International Standard Serial Number (ISSN): 2249-6807

International Journal of Institutional Pharmacy and Life Sciences 3(6): November-December 2013

INTERNATIONAL JOURNAL OF INSTITUTIONAL PHARMACY AND LIFE SCIENCES

Life Sciences **Research Article……!!!**

Received: 21-02-2013; Revised; Accepted: 04-11-2013

IN SILICO CHARACTERIZATION OF HUMAN CYCLOOXYGENASE USING COMPUTATIONAL TOOLS AND SERVERS

Sen Gupta Parth Sarthi[1], Mandal Buddhadeb[2] and Bandyopadhyay Amal Kumar*[1]

1. Department of Biotechnology, The University of Burdwan, Golapbag, Burdwan, 713104, West Bengal, India
2. Burdwan Raj College, Burdwan university, Burdwan, 713104, West Bengal, India

Keywords:

Cyclooxygenase; computational analysis; disulphide bridges; homology modeling; proteomics tools

For Correspondence:

Dr. Bandyopadhyay A. K.

Department of Biotechnology, The University of Burdwan, Golapbag, Burdwan, 713104, West Bengal, India

E-mail:

akbanerjee40@gmail.com
parth.biotech@gmail.com

ABSTRACT

In this paper, sequences of twelve human cyclooxygenases retrieved from Swiss -Prot database are analyzed and characterized using *In silico* tools. Primary structure analysis shows that most of the cyclooxygenase are hydrophobic in nature due to the high content of non -polar residues. The presence of cysteines in the most of the cyclooxygenase sequences infer that these proteins may form disulphide (SS) bonds, which are regarded as a positive factor for stability. The aliphatic index computed by Ex-Pasy's ProtParam infers that cyclooxygenases may be thermolabile for a wide range of temperature. Secondary structure analysis shows that some of the cyclooxygenases have predominant α-helical structures and rest of the cyclooxygenases have mixed secondary structure. The very high coil structural content of most of the sequences are due to the rich content of more flexible glycine and hydrophobic proline amino acids. Proline has a special property of creating kinks in polypeptide chains and disrupting ordered secondary structure. SOSUI server predicts one transmembrane region in P98187 and P23219 cyclooxygenase. The predicted transmembrane regions were visualized and analyzed using helical wheel plots generated by EMBOSS pepwheel tool. The presence of disulphide (SS) bonds in the cyclooxygenases P98187, Q99988, P18054, P09917, P19838, P23219, P35354, Q96EB6 and P15692 are predicted by CYS_REC tool, and the presence of disulfide bond in P23219 is also identified from the three-dimensional structure using Rasmol & Swiss-Pdb Viewer (SPDBV) tools. The disulphide bonds identified from the three-dimensional structure using the Swiss-Pdb Viewer tool might be correct as the evaluation parameters are within the acceptable limits for the modeled 3D structures.

INTRODUCTION

Computational packages and online servers are the current tools used in the protein sequence analysis and characterization.[1] The physicochemical and the structural properties of the proteins are well under-stood with the use of computational tools. Today, number of computational tools has been developed for making predictions regarding the identification and structure prediction of proteins. The statistics about a protein sequence such as number of amino acid, sequence length, and the physicochemical properties of a proteins such as molecular weight, atomic com-position, extinction coefficient, GRAVY, aliphatic index, instability index, etc. can be computed by computational tools for the prediction and characterization of protein structure. The amino acid sequence provides most of the information required for determining and characterizing the molecule's function, physical and chemical properties. Sequence analysis and physicochemical characterization of proteins using biocomputation tools have been done by many researchers and reported.[2–8] Cyclooxygenase (COX), first purified in 1976 and cloned in 1988, is the key Enzyme in the synthesis of prostaglandins (PGs). In 1991, several laboratories identified a product from a second gene with COX activity and called it COX-2. Prostaglandin endoperoxide H synthases 1 and 2, also known as cyclooxygenases (COXs) 1 and 2, are targets of nonspecific nonsteroidal anti-inflammatory drugs and COX-2-specific inhibitors called coxibs. The two isoforms of COX (COX-1 & COX-2) are almost identical in structure but have important differences in substrate and inhibitor selectivity and in their intracellular locations. Protective PGs, which preserve the integrity of the stomach lining and maintain normal renal function in a compromised kidney, are synthesized by COX-1. In addition to the induction of COX-2 in inflammatory lesions, it is present constitutively in the brain and spinal cord, where it may be involved in nerve transmission, particularly that for pain and fever. PGs made by COX-2 are also important in ovulation and in the birth process. The discovery of COX-2 has made possible the design of drugs that reduce inflammation without removing the protective PGs in the stomach and kidney made by COX-1.Numerous structure and function studies have been reported from time to time from all over the world.[9-18] However, physicochemical characterization of cyclooxygenase has not been done so far. In this paper, we report the *In silico* analysis and characterization studies on 12 cyclooxygenases of human.

