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Comparison of Sequence with Cluster based analysis for molecular properties and composition of Glutathione Peroxidase family proteins

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ABSTRACT

Glutathione peroxidase (GPx) is very important protein helps in eradication of exogenous materials from the body of human reported to do the same work in other organisms. The selenocysteine amino acid contributes to the structure of this protein. Here a comparative study on sequence based methods and property based methods is carried out on all curated sequences of Glutathione Peroxidase for Human. Swiss Prot database was explored and only 65 protein of GPx family was obtained out of which 18 curated sequences were used for further analysis. Sequence based Distance method of Multiple Sequence Analysis is used for finding similar groups. Then all 18 sequences were studied for number of cleavage sites analysis followed by hierarchical clustering which represents cleavage sites based similar groups. Further, these sequences were computed to find amino acid composition and various properties like theoretical PI, Instability Index, Alipahtic Index and Hydropathy, followed by Hierarchical clustering. The interesting fact obtained in this study is that on comparison with the cleavage sites based clustering and amino acid composition with properties based clustering is having similar type of groups. These similar groups are having no relation sequence based methods groups. Hence, we conclude that for finding functional similarity between various sequences clustering of property based methods are more reliable as compared to sequence based methods phylogenetic methods.

Keywords: Glutathione Peroxidase; computational analysis; hierarchical clustering; curated; hydropathy.

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Introduction:

Glutathione peroxidase (GPx) is very important protein for maintaining the redox balance of the body [1]. GPx protein is a family of multiple isozymes functionally responsible for Protection of hemoglobin in erythrocytes from oxidative breakdown in humans [2]. These isozymes also catalyzes the reduction of H_2O_2 or organic hydro peroxides (exogenous as well as endogenous) to water or corresponding alcohols by reducing glutathione which works as an electron donor [3]. Glutathione peroxidases are the enzymes that are responsible for eradication of free radicals from human body. They not only help in the removal of endogenous free radicals only they are also working for exogenous free radical eradications. Various studies on number of diseases represent about active or passive involvement GPx family of proteins. The altered activity of GPx proteins in human body is reported to cause various diseases like hypertension, cardiac dysfunction, and cancer which are very common and fatal as well. Apart from this if the gene ontology of this class of enzymes are studied it was observed that this enzyme having wide range of activity like, UV protection activity [4], heart contraction [5], regulation of apoptotic genes like Bax and Bcl-2 [6]. The Metazoan study reveals that some GPx's have a selenium-dependent glutathione peroxidase activity, with selenocysteine being encoded by an opal codon TGA. In our previous in-silico study on Gpx family of enzymes reveals that there are significant differences in the sequence composition and structure of all eight variants of GPx whereas the function of all the variants is same [7]. This creates a question in our mind that what are the various physiochemical characteristics of these enzymes that makes them so specific and how they are different from each other. In current world, number of computational tools has been developed for making predictions with great accuracy regarding the identification and structure prediction of proteins. In this study we used computational tools and online servers for protein sequence analysis and their characterization. The physicochemical and the structural properties of this family of proteins are also well understood with the use of computational tools. Here, in this study the sequence based and property based methods are used to compare the difference and similarity of all protein of GPx family. The major focus was to find that, on which method one should rely to find similarity in proteins sequence and their evolutionary relation i.e. sequence based method or properties based methods.

Materials and Methods:

Glutathione Peroxidase sequences

Glutathione Peroxidase sequences were retrieved from the manually curated public protein database Swiss-Prot [8]. From Swiss prot we retrieved 65 Glutathione Peroxidase sequences of *Homo sapiens* were retrieved. From total 65 sequences, only reviewed 19 sequences are found are listed in Table 1. One sequence IARS is not used for further analysis to avoid variation in results, thus, only 18 unique GPx family proteins are used for analysis. The sequences are retrieved in FASTA format. The average length of Gluathione Peroxidase is found to be of 251 AA. The statistics used for a protein sequence such as number of amino acid, sequence length, and the physicochemical properties of a proteins such as molecular weight, atomic composition, extinction coefficient, GRAVY, aliphatic index, instability index, etc. The amino acid sequences 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 bio computation tools have been done by many researchers and reported.

Entropy of GPx sequences:

The entropy of each sequence is calculated as Shannon Entropy. The Shannon Entropy H(X)

of the Sequence was calculated to check the randomness in the sequence [9][10]. Entropy is defined as sum of product of individual probabilities and their log for all events.

$$H(X) = \sum_i P(x_i) I(x_i) = -\sum_i P(x_i) \log_b P(x_i)$$

Sequence analysis:

Basic phylogenetics methods were used for sequence comparison i.e. multiple sequence alignment, distance calculation and tree preparation on the basis of distance. Windows platform based MEGA6.6 [11] software is used for sequence comparison and distance calculation. This software is providing facility of various genomic and proteomic analysis.

