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TFT1 and TFT10 Most Likely Interact with Sucrose-Phosphate Synthase (SPS) in Tomato

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ABSTRACT

SPS is a target for 14-3-3s and there are 12 isoforms (TFT1-TFT12) of 14-3-3s in tomato (*Solanum lycopersicum*). Bornke's research showed that T14-3d and T14-3g, isoforms of 14-3-3s, can interact with SPSA in tobacco (*Nicotiana tabacum*). Analyzing the physical and chemical properties of 14-3-3s, 3-D structures and motifs of tomato 14-3-3s and SPS with bioinformatics softwares, we predicted, which isoform(s) has (have) the ability to bind with SPS based on Bornke's research. The results showed that aliphatic indexes are all greater than 70 and the grand average of hydrophobicity ranged from -0.701 to -0.269 of 14-3-3s in tomato, indicating that tomato 14-3-3s are hydrophilic proteins with high thermal stability. The similarities between TFT1 and T14-3d, TFT10 and T14-3 g had 95% and 94% identity, respectively. By constructing a phylogenetic tree, TFT1\T14-3d\TFT10\T14-3 g are found to be on the same branch. This indicated that *tft1* and *t14-3d*, *tft10* and *t14-3 g* are 2 groups of orthologous genes. The 3-D structure modeling showed that TFT1 and T14-3d, TFT10 and T14-3 g have similar structures. Likewise, structures of tomato SPS and tobacco SPSA were very similar. Moreover, all tomato 14-3-3s had an auto-inhibitory domain in C-terminal variable region to prevent the interaction between 14-3-3s and SPS with the exception of TFT1 and TFT10. From these comprehensive analyses, we clearly deduced that TFT1 and TFT10 are those most likely to interact with SPS in tomato.

Key Words: Tomato; 14-3-3s; SPS; Protein interaction; Prediction
Abbreviations: 14-3-3s-14-3-3 proteins, SPS-sucrose phosphate synthase,SPSA-A isoform of tobacco SPS

INTRODUCTION

The 14-3-3s (a family of regulatory proteins) are phosphoserine-binding proteins that play important roles in a variety of biological processes(Aitken, 2006). The 14-3-3s are acidic in nature with molecular weight of approximately 30 kD, which exist primarily as homo-or hetero-dimer within all eukaryotic cells (Isobe *et al*., 1991; Yaffe, 2002). Moore and Perez (1967) were the first to isolate these proteins from bovine brain (Moore & Perez, 1967). Later these proteins were isolated from a variety of eukaryotes, successively. Up to 1992 (Chevalier *et al*., 2009), 14-3-3s have been identified from plants, such as Arabidopsis (*Arabidopsis thaliana*), barley (*Hordeum vulgare*), maize (*Zea mays*), spinach (*Spinacia oleracea*), evening primrose (*Oenothera hookeri*), potato (*Solanum tuberosum*), tomato (*So. lycopersicum*) and so on (Brandt *et al*., 1992; Hirsch *et al*., 1992; Lu *et al*., 1992; Roberts & Bowles, 1999; Rosenquist *et al*., 2001; Chen *et al*., 2006; Yao *et al*., 2007; Wang *et al*., 2008). So far, researchers have discovered 13, 11 and 12 isoforms of 14-3-3s in Arabidopsis,tobacco (*Nicotiana tabacum*) (lancs, 2010) and tomato (Xu & Shi, 2006), respectively.

These proteins function by interacting directly with numerous target proteins and thereby altering their activity, 14-3-3s can directly alter protein activity (either positively or negatively), control nuclear-cytoplasmic shuttling, mediate protein import into mitochondria and chloroplasts, modulate the stress response, form a scaffold to permit interactions between two different binding proteins and so on(Van Hemert *Et Al*., 2001; Muniz Garcia *et al*., 2011). The target proteins interacting with 14-3-3s are various in different cells. In cancer cells (Pozuelo Rubio *et al*., 2004), there are more than 200 kinds of target proteins;in barely leaves, 100 and 50 (Schoonheim *et al*., 2007).