MATERIALS AND METHODS

Cyclooxygenase sequences

Cyclooxygenase sequences were retrieved from the manually curated public protein database Swiss-Prot.[19] Swiss-Prot is scanned for the key word cyclooxygenase and then click on the

taxonomy, the search result yielded various taxonomic groups. From this we retrieved 12 reviewed sequences of human cyclooxygenase and have organized a non-redundant data set (table 1). The cyclooxygenases were retrieved in FASTA format and used for analysis.

Table 1. Human cyclooxygenase sequences retrieved from Swiss-Prot database.

Accession number	Sequence description	organism
P00918	Carbonic anhydrase 2	Homo sapiens (Human)
P49716	CCAAT/enhancer-binding protein delta	Homo sapiens (Human)
P98187	Cytochrome P450 4F8	Homo sapiens (Human)
Q99988	Growth/differentiation factor 15	Homo sapiens (Human)
P18054	Arachidonate 12-lipoxygenase, 12S-type	Homo sapiens (Human)
P09917	Arachidonate 5-lipoxygenase	Homo sapiens (Human)
P19838	Nuclear factor NF-kappa-B p105 subunit	Homo sapiens (Human)
P23219	Prostaglandin G/H synthase 1	Homo sapiens (Human)
P35354	Prostaglandin G/H synthase 2	Homo sapiens (Human)
Q96EB6	NAD-dependent protein deacetylase sirtuin-1	Homo sapiens (Human)
Q15185	Prostaglandin E synthase 3	Homo sapiens (Human)
P15692	Vascular endothelial growth factor A	Homo sapiens (Human)

Computational tools and servers

The amino acid composition (table 2) of cyclooxygenase sequences were computed using the tool CLC *free Workbench.*[20] Percentages of hydrophobic and hydrophilic residues were calculated from the primary structure analysis results and tabulated in table 3. The physico-chemical parameters, theoretical isoelectric point (pI), molecular weight, total number of positive and negative residues, extinction coefficient,[21] half-life,[22–25] instability index,[26] aliphatic index[27] and grand average hydrophathy[28] (GRAVY) were computed using the Expasy's ProtParam (http://us.expasy.org/tools/ protparam.html) prediction server and tabulated in table 4. The tools SOPM, SOPMA[29] and Secondary Structural Content Prediction (SSCP method-I) server[30] were used for the secondary structure prediction. The SOSUI[31] server performed the identification of transmembrane regions (table 5). The predicted trans-membrane helices were plotted using Kite and Doolittle score (figure 1). Predicted trans-membrane helices were visualized and analyzed using helical wheel plots (figure 2) generated by the program Pepwheel[32] included in the EMBOSS 2.7 suite. cyclooxygenases P98187, Q99988, P18054, P09917, P19838, P23219, P35354, Q96EB6 and P15692 are predicted by CYS_REC.[33] CYS_REC identifies the positions of cysteines, total number of cysteines present and predicts the most probable SS bond pattern of pairs in the protein sequence. The visualization and identifications of SS bonds in P23219 using

the three-dimensional structure of protein (3D coordinate's data) is done. The 3D structure of cyclooxygenase P23219 was generated by homology modeling using Esypred[34] server. The similar 3D structure (for the cyclooxygenase P23219 sequences) in the Protein Data bank (www.rscb.org) was identified by the BLASTP analysis (http://www.ncbi.nlm.nih.gov:80 /BLAST/) in table 6.

Table 2. Amino acid composition (in %) of human cyclooxygenase computed using CLC free Workbench tool.