Results and Discussion:

Entropy of information:

The randomness found in GPx sequences is similar in all proteins ranging from 4.21978 to 3.64285 as shown in Table 1. The average entropy of the sequences is 4.08548, which represents that there no such variation in the information content in the sequences. The curve in Figure 1 shows stability in the information content of sequences i.e. the sequences used in the study are stable and representing to common specific category.

Table 1: SwissProt/Uniprot retrieved sequences of Glutathione Peroxidase

S.N.	Gene names	Entry	Organism	Length	Shannon entropy H(X)
	GPX1	P07203	Human	203	4.08433
	GPX2	P18283	Human	190	4.17454
	GPX3	P22352	Human	226	4.17409
	GPX4	P36969	Human	197	4.21978
	GPX5	O75715	Human	221	4.14397
	GPX6	P59796	Human	221	4.1005
	GPX7	Q96SL4	Human	187	4.12986
	GPX8	Q8TED1	Human	209	4.02618
	GSTK1	Q9Y2Q3	Human	226	4.11617
	GSTT1	P30711	Human	240	4.05993
	GSTZ1	O43708	Human	216	4.12569
	HPGDS	O60760	Human	199	4.18639
	IARS	P41252	Human	1262	4.17552
	LTC4S	Q16873	Human	150	3.64285
	MGST1	P10620	Human	155	4.04231
	MGST2	Q99735	Human	147	4.03208
	MGST3	O14880	Human	152	4.05916
	PRDX6	P30041	Human	224	4.0519
	SOD1	P00441	Human	154	3.95354

Sequence Analysis:

For protein sequence analysis the data is rectified for outliers hence, protein id P41252 in Table 1 is not used for further Sequence and proteolytic analysis because the sequence length is very large as compared to other GPx proteins. This protein id P41252 may cause problem in further calculation so to maintain the

stability in the analysis this sequence is marked as outlier in the data set. A distance matrix is calculated on the basis of positional sequence differences between all pairs of sequences. By the help of this distance matrix a neighbor joining approaches [12] was used to find phylogenic relation among all GPx proteins. The tree in Figure 2(B) shows various groups of proteins. In common practice for finding functional relation between various proteins researcher use a phylogenetics based methods.