However, in tomato, different isoform (TFT1-TFT12) appears to interact with different target proteins and perform various function. Oh *et al.* (2010) discovered that TFT7 can bind to the C-terminal region of MAPKKKα and positively regulates immunity-associated programmed cell death by enhancing protein abundance and signaling ability (Oh *et al*., 2010; Oh & Martin, 2011). Kim *et al.* (2009) reported that TFT1\TFT3\TFT5\TFT6 interact with HopM1 and XopN of tomato bacterial scab and have functional roles with modulating disease resistance (Kim *et al*., 2009). There is evidence that 14-3-3s can combine with SPS and regulate

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its activity (Toroser *et al*., 1998; Cotelle *et al*., 2000). SPS is one of the key enzymes that regulates carbon assimilation and allocation in the plant and the activity level of SPS affects the synthesis of sucrose and the distribution of carbon between starch and sugar (Baxter *et al.*, 2003). SPS not only controls the carbon metabolism in tomato leaves, but also regulates the ability of the composition and sugar content of fruits (Sun *et al*., 2011). In tobacco, Bornke (2005) screened two 14-3-3s (namely T14-3d, T14-3 g) that could interact with SPSA, using a yeast two-hybrid system. But in tomato, any interaction between SPS and 14-3-3s has not been reported, while studies on 14-3-3s around metabolic pathways have some evidences. Through examining the physical-chemical properties, 3-D structures, sequence similarity and search of auto-inhibitory domain using bioinformatics soft wares, we predicted the possible isoforms of 14-3-3s that might interact with SPS (EC2.4.1.14) in tomato, which could provide the theoretical basis for metabolic interactions of 14-3-3s and SPS.

MATERIALS AND METHODS

Major databases and tools: Major databases and tools were listed in Table I.

Data search: From the EXPASY (Expert Protein Analysis System) UniProtKB database(updated June 2010) of the Swiss Institute of bioinformatics, 10 tomato 14-3-3 protein sequences, 1SPS sequence and 2 tobacco 14-3-3s sequences (T14-3 g, T14-3d; Table II), 1SPSA sequence were downloaded. Sequences of TFT11 and TFT12 were not published in the UniProtKB database and NCBI, they were downloaded from the University of Lancaster website(Lancaster, 2010).

Physical and chemical properties of tomato 14-3-3s: Results presented in Table III showed Physical and chemical properties of 12 tomato 14-3-3 isoforms predicted by Prot Param.

Amino acid-sequence-similarity analysis: T14-3 g and T14-3d in tobacco were used as local library and tomato 14-3-3s as query sequences to compare their similarity by LOCAL BLAST. Simultaneously, phylogenetic tree was constructed by MEGA4.0, and the local database was built with tobacco SPS sequence, to compare tomato and tobacco SPS sequence similarity using LOCAL BLAST.

Three-dimensional structure of the candidate protein sequences: Using TFT1 as a probe to query the Protein Data Bank (PDB) Database with Blastp at NCBI website, the template sequence of TFT1 was downloaded. Then TFT1 was used as target sequence to make multiple sequence alignment with template sequence. The multiple-sequence alignment file was uploaded to the Swiss-Model (Schwede *et al*., 2000; Schwede *et al*., 2003; Arnold *et al*., 2006) for three-dimensional structure modeling using the alignment mode of the Swiss-Model. For quality inspection, the native ANOLEA atomic average potential energy of the Swiss-Model web interface was used. The modeling results were downloaded to a local disk and the 3-D structure of the protein was viewed and edited with the RasMol software. Similar treatments were delivered to TFT10, T14-3d, T14-3 g, SPSA and SPS.

RESULTS AND DISCUSSION

Physical and chemical properties of 14-3-3s in tomato: In Table III. The physical and chemical properties of 14-3-3s in tomato were shown. The isoelectric point (PI) of tomato 14-3-3s ranged from 4.63 (TFT8) to 4.96(TFT7), indicating that 14-3-3s have been discovered currently in tomato were acidic protein. The relative molecular weight of these proteins ranged from 28.2 kD (TFT1) to 32.2 kD (TFT12) and the average molecular weight was 29.3 kD, this is consistent with the assumption that 14-3-3s are acidic protein whose average molecular weight is about 30 kD (Isobe *et al*., 1991; Yaffe, 2002). The grand average of hydropathicity (GRAVY) of tomato 14-3-3s ranged from -0.701 (TFT12) to -0.269 (TFT1), indicating that tomato 14-3-3s are all hydrophilic protein.

