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In-silico analysis predicting the best model for photosystemIID2 Protein of Spinaciaolearacea using multiple templates

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Abstract Spinach is a natural medicine against diabetes, prostate cancer, asthma, constipation, high blood pressure. Spinach acts as anti-inflammatory, antiproliferative, antioxidative. In this study the PHOTOSYSTEMII D2 protein has considered for in-silico analysis. Models of the protein were generated using 1IZLD, 3A0B, 3WU2, 4IL6 templates. The sequence retrieved from uniprot, templates were predicted by usingblastP tool, physico-chemical analysis showed the properties of protein using prot-param tool , secondary structure prediction showed helices, turns and sheets using CFFSP server, homologous models were generated using modeller9.12 tool, backbone confirmation was performed by using Rampage server and finally the best model generated by using 3A0B template having 94.0% of residues lying in favored region, 2.0% residues lying in outlier region, with 91% of query coverage and 95% of identity with photosystemQ (B) protein of Thermosynechocuccus vulcanus.

Keywords PHOTOSYSTEMII D2 protein; Template prediction; Homology modeling; Model validation; Best model prediction

Introduction

The common name of Spinaciaolearacea is spinach which belongs to the family *Amaranthaceae-Chenopodiaceae* and plays an important role as a source of energy. Most commonly this green leavesare used as food . from a research it has been proved that the spinach is full of vitamin C, which helps to protect all of the oxygen-sensitive phytonutrients in the spinach leaves for which the leaves look vibrant and alive. The main health-supportive nutrients found in spinach isglycerolipids. Naturally spinach is anti-inflammatory (Lomnitski et al., 2000), antiproliferative (Bergman et al., 2011), antioxidative (Sani et al., 2004). From a research among broccoli, spinach, cauliflower, cabbage, mustard greens, collard and kale the spinach showed significant protection against the occurrence of aggressive prostate cancer in male. The spinach is blessed with a natural anti-cancer carotenoid i.e, epoxyxanthophylls. It contains carotenoids i.e, beta carotene, lutein, zeaxanthin along with antioxidants i.e, flavonoid. Spinach is quite healthy as it is composed of vit.K, vit.A, vit.B1, vit.B3, vitB2, vit.E, vit.B6, iron, copper, folate, manganese, calcium, fiber, potassium, zinc, protein, choline, omega-3 fats, selenium, pantothenic acid etc. the vit.K1 and vit.K2 helps in activating the osteocalcin leading to bone-up. However spinach is full of oxalate too which is dangerous to health if taken in a huge amount. Spinach contains natural purine which causes kidney stone and gout disease if taken in a huge a amount. Basically spinach helps to fight against weak bone, high blood pressure, diabetes, asthma, prostate cancer in male, constipation,human pancreatic cancer cells (Lomnitski et al., 2000). Spinach is also helpful for energy metabolism, maintaining muscle and nerve function, heart rhythm, a healthy immune system and maintaining blood pressure. In this study we have considered the Photosystem II D2 of spinach. This protein is a plastoquinone oxidoreductase that uses light energy to abstract electrons from H_2O producing oxygen and proton gradient in order to produce ATP. Photosystem II D2 is a membrane protein. PSII is composed of 1 copy each of membrane proteins PsbA, PsbB, PsbC, PsbD, PsbE, PsbF, PsbH, PsbI, PsbJ, PsbK, PsbL, PsbM, PsbT, PsbX, PsbY, PsbZ, Ycf12. In the study the physico-chemical analysis of protein has been done along with homology modeling, model validation and optimization leading in prediction of

the best model using mu; tiple templates.

1 Material and Methods

1.1 Sequence retrieval

The amino acid sequence of photosytemII D2 protein was retrieved from uniprot in fasta format. The detail information is given in Table 1.

1.2 Physico-chemical characterization of photosytemII D2 protein

The physico-chemical properties of the protein were studied by using protparam tool. From this analysis the theoretical PI, molecular weight, aliphatic index, extinction coefficient, number of amino acids, total number of positively and negatively charged residues, atomic position, chemical formula, instability index and GRAVY (Grand average of hydropathicity) of the protein. The detail information is given in Table 2 and Table 3.