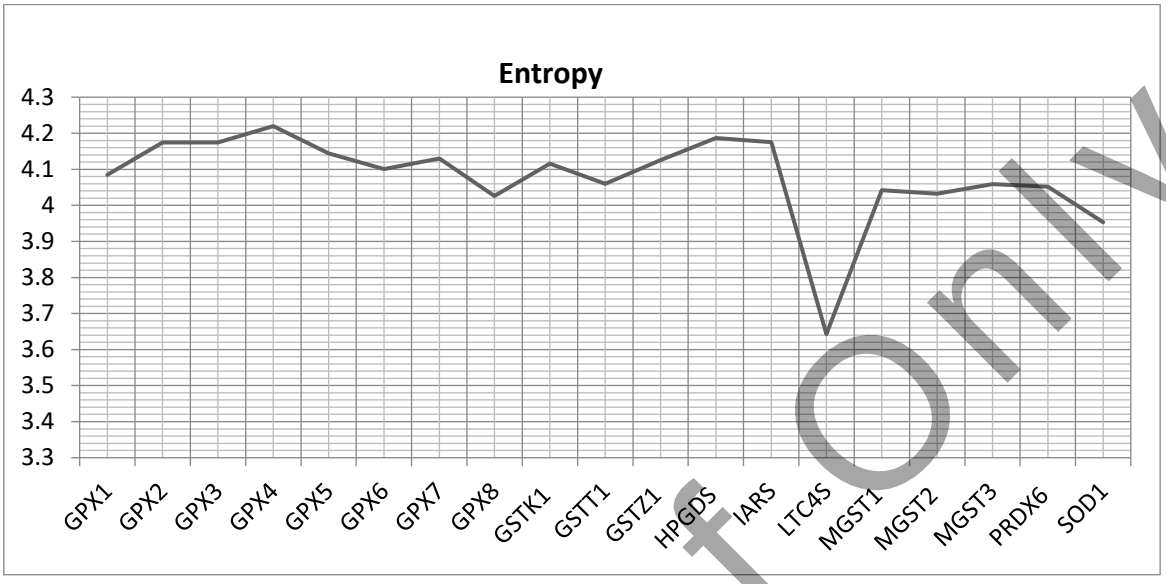
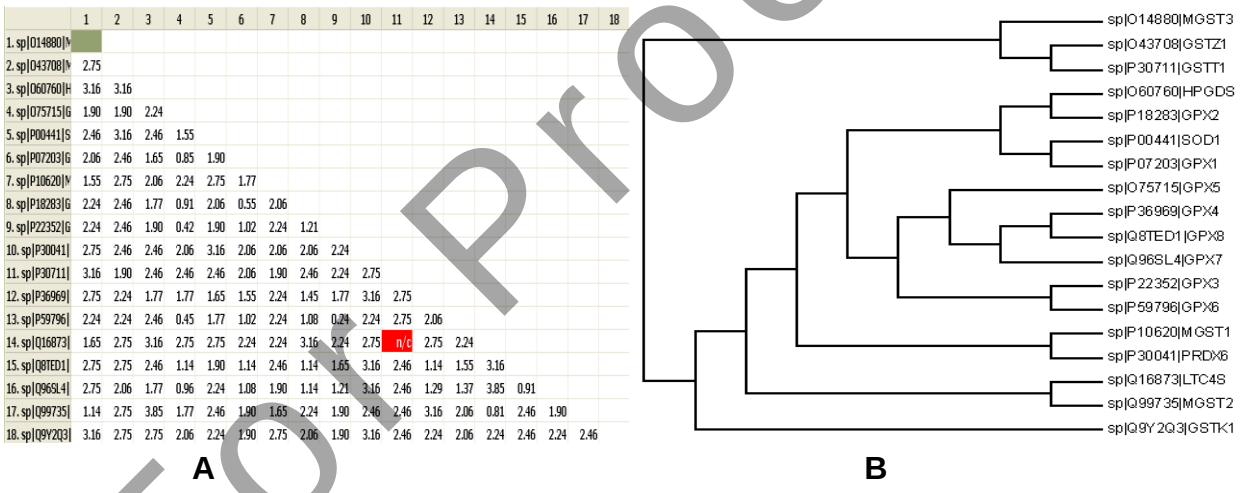


Figure 1: Entropy of GPx Family Proteins



A **B**

Figure 2: Distance Matrix (A) and Phylogenetics Tree (B) after MSA of 18 GPx Sequences.

Protein Digestion:

The number of proteolytic cleavages sites by various proteolytic enzymes is calculated by the ExPasy's peptide cutter tool **[13]** for all the sequences of glutathione peroxidase. The number of fragments produced by each enzyme is tabulated in Table 2. The graph in Figure 3 represents the similar pattern of fragmentation by proteolytic enzymes. These fragments are then subject to hierarchical clustering **[14]** to find group of similarly fragmented proteins. The cluster tree represented in Figure 4(A) shows groups by using proteolytic cleavage site information. A similar patter of cleavage is reported in the various GPx proteins. Figure 4(B) shows scores found in clustering each dot in the graph presents a node and its distance in the tree.

Table 2: Number of proteolytic sites available on Glutathione peroxidases family proteins.

GPx Type / Enzyme	GPX1	GPX3	GPX2	GPX4	GPX5	GPX6	GPX7	GPX8	GSTK1	GSTT1	GSTZ1	HPGDS	LTC4S	MGST1	MGST2	MGST3	PRDX6	SOD1
Arg-C proteinase	14	10	13	9	5	2	9	9	12	12	12	10	13	11	8	6	12	4
Asp-N endopeptidase	8	7	10	11	9	6	8	6	7	15	10	15	1	8	2	1	16	11
Asp-N endopeptidase + N-terminal Glu	21	23	22	21	23	20	18	23	23	28	20	27	6	13	7	6	32	21
BNPS-Skatole	2	3	2	4	3	3	5	3	5	5	2	6	3	0	3	2	3	1
CNBr	4	6	2	7	4	3	2	1	11	5	6	6	1	7	2	3	4	1
Chymotrypsin -high specificity	16	26	20	18	24	29	20	22	14	20	13	21	19	19	21	17	16	5
Chymotrypsin -low specificity	43	59	40	45	53	58	42	48	60	65	43	52	52	49	45	40	41	23
Clostripain	14	10	13	9	5	2	9	9	12	12	12	10	13	11	8	6	12	4
Formic acid	8	7	10	11	9	6	8	6	7	15	10	15	1	8	2	1	16	11
Glutamyl endopeptidase	13	16	12	10	14	14	10	17	16	13	10	12	5	5	5	5	16	10
Hydroxylamin e	1	1	1	2	1	2	0	0	0	0	0	0	0	0		1	0	1
Iodosobenzoic acid	2	3	2	4	3	3	5	3	5	5	2	6	3	0	3	2	3	1
LysC	6	15	10	17	23	16	11	24	13	16	12	12	1	7	7	8	18	11
LysN	6	15	10	17	23	16	11	24	13	16	12	12	1	7	7	8	18	11
NTCB (2-nitro-5-thiocyanobenz oic acid)	5	4	4	9	5	5	3	4	2	3	5	3	2	1	3	4	2	4
Pepsin (pH1.3)	50	63	45	38	53	73	43	57	53	77	41	50	63	43	52	39	47	18
Pepsin (pH>2)	58	82	55	52	70	90	61	69	67	94	55	73	77	58	72	54	56	19
Proline-endopeptidase [*]	3	1	2	2	3	1	2	4	2	2	3	3	0	0	0	1	1	0
Proteinase K	103	111	95	90	108	113	102	111	114	136	107	108	102	87	89	81	118	65
Staphylococca l peptidase I	12	14	11	8	13	12	9	16	16	13	8	12	5	5	5	5	13	8
Thermolysin	67	65	53	60	62	71	64	70	73	82	69	53	76	64	63	59	65	42
Trypsin	18	24	22	25	26	17	19	30	23	27	23	20	14	18	15	14	30	14
GranzymeB	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Thrombin	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0