Aliphatic index is an indicator reflecting the thermal stability of a protein. The aliphatic index of tomato 14-3-3s was all greater than 70, indicating that they have relatively high thermal stability. Lu *et al.* (1998) compared the amino acid composition between thermophilic and mesophilic microbial protein, and reported that the content of leucine (Leu) and glutamic acid (Glu) in thermophilic bacteria were all higher than that in bacteria that exist at normal temperature(Lu *et al*., 1998). This is mainly due to the fact that Leu has stronger and larger hydrophobic side chain and Glu has stronger side chain than other amino acids with the same charge. These facts indicate that the high content of Leu and Glu can enhance the thermal stability of protein. The analysis of amino acid composition in tomato 14-3-3s showed that the content of glutamic acid (Glu) content was the highest for all tomato 14-3-3s with the exception of TFT10, in which leucine (Leu) was the highest (13.1%), the results further confirmed that the high content of Leu and Glu played an important role in enhancing the thermal stability of the protein.

Amino acid-sequence-similarity analysis: In Table Ⅳ, results of sequence alignment by BLAST were presented. TFT1 had the highest similarity to T14-3d and TFT10 to T14-3 g. Similarities between the remaining tomato 14-3-3s and T14-3d, T14-3 g were ranged from 60% to 80%, respectively. It indicated that tomato 14-3-3s may be homologous to T14-3d and T14-3 g. The sequence similarity between tomato and tobacco SPS was 93%, indicating a high degree of similarity and homology. Bornke (2005) confirmed that T14-3d, T14-3 g can interact with SPS. Based on initial speculation, all tomato 14-3-3s may interact with SPS.

The phylogenetic tree (Fig. 1) showed that TFT1\TFT10\T14-3d\T14-3 g were on the same branch. This fact clearly indicated that TFT1 and T14-3d, TFT10

Table I: Major databases and tools

Databases or tools	Websites	Functions
UniProtKB	http://www.expasy.org/	Obtain protein sequences
ProtParam	http://www.expasy.org/	Predict the physical and chemical properties of proteins
Swiss-Model	http://swissmodel.expasy.org/	three-dimensional structure modeling
MEGA4.0	http://www.megasoftware.net/	Construct phylogenetic tree
Blast+	ftp://ftp.ncbi.nlm.nih.gov/blast	Compare similarity among proteins
RasMol	http://rasmol.org/	Edit 3D structure
Blast online	http://blast.ncbi.nlm.nih.gov/Blast.cgi	Data retrieval
DNAMAN	http://www.lynnon.com/	Multiple sequence alignment
NCBI	http://www.ncbi.nlm.nih.gov/	Data retrieval
Prosite	http://www.expasy.ch/prosite/	Motif retrival

Table II: Accession number of protein sequence in UniProtKB

Tobacco		Tomato	
Name of proteins	Accession number	Name of proteins	Accession number
T14-3d	Q5KTN4	TFT1	P93206
T14-3g	Q947K7	TFT2	P93208
SPSA	Q9SNY7	TFT3	P93209
		TFT4	P42652
		TFT5	P93210
		TFT6	P93211
		TFT7	P93212
		TFT8	P93213
		TFT9	P93214
		TFT10	P93207
		TFT11	—
		TFT12	—
		SPS	Q9FXK8

and T14-3 g were 2 groups of orthologous genes. Thus TFT1 and TFT10 are most likely to interact with SPS. Other isoforms of tomato 14-3-3s are on different branches and distant on genetic relationship with T14-3d and T14-3 g. The high similarity between other tomato14-3-3s and T14-3d, T14-3 g may be owed to they are belonged to the same protein family.