Amino Acid	ACCESION ID											
	P00918	P49716	P98187	Q99988	P18054	P09917	P19838	P23219	P35354	Q96EB6	Q15185	P15692
Ala	5	15.2	6.2	9.7	7.8	6.4	7.5	4.2	5.1	8.8	2.5	3.4
Cys	4	1.1	2.3	2.9	2.6	1.9	1.1	2.2	2.2	2.5	3.1	7.8
Asp	7.31	5.2	6.0	4.5	5.7	6.5	7.1	4.0	4.3	7.8	16.9	3.0
Glu	5	6.7	5.2	4.5	6.6	7.1	6.6	6.0	6.0	10.2	8.1	6.9
Phe	4.6	3.0	5.8	1.3	3.9	5.2	3.3	6.5	6.3	2.9	5.6	2.6
Gly	8.5	9.7	4.6	5.2	5.9	4.9	9.3	7.5	6.1	7.0	4.4	6.0
His	4.6	1.1	3.1	3.9	2.7	2.5	3.1	3.0	3.1	1.6	1.9	4.7
Ile	3.5	1.1	5.6	1.9	4.4	6.5	3.8	4.2	5.6	5.8	2.5	3.4
Lys	9.2	4.1	4.8	1.6	4.1	5.6	6.0	4.2	5.6	4.6	7.5	9.1
Leu	10	8.9	12.9	14.9	12.5	8.8	11.4	12.4	9.4	7.6	6.9	6.9
Met	0.8	1.5	1.9	1.6	2.7	2.4	2.3	3.0	2.5	1.3	3.8	3.4
Asn	3.8	2.2	3.3	1.9	2.7	3.9	3.6	3.2	4.8	3.5	6.9	3.0
Pro	6.5	13.8	6.5	8.8	6.5	5.0	5.2	7.7	6.6	8.6	3.1	6.5
Gln	4.2	4.5	3.5	4.5	6.0	4.6	4.2	4.5	5.1	3.7	1.9	5.6
Arg	2.7	8.6	7.7	11.4	6.3	5.3	3.6	5.5	4.5	5.5	5.0	8.2
Ser	6.9	5.9	5.4	9.4	4.4	4.9	6.2	5.5	5.8	7.6	9.4	6.5
Thr	4.6	2.6	5.6	4.2	4.5	4.5	6.3	5.0	5.6	3.3	1.9	2.6
Val	6.5	2.6	5.8	4.9	5.1	7.3	6.2	5.3	5.8	5.0	4.4	4.7
Trp	2.7	0.07	2.5	1.6	2.9	2.2	4.0	10.0	1.0	0.5	3.1	2.2
Tyr	3.1	1.5	2.1	1.0	2.6	4.5	2.7	4.5	4.5	2.1	1.2	3.0

Table 3. Hydrophilic and hydrophobic residues content.

Accession Number	Percentage of hydrophobic residues	Percentage of hydrophilic residues	Net hydrophobic residues content
P00918	48.5	51.41	low
P49716	56.97	42.4	very high
P98187	54	46	very high
Q99988	52.8	46.9	very high
P18054	54.3	45.6	very high
P09917	50.6	49.4	-
P19838	50.5	49.4	-
P23219	54.7	45.4	very high
P35354	50.6	49.3	-
Q96EB6	50	49.9	-
Q15185	39.4	60.7	very low
P15692	46.9	53	very low

Table 4. Parameters computed using Expasy's protParam tool.

Accession Number	Sequence length	M. wt	pI	-R	+R	EC	II	AI	GRAVY
P00918	260	29246.0	6.87	32	31	50420	21.68	76.46	-0.579
P49716	269	28467.0	8.44	32	34	16960	62.11	61.93	-0.669
P98187	520	59994.6	8.73	58	65	87890	52.23	94.88	-0.160
Q99988	308	34140.2	9.79	28	40	31970	63.89	89.71	-0.363
P18054	663	75694.0	5.82	82	69	129830	43.32	88.60	-0.268
P09917	674	77983.2	5.51	92	74	127200	42.10	87.06	-0.278
P19838	968	105356.0	5.20	133	93	60740	38.15	84.74	-0.339
P23219	599	68686.3	6.81	60	58	95230	44.50	84.12	-0.181
P35354	604	68996.1	7.02	62	61	73230	37.67	80.70	-0.287
Q96EB6	747	81681.0	4.55	134	75	45840	53.88	75.41	-0.551
Q15185	160	18697.3	4.32	40	20	30480	51.60	51.75	-1.049
P15692	232	27042.3	9.21	24	40	37930	52.30	57.54	-0.783

M. wt., Molecular weight; pI, Isoelectric point; –R, Number of negative residues; +R, Number of positive residues; EC, Extinction coefficient at 280 nm; II, Instability index; AI, Aliphatic index; GRAVY, Grand Average Hydropathy.