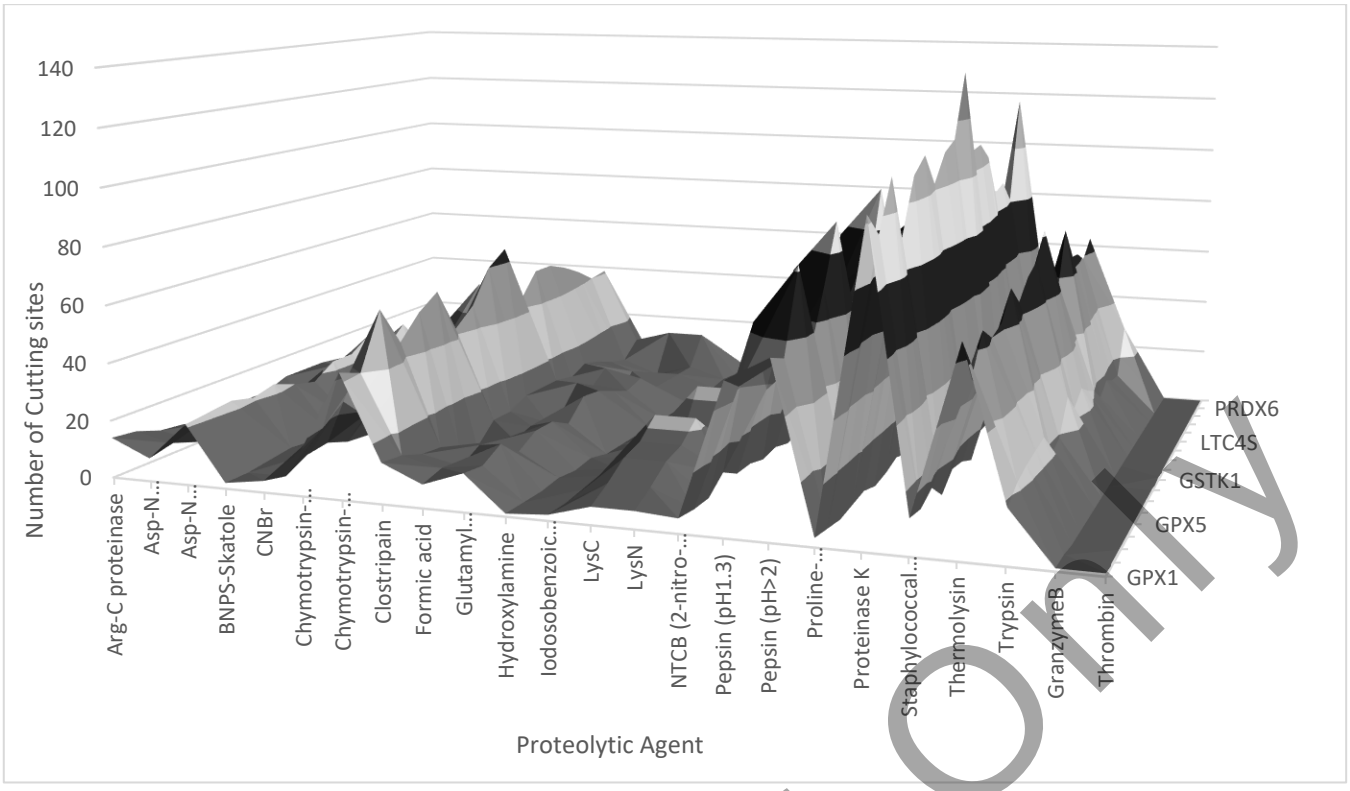


Figure 3: Proteolytic Graph of Various protein of GPx Family.