Auto-inhibitory domain search of tomato 14-3-3s: The C-terminal variable region in tomato 14-3-3s were compared with those of T14-3d and T14-3 g,the length of C-terminal amino acid residues of TFT1 and T14-3d, TFT10 and T14-3 g were identical respectively, while other tomato 14-3-3s were longer than those of T14-3d and T14-3 g (Fig. 2). It is of note that the study of Bornke (2005) reported that the C-terminal variable region of tobacco 14-3-3s has an auto-inhibitory function, thus preventing the interaction with SPS in yeast. Comparisons of T14-3d, T14-3 g and the remaining 14-3-3s of tobacco showed that the length of C-terminal end of the remaining 14-3-3s in tobacco was longer than T14-3d and T14-3 g. Certain insertion sequence (autoinhibitory domain) that exist here inhibit the interaction between 14-3-3s and SPSA. However, when this insertion sequence of T14-3c which is an isoform of tobacco 14-3-3s, was deleted, it interacted with SPSA. This indicated that the insertion sequence can inhibit the interaction between tobacco 14-3-3s and SPSA in yeast.

The results indicated that the C-terminal variable region of TFT1 and TFT10 do not have an autoinhibitory domain, which prevents the interaction between TFT1, TFT10 and SPS in tomato.

The Three-dimensional Structure Prediction of Candidate Sequences

Structure modeling of 14-3-3s: A total of 23,23,20,24 sequences were matched to TFT1\TFT10\T14-3d\ T14-3 g, respectively following BLAST in the PDB library, in which the best matching template sequence was the same one (PDB accession number: 1O9CA). With the exception of 75% identity between T14-3 g and 1O9CA, the other three had 77% identities. By aligning the template sequence 1O9CA with TFT1\TFT10\T14-3d\T14-3 g, respectively the multiple sequence-alignment files were obtained. Then, the multiple sequence-alignment file was uploaded to the Swiss-Model and the three-dimensional structure was modeled with the Alignment Mode of the Swiss-Model. Meanwhile, the quality of the 3D structure was checked. Results were presented in Fig. 3A-D.

The fundamental structure of 14-3-3s is a central, highly conserved core that is flanked by variable termini. Fourteen-three-three proteins form a homo-or hetero-dimer, with each subunit consisting of a little more than nine α-helices in an antiparallel arrangement. This arrangement creates a groove large enough to house a random-coil peptide of the target protein(Chevalier *et al*., 2009). The predicted results of 3D structure indicated that TFT1, TFT10, T14-3d and T14-3 g all could create grooves, suggesting that the former two can identify and combine with the phosphorylated target proteins.

Structure modeling of SPS: With blast in PDB library, the best matching template sequence of SPS and SPSA was the same one (PDB accession number: 2R60A). The similarities between each one of SPSA, SPS and 2R60A were 32%, 31%, respectively. The procession of simulating the 3D structure was the same to above mentioned procession. Results were presented in Fig. 3E-F. The three-dimensional structure of SPS is very similar to the one of SPSA.

The interaction between 14-3-3s and the target proteins, which are phosphorylated by kinase changes the conformation of target proteins, thereby affecting their function (Chevalier *et al*., 2009). There are many methods, such as yeast two-hybrid, co-immunoprecipitation and so on, for determining the interaction between two proteins. If there are more than one objective proteins that need to be screened for interaction with a certain protein, it appears particularly important to predict the possible results using bioinformatics tools, and then using experimental methods to verify the prediction. We predicted the interaction between tomato 14-3-3s and SPS based on Bornke's yeast two-hybrid result (Bornke, 2005) and our results indicated

