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BIOINFORMATICS ANALYSIS ON SQUALENE EPOXIDASE 2 IN *PANAX NOTOGINSENG*

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ABSTRACT

Panax notoginseng is a commonly used medicinal herb. In this paper, from the perspective of bioinformatics, the main physical characteristics of Squalene Epoxidase 2 in Panax notoginseng are studied. The isoelectric point, theoretical pI and hydrophobicity etc, of the polypeptide are studied and the results show that the theoretical isoelectric point of Squalene Epoxidase 2 in Panax notoginseng is 9.13, its molecular weight is 60263.16, its instability index is 29.87, there are three transmembrane regions in the Squalene Epoxidase 2 sequence, last, the secondary structure of Squalene Epoxidase 2 shows that there are many secondary structure kinds, rate of alpha helix are 173(31.74%), rate of beta sheet are 133(24.40%), rate of beta turn are 69(12.66%) and rate of coil are 170(31.19%) respectively. Finally, this paper also discussed the three-dimensional structure and phosphorylation site of Squalene Epoxidase 2 in *Panax notoginseng.*

KEYWORDS: Squalene Epoxidase 2; *Panax notoginseng*, Bioinformatics analysis; traditional Chinese medicine.

INTRODUCTION

Panax notoginseng is a good medicinal plant for human body in traditional Chinese medicine, the medicinal parts are the root. Recent studies show that the *Panax notoginseng* has a hemostatic effect. Squalene epoxidase in *Panax notoginseng* has attracted the attention of many scholars.[1, 2] The authors also identified experimentally Squalene Epoxidase 2 from the perspective of bioinformatics, for example, Rekha, et al. studied the dynamics of squalene content in different tissues of Ashwagandha in its different growth stages.[3] Athar Ali, et al.

studied the characterization and expression of squalene synthase gene in different tissues of Artemisia annua L. plants.[4] Huihong Guo, et al. studied the molecular characterization and the regulation of Gynostemma pentaphyllum squalene epoxidase gene 1[5] and Ke Gao, et al. explored the molecular cloning and expression of squalene epoxidase of *bupleurum chinense.*[6] Anita Chugh, et al. tended to think that the squalene epoxidase could be used as hypocholesterolemic drug target revisited.[7] Masae Sawada, et al. studied the synthesis and biological activity of squalene epoxidase inhibitor.[8] Shi-hao Yang, et al. studied the antifungal effects of Aspidin BB against Trichophyton rubrum Involved Inhibition of Ergosterol Biosynthesis.[9] Ramiro, et al. Studied a squalene epoxidase involved in biosynthesis in both sterols in the Basidiomycete H. sublateritium and in the antitumor compound clavaric acid.[10] Masae Sawada, et al, explored the effect of a potent inhibitor of squalene epoxidase on cholesterol metabolism in HepG2 cells.[11] When the bioinformatics is concerned, B Favre, et al., studied the cloning and expression characteristics of squalene epoxidase in the pathogenic yeast Candida albicans.[12] Kenichi Kosuga, et al., studied the nucleotide sequence of cDNA for squalene epoxidase in the year 1995.[13] Many functions or characteristics of squalene epoxidase are all explored by lots of scholars.[14-18] In this paper, main physical characteristics of squalene epoxidase 2 in *Panax notoginseng* is studied from the bioinformatics view in the following paper.

MATERIALS AND METHODS

In order to study the characteristics of squalene epoxidase 2, the squalene epoxidase 2 *Panax notoginseng* is studied including the component, the signal peptide and the secondary structure information in sequences data. All the sequence is from NCBI and the accession number is AFV92748.1. All the prediction system is shown in the table 1.

Table 1. Some prediction system used for studying the characteristics of squalene epoxidase 2.

Item	**Web system**	**Ref**
Basic property	http://web.expasy.org/protparam/	[19]
Hydrophobicity	http://web.expasy.org/protscale/.	[20]
Transmembrane region	http://www.cbs.dtu.dk/services/TMHMM/.	[21]
Signal peptide	http://www.cbs.dtu.dk/services/SignalP/	[22]
Secondary structure	http://bioinf.cs.ucl.ac.uk/psipred/	[23]
Homology modeling	https://swissmodel.expasy.org	[24]

RESULTS AND DISCUSSION

Protparam system is a famous tool for studying the protein physical and chemical properties. Some physical and chemical properties such as amphoteric ionization, isoelectric point, etc., of squalene epoxidase 2 are predicted via the Protparam system. The results show that the N-terminal of the sequence considered is M (Met), the estimated half-life is 30 hours (mammalian reticulocytes, in vitro) and its formula is $C_{2713}H_{4299}N_{739}O_{756}S_{28.}$ Other physical and chemical properties squalene epoxidase 2 are shown in Table 2 and Figure 1.