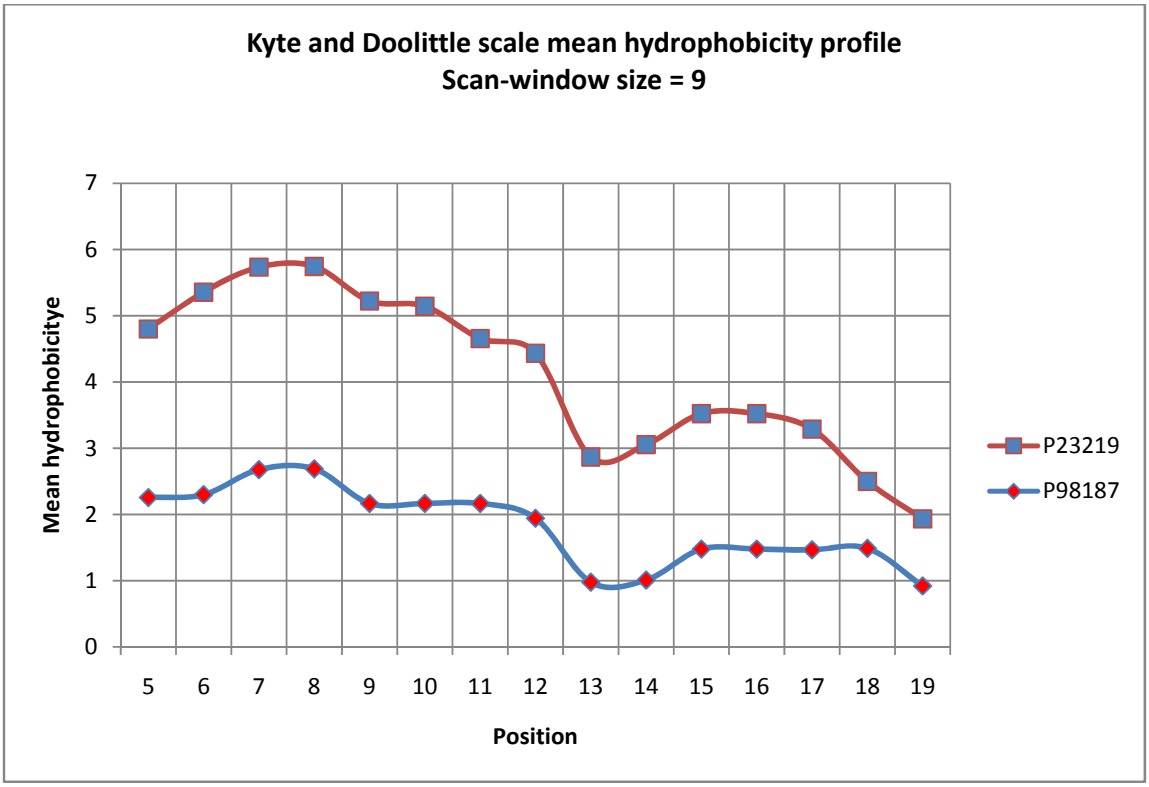


Figure 1. Kyte and Doolittle mean hydrophobicity profile computed for the transmembrane regions of Cyclooxygenase P23219 and P98187 (primary helices).

Table 5. Transmembrane regions identified by SOSUI server.

Accession number	Transmembrane region	Type	Length
P98187	SPWLLLLVVGASWLLARILAWTY	PRIMARY	23
P23219	SLLLWFLLFLLLLPPLPVLLADP	PRIMARY	23

Table 6. PDB templates (first 2 hits with maximum % identity) using BLASTP search against the Protein Data Bank.

Accession number	PDB code	
P23219	1CQE_A	2OYE_P

The modeled 3D structures were evaluated using the online servers Rampage,[35] and ProQ[36] (Protein Quality server). The tool Rasmol (http://openrasmol.org/) and swisspdbviewer [37](http://spdbv.vital_it.ch/) are used to visualize the modeled 3D structures and to identify the SS bonds. The three dimensional structure of cyclooxygenase P23219 modeled using the PDB template 1CQE_A are shown in figures 3 and 4 respectively. The nine cyclooxygenase sequences with number of CYS and disulphide bond pattern predicted by CYS_REC are shown in table 7(a).

Table 7(a). No. of CYS and Disulfide (SS) bond pattern of pairs predicted, by CYS_REC (using primary structure)

Accession number	No. of CYS	CYS_REC
P98187	12	Cys50-Cys260 Cys214-Cys402
Q99988	9	Cys203-Cys210 Cys211-Cys244 Cys240-Cys274 Cys273-Cys305
P18054	17	Cys89-Cys536 Cys140-Cys508 Cys499-Cys526 Cys559-Cys656
P09917	13	Cys32-Cys241 Cys265-Cys562 Cys301-Cys311 Cys419-Cys599
P19838	11	Cys118-Cys123
P23219	13	Cys35-Cys46 Cys36-Cys158 Cys40-Cys56 Cys58-Cys68 Cys568-Cys574
P35354	13	Cys21-Cys32 Cys22-Cys145 Cys26-Cys42 Cys44-Cys54 Cys555-Cys561
Q96EB6	19	Cys67-Cys268 Cys371-Cys374 Cys490-Cys502 Cys501-Cys680 Cys623-Cys671
P15692	18	Cys52-Cys94 Cys77-Cys172 Cys83-Cys128 Cys86-Cys171 Cys87-Cys130 Cys184-Cys202 Cys187-Cys204 Cys206-Cys225 Cys213-Cys227

Table 7(b). Disulfide (SS) bond pattern of pairs predicted, by CYS_REC (using primary structure) and identified by SPDBV (using 3d structure modeled).