Table 1 Information about PHOTOSYSTEMII D2 protein

UniprotID	Gene	Function	Catalytic activity	Subcellular location	Amino acid length
P06005	psbD	ATP formation	2 H_2O + 2 plastoquinone + 4 light = O_2 + 2 plastoquinol	Transmembrane 41-61 125-141 153-166 208-228 279-295	353

Table 2 Physico-chemical properties ofPHOTOSYSTEMII D2 protein

Molecular weight	Theoretical pI	Total number of negatively charged residues	Total number of positively charged residues	Total number of atoms	Aliphatic index	Grand average of hydropathicity (GRAVY)
39507.4	5.46	28	20	5514	88.78	0.358

Table 3 Amino acid composition result

Amino acids	symbols	Number of residues	In percentage
Ala	A	39	11.0%
Arg	R	15	4.2%
Asn	N	15	4.2%
Asp	D	12	3.4%
Cys	C	4	1.1%
Gln	Q	11	3.1%
Glu	E	16	4.5%
Gly	G	31	8.8%
His	H	8	2.3%
Ile	I	13	3.7%
Leu	L	41	11.6%
Lys	K	5	1.4%
Met	M	9	2.5%
Phe	F	39	11.0%
Pro	P	14	4.0%
Ser	S	18	5.1%
Thr	T	20	5.7%
Trp	W	13	3.7%
Tyr	Y	8	2.3%
Val	V	22	6.0%
Pyl	O	0	0.0%
Sec	U	0	0.0%

1.3 Prediction of templates

The similarity search is generally done by using BLAST tool and the protein-protein similarity search is carried out by using the blastP tool. So the retrived amino acid sequence was subjected to blastP against PDB. From the result the suitable templates were found for further study. The selected templates for model building are given in Table 4.

Table 4 List of four templates used for homology modelling

Templates	Chain	Identity	Query cover	E-value	Organism	Molecule
1IZL	D	89%	100%	0.0	Thermosynechocuccus vulcanus	PhotosystemII subunit psb A
4IL6	D	90%	96%	0.0	Thermosynechocuccus vulcanus	photosystemQ (B) protein
3A0B	D	95%	91%	0.0	Thermosynechocuccus vulcanus	photosystemQ (B) protein
3WU2	D	96%	90%	0.0	Thermosynechocuccus vulcanus	photosystemQ (B) protein

1.4 Secondary structure prediction of protein

The secondary structure of protein was predicted by using CFFSP server from where the percentage of helix, sheets and turns were found (Figure 1). The detail information is given in Table 5.

Table 5 composition ofhelices, sheets, turns

Templates	Helices	Sheets	Turns
1IZL	36.8%	0.0	5.7%
4IL6	51.3%	0.0	3.4%
3A0B	55.2%	0.0	1.1%
3WU2	54.1%	0.0	6.2%

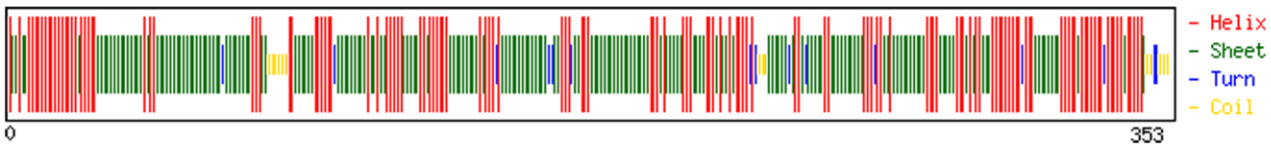

Figure 1Secondary structure ofPHOTOSYSTEMII D2 protein

1.5 Homology modelling

The 3D structures of the photosystemII D2 protein was generated by using homology modelling concept, in which four different templates were selected for model building.The models were generated by using modeller 9.12 tool(Bilal et al., 2013, Singh et al., 2009). The align2d.py, model-single.py and evaluate-model.py files were rum on the python script by setting the target, template and number of models to be generated. Here 5 models for each template were generated and the best model was selected on the basis of lowest DOPE score. The properties which were found after structure visualisation is given in Table 6 and Table 7. the tertiary atructures of protein is given in Figure 2-Figure 5.