Table 2. Physical and chemical property analysis of Squalene Epoxidase 2.

Parameter	**Results**
Number of amino acids	545
Molecular weight	60263.16
Theoretical pI	9.13
Total number of atoms	8535
Aliphatic index	93.78
Negatively residues (Asp + Glu)	51
Positively residues (Arg + Lys)	64
Instability index	47.14

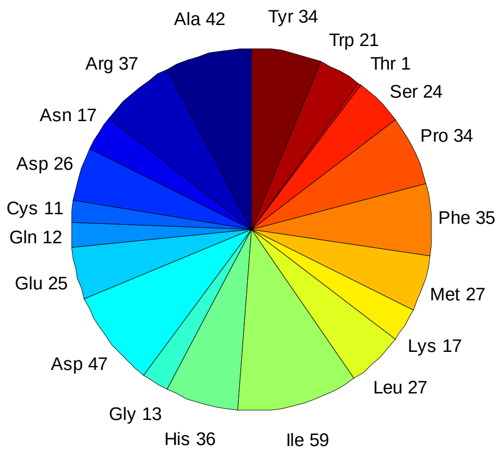

Figure 1. Amino acid composition of Squalene Epoxidase 2.

The hydrophobicity map of Squalene Epoxidase 2 was calculated using the Hphoh. / Kyte & Doolittle scale in ExPASy's ProtScale program (see Figure 2). The prediction Window size is 9. Relative weight for window edges is 100%, the weight variation model is linear. Vertical value above 0 denotes the hydrophobic area; below value 0 denotes the hydrophilic area, and the result shows that MIN value is -4.322 and the MAX value is 2.711.

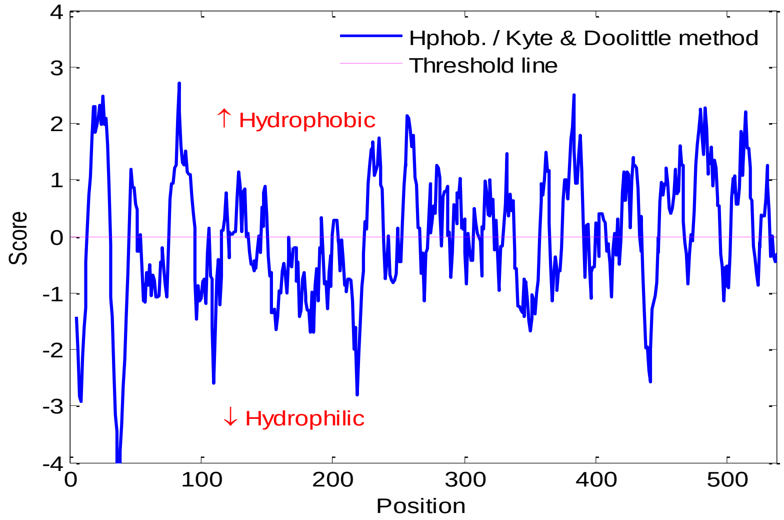

Figure 2. Hydrophobic characteristics of Squalene Epoxidase 2.

TMHMM is a kind of transmembrane helix prediction system based on hidden Markov model. The transmembrane region of Squalene Epoxidase 2 predicted result is shown in Figure 3. The results show that there are 3 transmembrane regions in Squalene Epoxidase 2. the predicted result also shows that the WEBSEQUENCE Exp number of AAs in TMHs is 63.23 and the Total prob of N-in is 0.393.