Accession number	CYS_REC	SPDBV
P23219	Cys35-Cys46 Cys36-Cys158 Cys40-Cys56 Cys58-Cys68 Cys568-Cys574	Cys35-Cys46 Cys36-Cys158 Cys40-Cys56 Cys58-Cys68 Cys568-Cys574

The five most probable SS bond pattern of pairs predicted by CYS_REC tool and the positions of SS bonds identified using Swiss-PDB Viewer in the cyclooxygenase P23219 is shown in table 7(b).

RESULTS AND DISCUSSION

The results of primary structure analysis suggest that most of the cyclooxygenase are hydrophobic in nature due to the presence of high non -polar residues content (tables 2 and 3). The presence of high Cys residues in cyclooxygenase P98187, Q99988, P18054, P09917, P19838, P23219, P19838 and P15692 indicates the presence of disulphide bridges (SS bonds) in these cyclooxygenases. Moreover, the primary structure analysis suggests that the cyclooxygenases has high residues of acidic and basic amino acid, this might be involves in salt bridge formation. The average molecular weight of cyclooxygenase calculated is 56332 Da. Isoelectric point (pI) is the pH at which the surface of protein is covered with charge but net charge of the protein is zero. At pI proteins are stable and compact. The computed pI value of P49716, P98187, Q99988, P35354 and P15692 (pI > 7) indicates that these Cyclooxygenases are basic and the pI of P00918, P18054, P09917, P19838, P23219, Q96EB6 and Q15185 (pI < 7) reveals that these are acidic in character. The computed isoelectric point (pI) will be useful for developing buffer systems for purification by isoelectric focusing method. Although Expasy's ProtParam computes the extinction coefficient for a range of (276, 278, 279, 280 and 282 nm) wavelength, 280 nm is favoured because proteins absorb strongly there while other substances commonly in protein solutions do not. Extinction coefficient of cyclooxygenases at 280 nm is ranging from 16960 to 129830 M^{-1} cm^{-1} with respect to the concentration of Cys, Trp and Tyr. The high extinction coefficient of P18054, P09917, P98187, P23219 and P35354 indicates presence of high concentration of Cys, Trp and Tyr. Similarly, the low extinction coefficient of P49716 indicates low concentration of Cys, Trp and Tyr. Rest of the cyclooxygenases have average extinction coefficient. The computed protein concentration and extinction coefficients help in the quantitative study of protein–protein and protein–ligand interactions in solution. The biocomputed half-life of most of the cyclooxygenases are 30 h in mammalian reticulocytes, invitro, greater than 20 h in yeast, in vivo and greater than 10h in E.coli, invivo. On the basis of instability index Expasy's ProtParam classifies the P00918, P19838 and P35354 cyclooxygenases as stable (Instability index < 40) and other cyclooxygenases as unstable (Instability index > 40). The

aliphatic index (AI) which is defined as the relative volume of a protein occupied by aliphatic side chains (A, V, I and L) is regarded as a positive factor for the increase of thermal stability of globular proteins. The lower thermal stability of most of the cyclooxygenases is indicative of a more flexible structure when compared to other cyclooxygenase (table 4). The low aliphatic index of most of the cyclooxygenases infers that AFPs may be thermolabile for a high range of temperature. Grand Average hydropathy (GRAVY) Index of Cyclooxygenases is ranging from –1.04 to -0.16. The very low GRAVY index of cyclooxygenases infers that these cyclooxygenases could result in a better interaction with water. The secondary structure predicted with the help of programs SOPM and SOPMA (table 8) infers that the some of the cyclooxygenases have rich alanine content and mostly α-helices. most of the cyclooxygenases have mixed secondary structure, i.e. α-helices β-strands and coils. The very high coil structural content of cyclooxygenases are due to the rich content of more flexible glycine and hydrophobic proline amino acids. Proline has a special property of creating kinks in polypeptide chains and disrupting ordered secondary structure.

Table 8. Secondary structure calculation (in %) of human cyclooxygenase computed using SOPMA.

Accession number	Secondary Structure			
	Alpha Helix	Beta Sheet	Random Coil	Others
P00918	16.54	25	50.38	8.08
P49716	32.34	6.32	60.22	1.12
P98187	43.65	11.92	38.85	5.58
Q99988	29.55	16.23	50	4.22
P18054	37.41	14.63	42.23	5.73
P09917	37.24	15.28	43.03	4.45
P19838	30.27	15.19	48.24	6.3
P23219	41.74	11.69	42.74	3.83
P35354	40.23	10.76	45.03	3.98
Q96EB6	36.41	7.90	51.27	4.42
Q15185	22.84	12.50	62.93	1.73
P15692	22.50	18.12	53.75	5.63

The server SOSUI classifies the cyclooxygenase P98187 and P23219 as membrane protein and other cyclooxygenase as soluble proteins. SOSUI server has identified one transmembrane region in both cyclooxygenase P98187 and P23219. The transmembrane regions and their length are tabulated in table 5. The transmembrane regions are rich in hydrophobic amino acids and it is also well documented by Kyte and Dolittle mean hydrophobicity profile (figure 1) in which all the points are above the 0.0 line. The helix of P98187 and P23219 are visualized using EMBOSS pep-wheel shown in figure 2(a) and 2(b) respectively.

