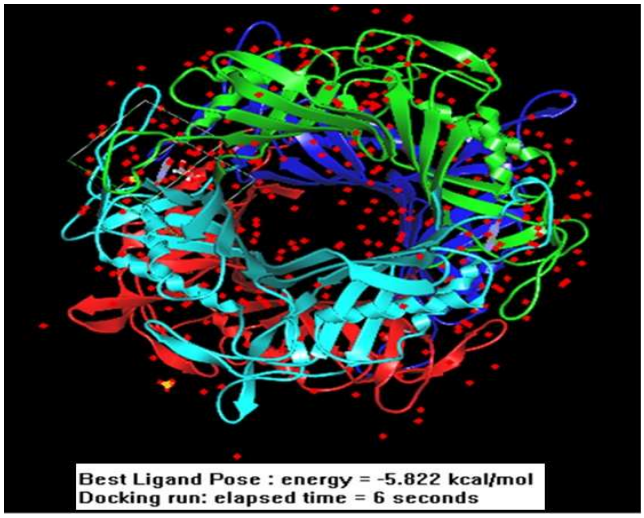

Fig. 7: 3D image of mPEG docked Uricase from *Bacillus sp.* (Strain TB-90)

Fig 7 indicates the docking results of mPEG molecule with uricase from *Bacillus sp.* (Strain TB-60). The docking studies revealed the possible sites for docking and the docked sites as show in **Fig 7**. From the **Fig 7**, it was observed that the cube on one of the subunits of uricase molecule indicates the region where the docking has occurred and the gray color in that cube indicate the mPEG molecule, which is attached to one of the amine group of the surface accessible amino acid residue. The other red dots in the 3D image are the possible sites for attachment of mPEG. Further, it was observed that the pose 1 is the best site of binding, as it requires very less mPEG binding energy of -5.82 kcal/mol.

The possible binding energies required for the other possible sites (120 – 129 possible binding sites) are listed in the **Fig 8,** as calculated by Argus lab 4.0.1

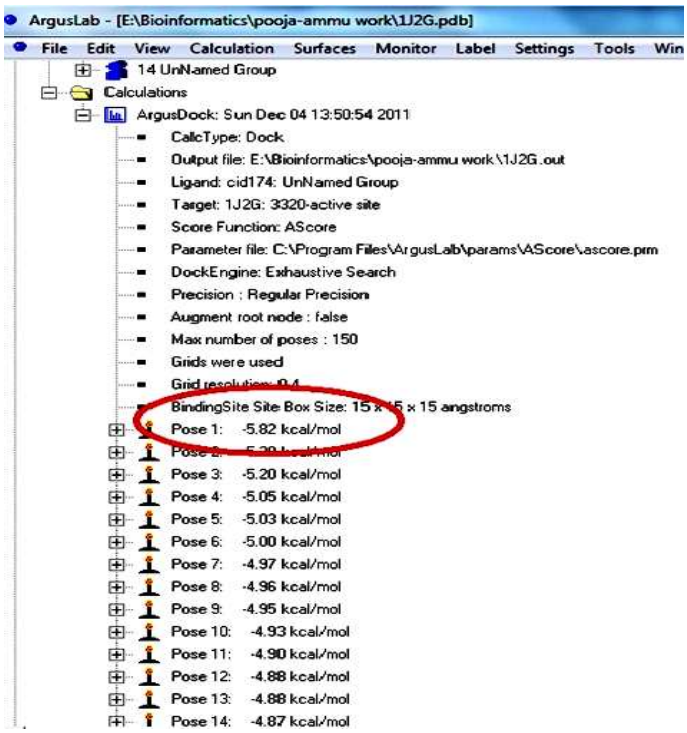

Fig. 8: Binding energy of different poses in different subunits of Uricase from *Bacillus sp.* (Strain TB-90)

CONCLUSION

In this present work, uricase was PEGylated with methoxyPEG-*p*-nitro phenyl carbonate. It was observed that, there were significant changes in the physio-chemical properties of the unmodified uricase after PEGylation. All the conjugates exhibited lower uricolytic activities compared to the unmodified uricase, due to the masking of active sites by the mPEG molecules. The Uc-mPEG-np conjugate retained 90.9% of its initial uricolytic activity, when Uc and mPEG-np were reacted in a concentration ratio of 1:17. The change in the molecular weight of the PEGylated uricase was determined through SDS-PAGE and found to be 79.4 kDa and the probable number of mPEG attached was found to be 9. Stability analysis based on different temperature and pH conditions indicated that unmodified uricase and Uc-mPEG-np bioconjugates were very stable at pH 9.0 and 32˚C. Docking studies were performed using bioinformatics tools like BLAST and Argus Lab 4.0.1 to predict the possible patterns of docking, which gave interesting results. This conjugate is being characterized and tested for its pharmacodynamic and pharmacokinetic properties for its therapeutic usage which will be reported soon.

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