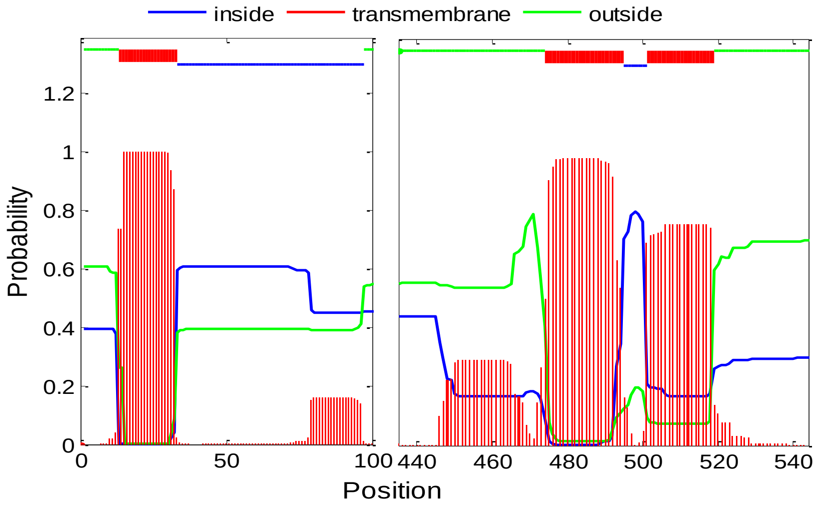

Figure 3. Transmembrane region prediction of Squalene Epoxidase 2.

The signal peptide is a short peptide that secures extracellularly proteins from cells to extracellular. Its typically length is 5-30 amino acids. Signal peptide characteristics of Squalene Epoxidase 2 is calculated via SignalP and the result is shown in Figure 4. C-score in the figure 4 denotes the signal peptide cleavage site value, S-score in the figure 4 is the signal peptide value; and Y-score in the figure 4 is the integrated score of shear point. Predictive results show that there is no signal peptide in Squalene Epoxidase 2 with the max. C is 0.116, max. S is 0.228 and the mean S is 0.151.

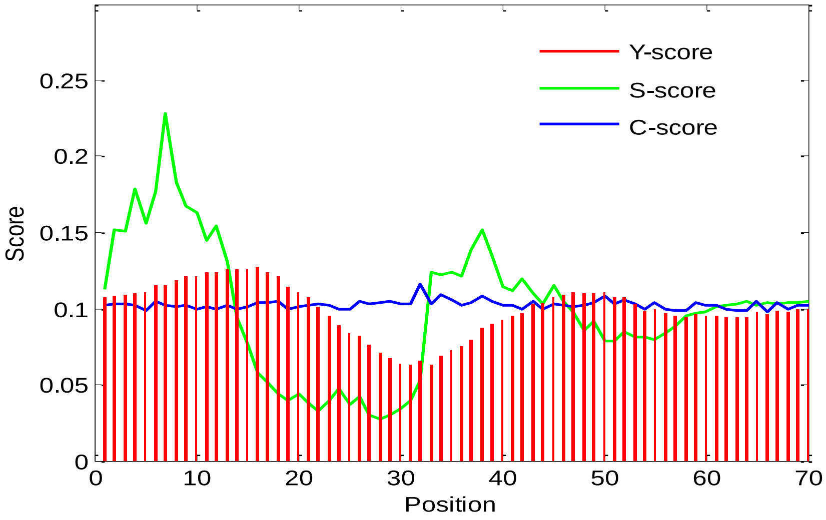

Figure 4. Signal peptide analysis of Squalene Epoxidase 2.

The Psipred prediction system is used for predicting the Secondary structure prediction for the Squalene Epoxidase 2. the predicted parameters are set as Window width is 17, threshold=8, Division factor is 4. The secondary structure predicted results of (via Psipred prediction system) Squalene Epoxidase 2 are shown in Figure 5. And the results show that the secondary state rates: Alpha helix=31.74%, Beta sheet=24.40%, Turn=12.66%, Coil=31.19%.