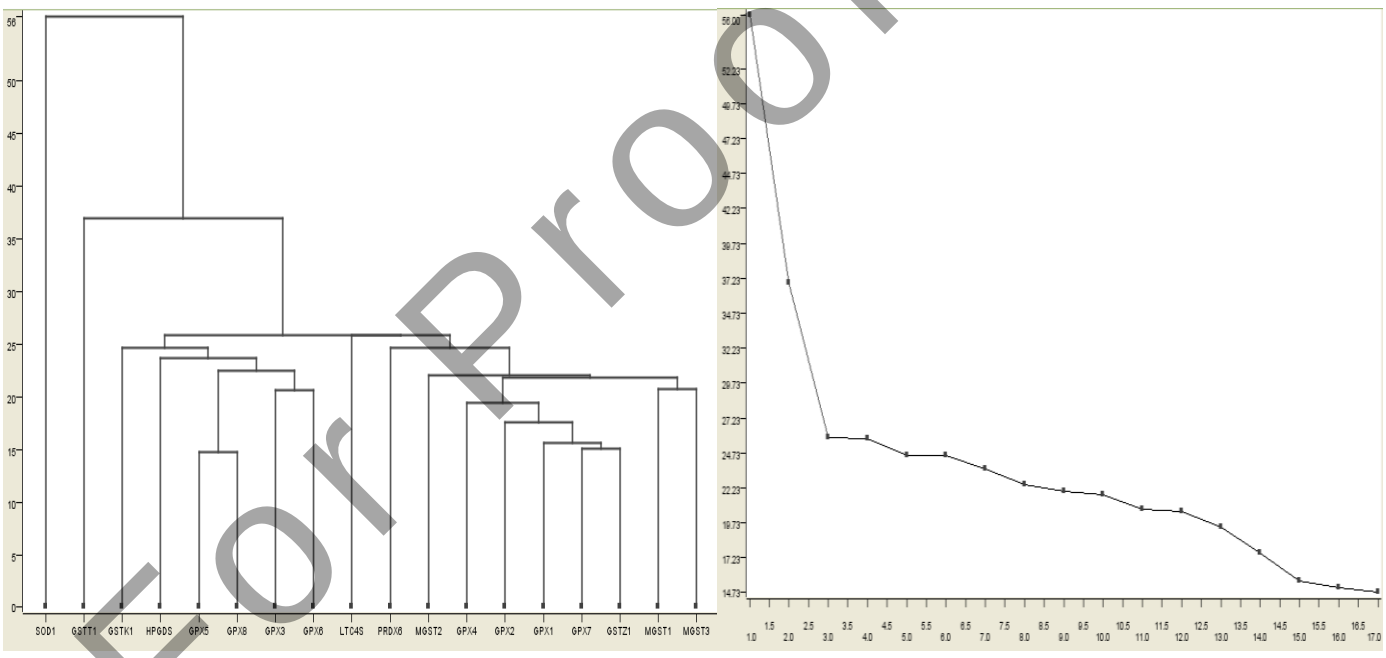


Figure 4: Hierarchical Clustering of GPx Proteins on the basis of proteolytic site, node are represented in dot form its distance in the tree.

Amino Acid Composition:

The amino acid composition of all proteins is calculated by ProtParam [15] tool(http://us.expasy.org/tools/ protparam.html) of ExPASy . This tool represents percentage composition of Amino Acids of individual protein is represented in Table 3 and Figure 5. ProtParam tool also calculates various other molecular properties like Molecular Weight, Theoretical pI (Isoelectric point), Instability index, aliphatic index, Grand average of hydropathicity (GRAVY) of a protein sequence.

Table 3: Percentage of Amino acid composition for all GPx proteins.