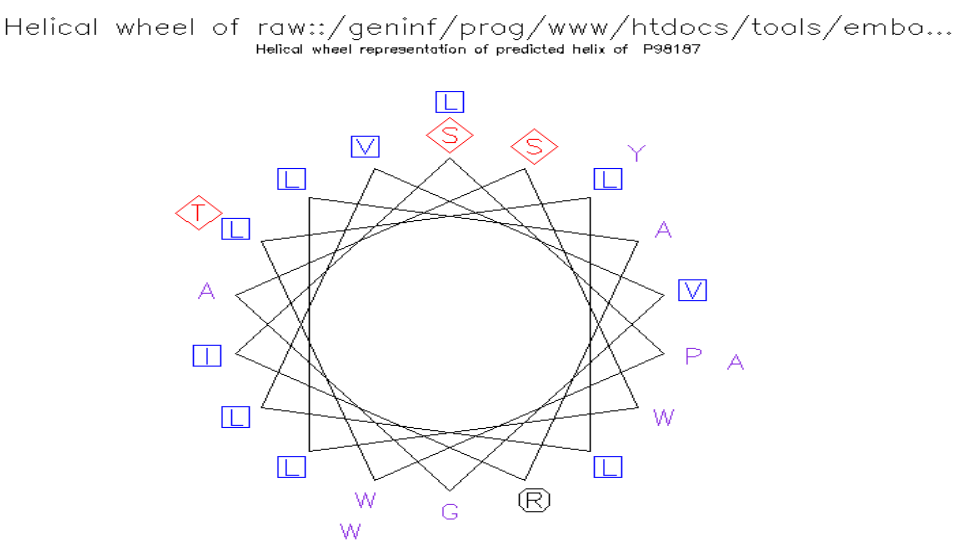


Figure 2(a). Helical wheel representation of predicted helix of P98187 Cyclooxygenase. Hydrophobic resi-dues (V, L, I) are represented as blue squares and violet letters (A, G, P, Y), polar residues (E, Q, S, T) as red dia-monds.

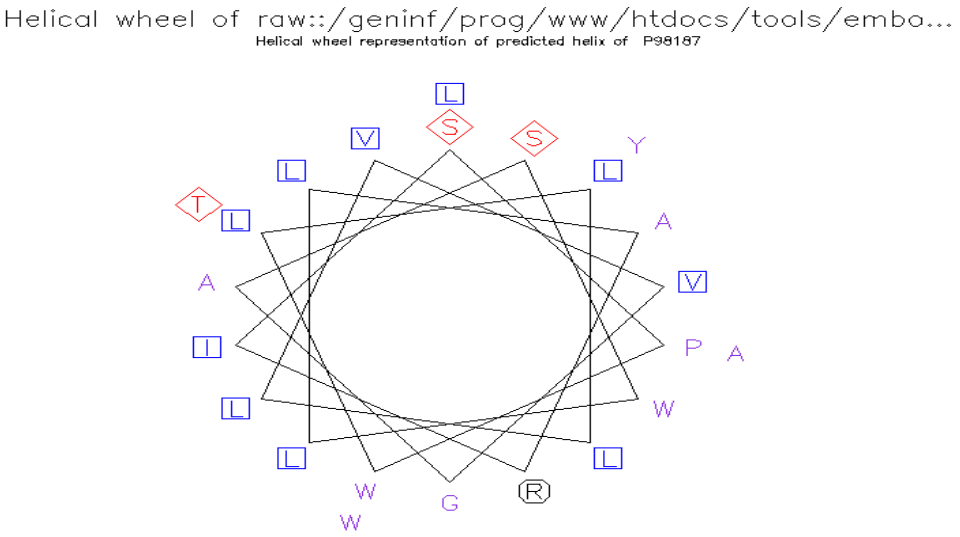


Figure 2(b). Helical wheel representation of predicted helix of P23219 Cyclooxygenase. Hydrophobic resi-dues (V, L, I) are represented as blue squares and violet letters (A, G, P, Y), polar residues (E, Q, S, T) as red dia-monds.

The tool CYS_REC recognizes the presence of 13 Cysteines in cyclooxygenase P23219 sequence and predicted five most probable SS bond pattern of pairs (as discussed in the primary structure analysis) in the protein. The positions of five most probable SS bonds predicted by CYS_REC and the five SS bonds identified using SPDBV in the cyclooxygenase P23219 is shown in table 7. The three dimensional structure of cyclooxygenase P23219 was modeled using PDB template (table 6) selected from the hits obtained through the BLASTP analysis and the modeled structures were evaluated. According to evaluation analysis, the Ramachandran plot and other parameters (table 9) were within the standard acceptable limits for the 3D structures modeled using the PDB template 1CQE_A for the (target) protein.