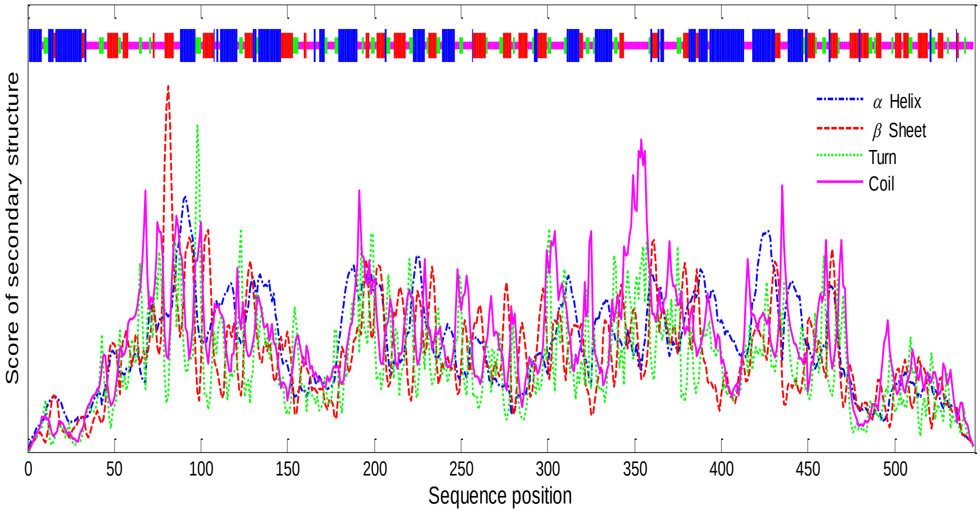

Figure 5. Secondary structure prediction of Squalene Epoxidase 2.

The Swiss-Model system provides an algorithm to predict the three-dimensional structure of the polypeptide. The algorithm is used to predict the three-dimensional structure of Squalene Epoxidase 2 (see figure 6). As can be seen from Figure 6, squalene epoxidase 2 is mainly composed of β-sheet and there are still some small helical structures. In the figure 6, it also can be seen that there is one signal peptide in the structure.

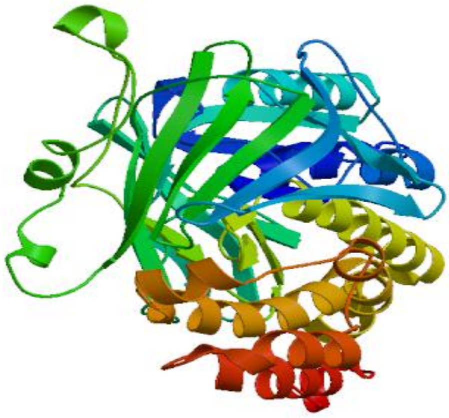

Figure 6. 3D model of squalene epoxidase 2 predicted via Swiss-Model system.

The NetPhos online system could be used for predicting the protein phosphorylation site. The phosphorylation sites of squalene epoxidase 2 prediction result is shown in Figure 7. From the prediction results, it can be seen that there are 4 phosphorylation sites for tyrosine, 17 phosphorylation sites for threonine and 25 phosphorylation sites for serine. The sites with the phosphorylation potential value less than 0.5 are neglected. So, all phosphorylation sites which phosphorylation potential value are more than 0.5.

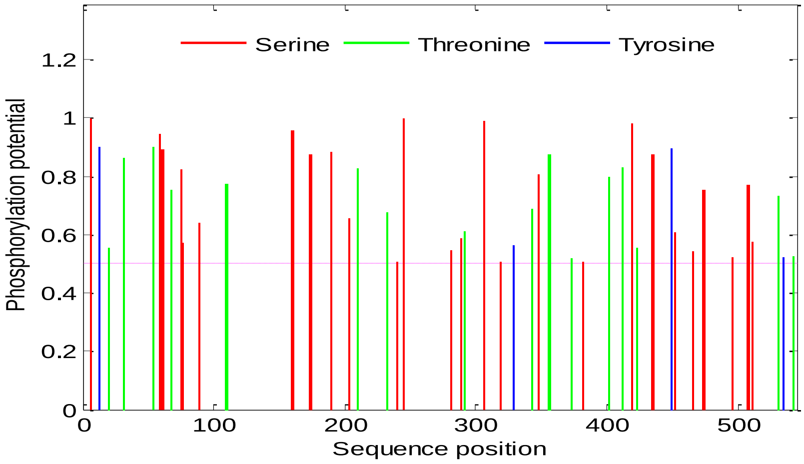

Figure 7. The phosphorylation potential value (threshold = 0.5).

CONCLUSION

Since it is valuable to determine or predict the characteristics in protein molecules via bioinformatics tools, the presence of predicted mechanisms can help us to understand the possible localization of the protein in the cell, the other characteristics of the protein sequence can also be studied to reveal their localization in the cell because a large number of protein sequences do not include a typical target peptide fragment. In this study, the bioinformatics characteristics contained in squalene epoxidase 2 are systematically studied, the basic physical and chemical properties of squalene epoxidase 2 are predicted, which provided a reference for understanding the value of *Panax notoginseng*. The 3D structure of the squalene epoxidase 2 is predicted too in order to understand the structural characteristics of squalene epoxidase 2 and reveal for further use of *Panax notoginseng* in medicine.

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