Type	Ala	Arg	Asn	Asp	Cys	Gln	Glu	Gly	His	Ile	Leu	Lys	Met	Phe	Pro	Ser	Thr	Trp	Tyr	Val	Sec
GPX1	11.8	6.9	4.9	3.9	2.5	3.4	6.4	8.4	1	3	11.3	3	2	5.4	7.4	5.4	3.4	1	2	6.4	0.5
GPX2	4.2	6.8	5.8	5.3	2.1	4.2	6.3	5.8	1.1	5.8	9.5	5.3	1.1	7.9	7.4	4.7	5.3	1.1	3.7	6.3	0.5
GPX3	4.4	4.4	4	3.1	1.8	4.9	7.1	10.2	1.8	4.9	11.1	6.6	2.7	6.2	5.3	5.8	4	1.3	4.4	5.8	0.4
GPX4	8.1	4.6	5.1	5.6	4.6	2.5	5.1	8.6	2.5	5.1	9.1	8.6	3.6	5.1	4.6	3.6	2.5	2	3	5.6	0.5
GPX5	3.6	2.3	3.6	4.1	2.3	5	6.3	7.2	3.2	4.1	10	10.4	1.8	6.3	6.3	5	4.5	1.4	4.1	8.6	0
GPX6	5	0.9	5.4	2.7	2.3	7.7	6.3	8.1	2.3	2.7	10.4	7.2	1.4	9.5	5.4	5	4.1	1.4	3.2	8.6	0.5
GPX7	11.2	4.8	3.2	4.3	1.6	6.4	5.3	5.3	2.1	2.7	9.1	5.9	1.1	5.9	4.8	5.9	4.8	2.7	3.2	9.6	0
GPX8	5.7	4.3	3.3	2.9	1.9	2.4	8.1	4.8	1.4	4.8	10.5	11.5	0.5	7.7	7.2	6.7	2.4	1.4	2.9	9.6	0
GSTK1	8	5.3	4	3.1	0.9	3.5	7.1	6.6	3.1	5.3	14.6	5.8	4.9	3.1	7.1	5.3	4	2.2	2.2	4	0
GSTT1	10	5	1.2	6.2	1.2	5.4	5.4	3.8	2.5	4.2	15	6.7	2.1	4.6	6.7	2.5	5	2.1	2.9	7.5	0
GSTZ1	6.5	5	4.5	7.5	1.5	4.5	6	3.5	2.5	7	11.6	6	3	4	5	2.5	7	3	4.5	4.5	0
LTC4S	18.7	8.7	0.7	0.7	1.3	3.3	3.3	5.3	1.3	1.3	21.3	0.7	0.7	6.7	5.3	4	3.3	2	4.7	6.7	0
MGST1	9	7.1	3.2	5.2	0.6	1.3	3.2	5.2	1.9	5.2	13.5	4.5	4.5	6.5	4.5	5.8	6.5	0	5.8	6.5	0
MGST2	10.9	5.4	3.4	1.4	2	4.8	3.4	7.5	0.7	6.8	14.3	4.8	1.4	7.5	2.7	5.4	4.1	2	6.1	5.4	0
MGST3	9.2	3.9	2.6	0.7	2.6	2	3.3	11.8	3.9	5.3	10.5	5.3	2	4.6	4.6	7.2	3.9	1.3	6.6	8.6	0
PRDX6	7.1	5.4	3.1	7.1	0.9	0.9	7.1	6.2	1.3	5.4	11.2	8	1.8	5.4	8.5	4	6.2	1.3	1.8	7.1	0
SOD1	6.5	2.6	4.5	7.1	2.6	1.9	6.5	16.2	5.2	5.8	5.8	7.1	0.6	2.6	3.2	6.5	5.2	0.6	0	9.1	0