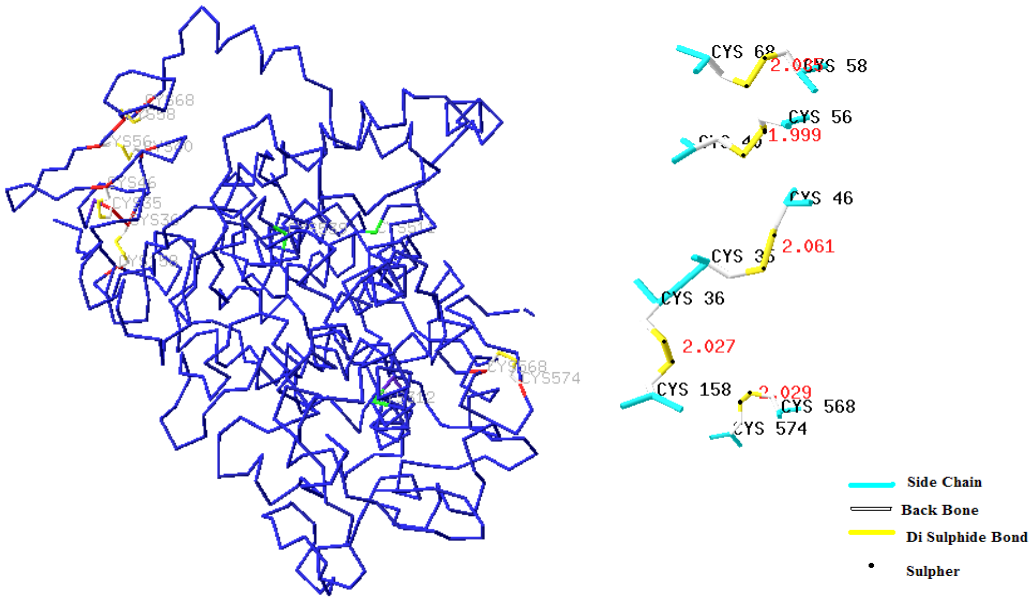
Table 9. Validation parameters computed for the built 3D structures of target P23219.

		Rampage	Procheck	ProQ	
		Percentage residues	Percentage of residues		
	Template	in favored region	In favored region	LG score	maxsub
Target	(PDB codes)				
P23219	1CQE_A	97.3	92.5	4.99	0.43

Table 10. Criteria for a good (model) 3D structure.

Rampage	Procheck	ProQ		
Percentage of residues	Percentage of residues			
In favored region	In favored region	LG score	maxsub	Quality of the model
98	90	>1.5	>0.1	Fairly good model
		>2.5	>0.5	Very good model
		>4.0	>0.8	extremely good model

A criterion for a good 3D structure is given in table 10. The cysteines and the SS bonds identified using the three-dimensional structure of cyclooxygenase P23219 is shown in figures 3 and 4 respectively. In the case of cyclooxygenase P23219 the five SS bond positions Cys35-Cys46, Cys36-Cys158, Cys40-Cys56, Cys58-Cys68, Cys568-Cys574 predicted by CYS_REC are correlating with the SS bond positions Cys35-Cys46, Cys36-Cys158, Cys40-Cys56, Cys58-Cys68, Cys568-Cys574 identified using SPDBV tool. We speculate that the SS bonds predicted from the primary structure (protein sequence) using CYS_REC tool and the SS bonds identified from the three-dimensional structure (3D coordinates) using the SPDBV tool is similar, therefore might be correct.



a **b**

Figure3 a) modeled structure with carbon backbone b) disulfide bond and cystiene(enlarge view) with distance.

The thirteen cysteines and five SS bonds present in the cyclooxygenase P23219 is shown in figures 3(a) and 3(b) respectively. The three unpaired cysteine in the protein is also shown in the figures. Helix and strand are also shown separately in the figure (5).the modeled structures are evaluated by Rampage, Procheck and ProQ software. The parameters are given in the table (9), which fulfills the criteria of very good model. Moreover, the ramachandran plot after evaluation with Procheck is shown in the figure4.