Table 6 Result obtained from yasara tool

Templates	Beta factor	Stability of object	Minimized energy	VDW radius
1IZL	140.0	930.7kcal/mol	-0.737849	60.267A^0
4IL6	110.9	723.92 kcal/mol	-2.8532	53.888 A^0
3A0B	105.4	607.54 kcal/mol	-2.21035	53.484 A^0
3WU2	99.8	591.03 kcal/mol	-1.38159	52.549 A^0

Table 7 Result obtained frompymol tool

Templates	Atom count	Formal Charge sum	Molecular surface area	Solvent accessible surface area
1IZL	2804	-9.0	34263.043 A^0	21350.953 A^0
4IL6	2804	-9.0	34034.191 A^0	21938.918 A^0
3A0B	2804	-9.0	34332.879 A^0	21257.391 A^0
3WU2	2804	-9.0	34461.117 A^0	21316.729 A^0

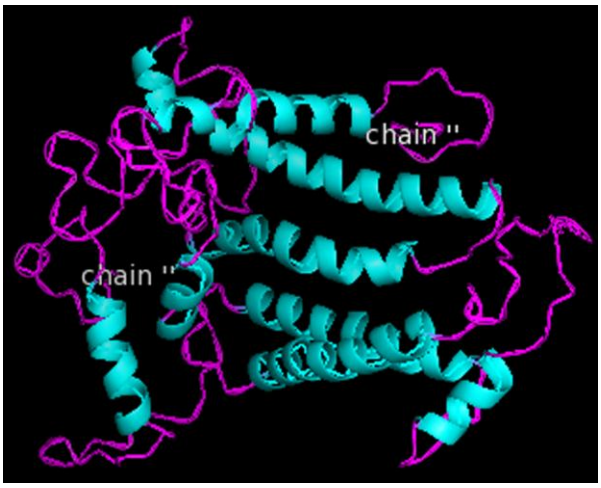

Figure 2 model generated using 1IZLD

Figure 3model generated using 3A0B

Figure 4 model generated using3WU2

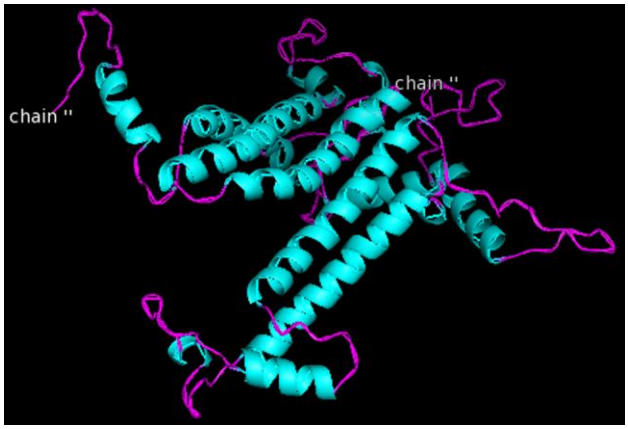

Figure 5 model generated using4IL6

1.6 Model validation

The final models were further subjected to Rampage server for the analysis of backbone confirmation of protein. The backbone confirmation for each models generated which showed the number of residues lying in allowed region, favoured region and in outlier regions. Depending upon these characters the best model is selected. The ANOLEA server was used to find out Z-score and Q-mean score. Least Z-score indicates the best model. The validated models information and backbone confirmation is given in Table 8 and Table 9. the backbone confirmtion of protein models are given in Figure 6-Figure 9.

1.7 Selection of best model

However the best model is selected on the basis of identity, query coverage, Z-score, Qmean score, E-value etc.