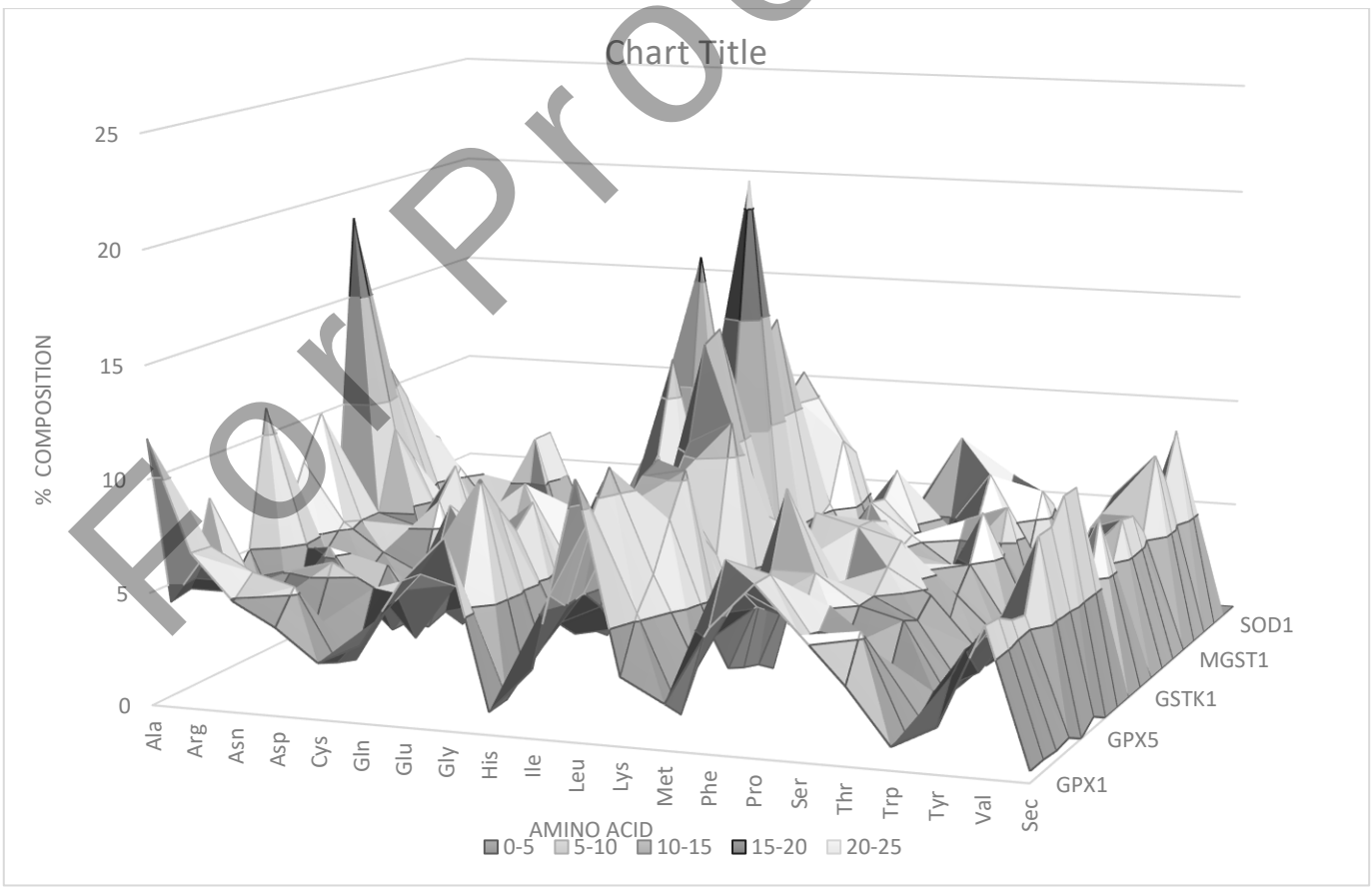
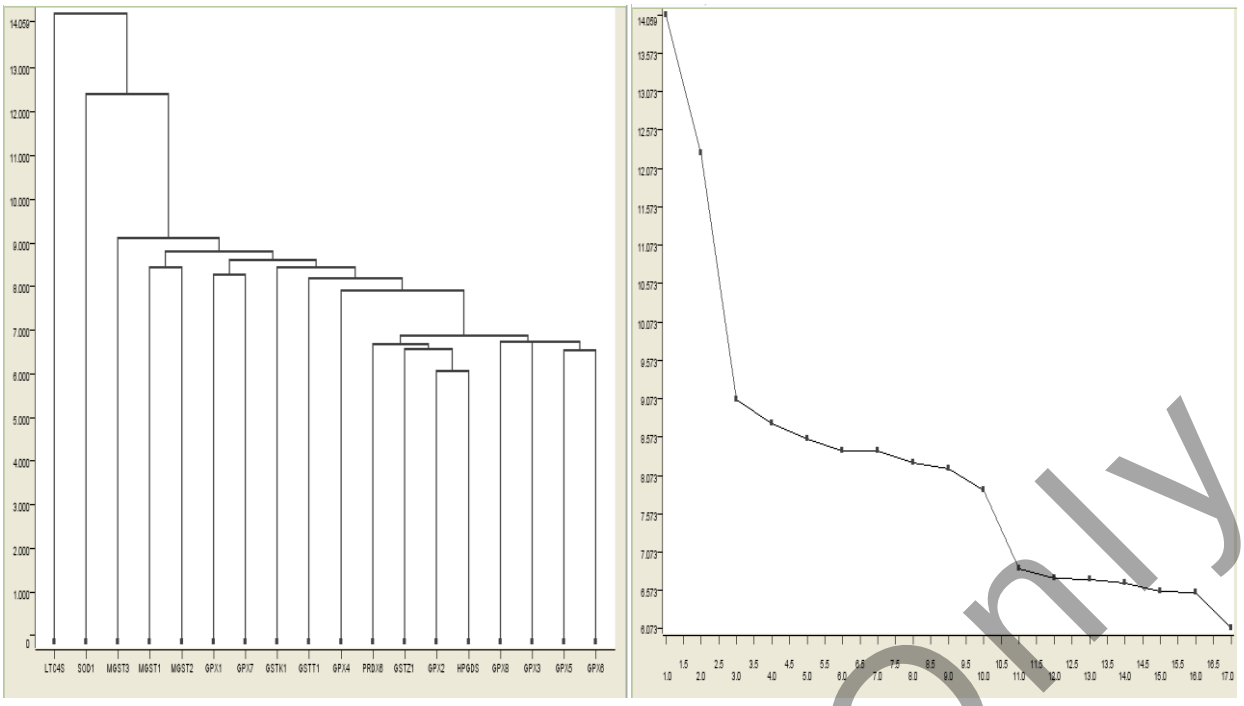