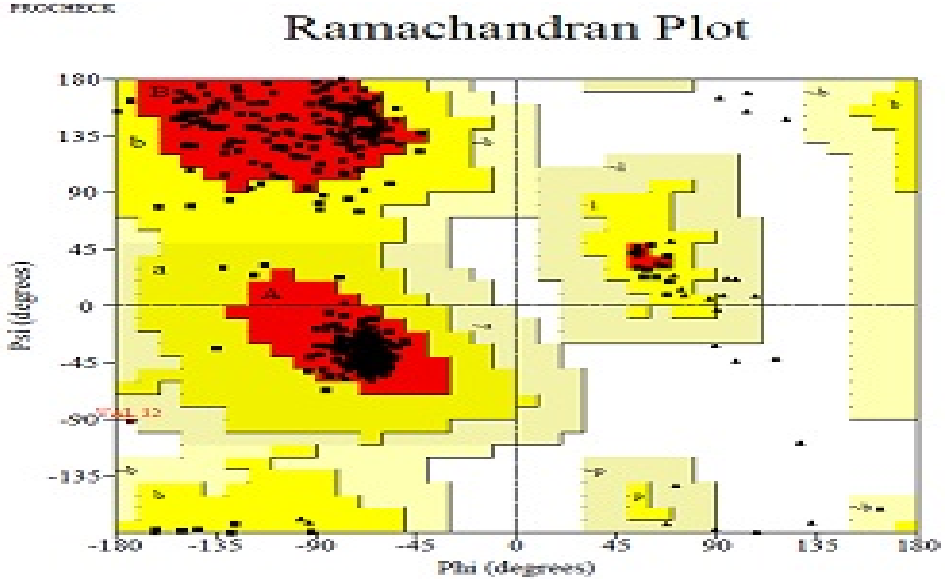


Figure 4. Ramachandran plot after evaluation by Procheck

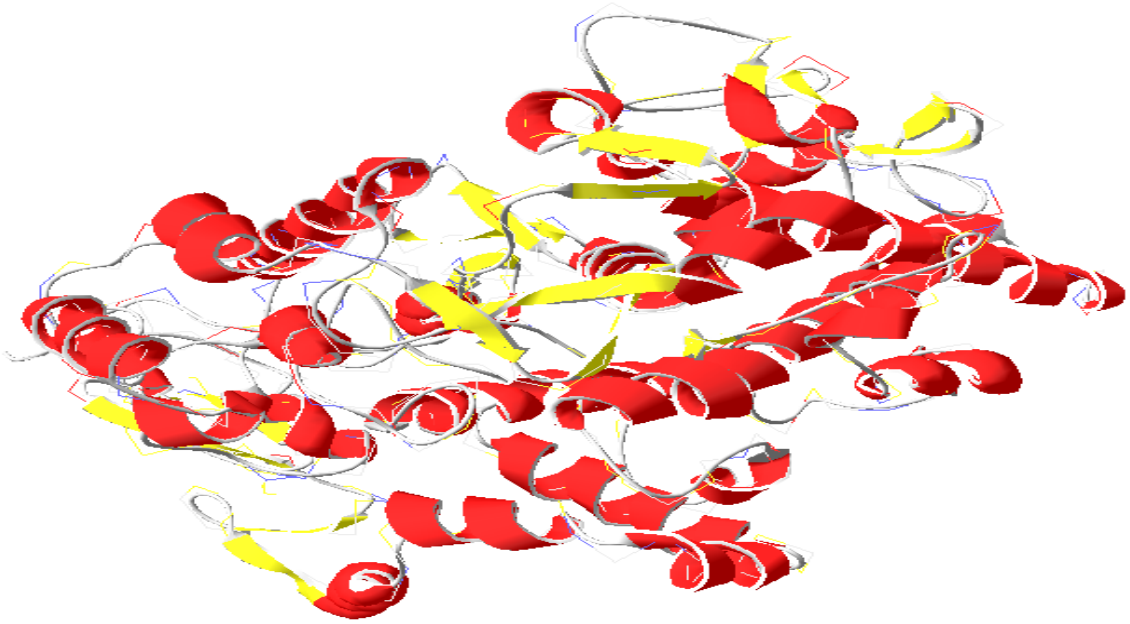


Figure 5. Modeled structure of cyclooxygenase P23219 showing helix and strand.

CONCLUSIONS

Twelve human cyclooxygenase have been chosen mainly to study their physico-chemical properties, primary and secondary structures by using computational tools and servers. Primary structure analysis reveals that most of the cyclooxygenases under study are hydrophobic in nature and nine of them contain disulphide linkages. Physico-chemical characterization studies give a good idea about the properties such as pI, EC, AI, GRAVY and Instability Index that are essential and vital in providing data about the proteins and their properties. Secondary structure analysis predicts that some of them contain α-helices and remaining of them contain mixed structure. The presence of 13 Cys residues in P23219 cyclooxygenase indicates the presence of disulfide bridges which is also confirmed using CYS_REC and SPDBV tools.

ACKNOWLEDGEMENT

Authors are grateful to the Department of Biotechnology (DBT), Govt. of India for the computational facility laboratory.

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