2 Result

2.1 Sequence retrieval result

The sequence of PHOTOSYSTEMII D2 protein was retrivred from uniprot with uniprotID of P06005. The function, location, catalytic activity is given below.

Table 8 Result obtained from ANOLEA-SWISS SERVER

Templates	QMEAN score	Z-score
1IZL	0.156	-7.194
4IL6	0.314	-5.356
3A0B	0.311	-5.382
3WU2	0.366	-5.439

Table 9 Backbone confirmation of models

Templates	Residues in favoured region	Residues in allowed region	Residues in outlier region
1IZL	290 (82.6%)	35 (10.0%)	26 (7.4%)
4IL6	330 (94.0%)	14 (4.0%)	7 (2.0%)
3A0B	333 (94.9%)	12 (3.4%)	6 (1.7%)
3WU2	330 (94.0%)	20 (5.7%)	1 (0.3%)

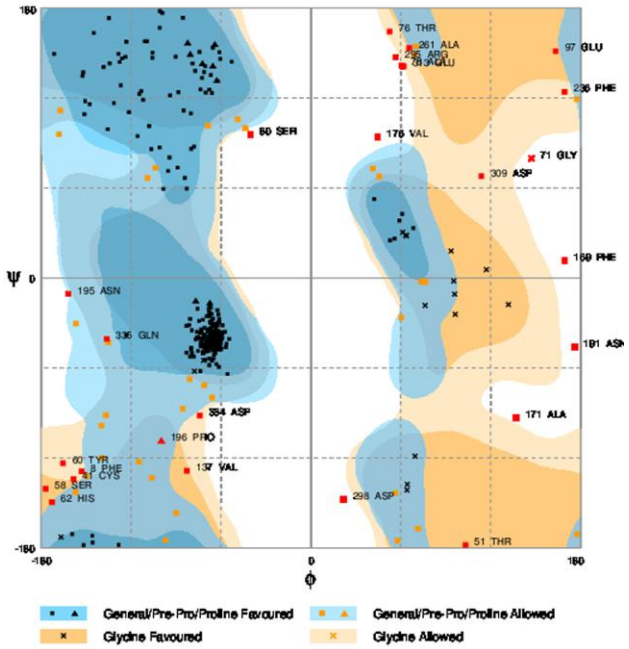

Figure 6 model with 1IZL template

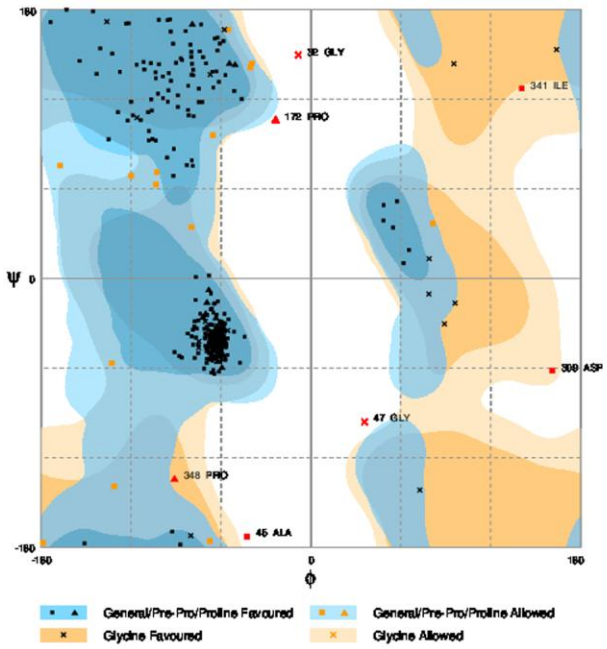

Figure 7 model with 3A0B template

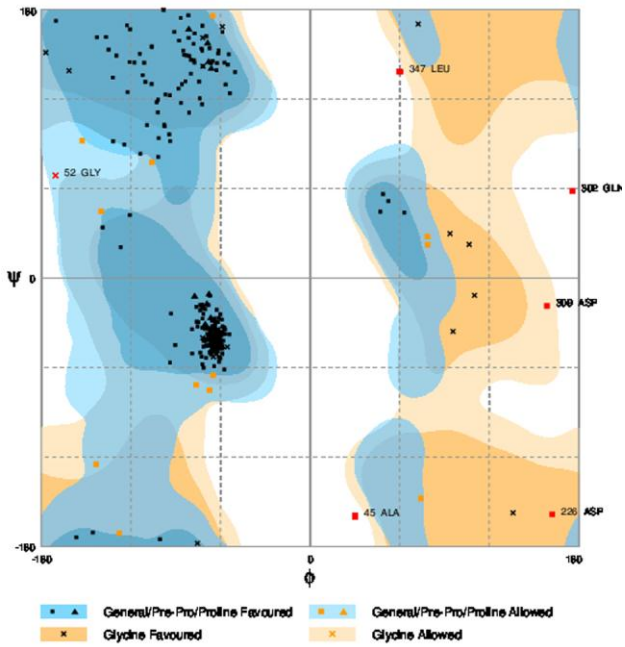

Figure 8 model with 3WU2 template

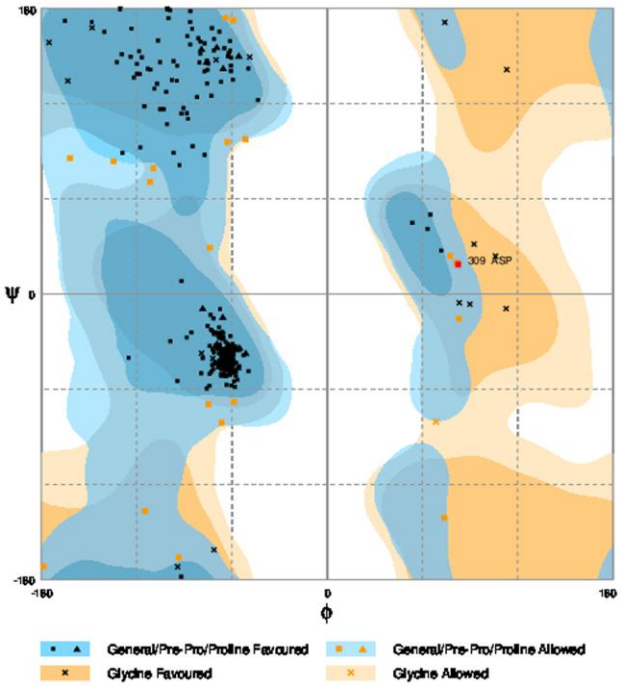

Figure 9 model with 4IL6 template

2.2Physico-chemical analysis result

From this analysis the amino acid composition, theoretical PI, number of positively and negatively charged residues, GRAVY, aliphatic index of the protein is found.

2.3 Selected templates

Four primer 1IZL, 4IL6, 3A0B, 3WU2 were selected after blastP run as the templates for the protein with following characters. Here more templates are selected in order to find out best model for protein with a suitable template.

2.4 Secondary structure prediction result

The secondary structure of protein which generated from CFFSP server showed the following result.

2.5 Homology modelling result

The finally generated models were visualised using PyMol visualiser. The helices were denoted with sky blue colurs and the loops were denoted with purple colours respectively. The atom count, formal charge sum, molecular surface area, solvent accessible surface area of the models were generated from PyMol and beta factor, stability of the models, VDW radius, minimized enegy were generated from Yasara tool.

2.6 Model validation analysis result

The finally generated models were submitted to Rampage server to find out the best protein. The best protein was predicted on the basis of residues lying in strong favoured region.

3 Discussions

From the above analysis the best model found for photosystemII D2 protein with the template 3A0B having 94.0% of residues lying in favoured region, 2.0% residues lying in outlier region, with 91% of query coverage and 95% of identity with photosystemQ (B) protein of Thermosynechocuccus vulcanus.

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