Figure 5: Amino Acids Composition represents a similarity in composition among all GPx Proteins.



(A) (B)

Figure 6: Cluster tree (A) based on composition and Cluster group and score graph (B).

Table 4: Various Parameters computed using Expasy's ProtParam tool of human glutathione peroxidase.

Composition	Amino Acid	Molecular Weight	Theoretical pI	Instability Index	Aliphatic index	Hydropathicity
GPX1	203	22088	6.15	47.96	86.11	-0.07
GPX2	190	21954	7.64	48.91	82.05	-0.32
GPX3	226	25552	8.26	53.55	83.23	-0.221
GPX4	197	22175	8.69	31.83	79.75	-0.194
GPX5	221	25202	8.83	48.73	83.26	-0.285
GPX6	221	24970	6.21	33.5	81.09	-0.096
GPX7	187	20996	8.42	42.29	85.03	-0.136
GPX8	209	23881	9.41	32.74	93.21	-0.136
GSTK1	226	25497	8.51	46.19	97.17	-0.135
GSTT1	240	27335	7.01	43.78	106.5	0.012
GSTZ1	199	23343	5.54	28.79	92.16	-0.312
LTC4S	150	16567	10.2	37.98	126.4	0.74
MGST1	155	17599	9.41	24.82	100.71	0.194
MGST2	147	16620	9.6	42.77	108.91	0.412
MGST3	152	16516	9.46	22.13	95.59	0.282
PRDX6	224	25034	6	43.24	92.28	-0.21
SOD1	154	15936	5.7	21.62	78.44	-0.344

The abundance of GPx proteins may vary from tissue to tissue in a human body but they have high similarity in their composition and functions. The main aim of this is to study to find relation between various different GPx proteins. In this study firstly we have calculated the Shannon Entropy of all the curated proteins to represent the randomness of the sequence. Randomness in the sequence information reveals that the sequences have same randomness level as shown in Figure 1. The information content of each GPx have low variation so the evolutionary difference between the sequences were calculated. To find the evolutionary differences and similarity we have used two different methods, one is Sequence based in figure 2 and other is property based Figure 3-6. The property based study method is further divided in to number cleavage site based and composition based studies. The results of sequence based analysis reveals that most of the glutathione peroxidase is forming similar groups but in property based methods showing wide variations in comparison to sequence based analysis. The similarity found in cluster analysis based on composition is between MGST3/MGST1, GPX1/GPX7, GSK1/GSTT1, and HPGDS/GPX8/GPX3/GPX5/GPX6. The similarities obtained by using number of proteolytic sites are between MGST1/MGST3, GPX1/GPX7, GSTT1/GSTK1, and HPGDS/GPX5/GPx8/GPx3/GPX6. These results obtained by both the methods are of high similarity. But when we are trying to compare both the property based tree with the Multiple sequence analysis base tree then there are lot of variations are obtained showing the non reliability of the sequence based methods. Table 4 represents property based differences between various GPx proteins.

Conclusion:

The compositional and cluster based analysis of GPx have high similarity and have immense potential to find similar acting GPx or any proteins. The MSA based methods are always supposed to be best for evolutionary studies but we found non-significant results in sequence based methods for GPx proteins. By using property clustering methods, we found good relation between sequences defining the similarity between sequence. On comparing the results obtained by both the methods we found that clustering based methods are working better than sequence based. This provide us evidence that sequence based methods are less relevant as compared to cluster and composition based for evolutionary studies of proteins. According to our finding the clustering and property based methods are more reliable and realistic and should be used for evolutionary studies of proteins.

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