Table III: Physical and chemical properties of tomato 14-3-3 protein family

Name of protein	Sequence length of amino acids	Molecular weight（kD）	Theoretical pI	Molecular formula	Grand average of hydrophobicity	The highest content of amino acid(%)	Aliphatic index
TFT1	249	28.2	4.76	$C_{1248}H_{1985}N_{323}O_{393}S_{13}$	-0.269	12.0	92.53
TFT 2	254	28.9	4.72	$C_{1257}H_{1997}N_{341}O_{417}S_{10}$	-0.591	12.6	82.68
TFT 3	260	29.3	4.78	$C_{1282}H_{2040}N_{346}O_{420}S_{9}$	-0.563	13.1	83.36
TFT 4	260	29.3	4.66	$C_{1284}H_{2042}N_{344}O_{426}S_{7}$	-0.517	13.1	87.54
TFT 5	255	28.8	4.76	$C_{1260}H_{2001}N_{339}O_{413}S_{9}$	-0.525	12.2	84.27
TFT 6	258	29.0	4.70	$C_{1266}H_{2009}N_{341}O_{417}S_{9}$	-0.508	12.4	83.72
TFT 7	252	28.8	4.96	$C_{1258}H_{1994}N_{346}O_{412}S_{8}$	-0.659	13.1	79.80
TFT 8	261	29.5	4.63	$C_{1286}H_{2038}N_{348}O_{426}S_{9}$	-0.531	11.1	84.87
TFT 9	261	29.4	4.76	$C_{1282}H_{2025}N_{349}O_{422}S_{11}$	-0.538	11.9	80.80
TFT 10	252	28.6	4.80	$C_{1265}H_{2012}N_{332}O_{402}S_{10}$	-0.356	13.1	92.58
TFT 11	258	29.1	4.69	$C_{1273}H_{2022}N_{344}O_{420}S_{8}$	-0.492	12.8	86.71
TFT 12	285	32.2	4.94	$C_{1404}H_{2241}N_{377}O_{467}S_{10}$	-0.701	13.3	74.70

Note: The highest-content amino acid of TFT10 was leucine (Leu), glutamic acid (Glu) was the the highest-content amino acid for other proteins

Table IV: Comparative analysis of tomato and tobacco 14-3-3s (%)

	TFT1	TFT2	TFT3	TFT4	TFT5	TFT6	TFT7	TFT8	TFT9	TFT10	TFT11	TFT12
T14-3d	95	78	77	76	75	76	66	63	68	83	76	67
T14-3g	82	76	76	75	75	76	67	60	66	94	76	67

that TFT1 and TFT10 are most likely to interact with SPS in tomato.

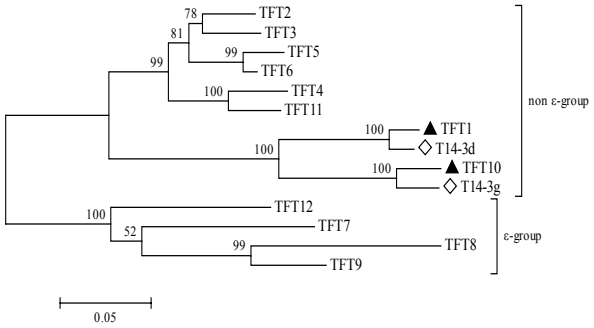
Results from previous studies indicated that SPS is phosphorylated by Ser\Thr protein kinase and that this inhibits the activity of SPS in the dark, however SPS can dephosphorylate and recover activity by protein phosphatase 2 (PP2A) (Huber & Huber, 1996) under illumination, indicating that SPS can exercise certain abilities at high temperature. The thermal stability of TFT1 and TFT10 is the highest among tomato 14-3-3s, indicating that TFT1 and TFT10 can interact with SPS and regulate its activity at high temperature.

The spatial structure of a protein determines its function. The predicted three-dimensional structure showed that the spatial structure of TFT1 and TFT10 are very similar to T14-3d and T14-3 g and thus they should have similar function. Additionally, 14-3-3s recognize the consensus sequences R(S/Ar)XpSXP and RX(Ar/S)XpSXP, in which pS denotes pSer/Thr and Ar denotes an aromatic residue(Yaffe & Elia, 2001). A peptide motif RQVpSAP surrounding Ser-229 from spinach (Toroser *et al*., 1998) have been confirmed. Although there are three SPS isoforms, namely A, B, C, we can determine that isoform A (SPSA)is the one that studied by Bornke (Bornke, 2005), whose results indicated that motifs (RQVpSSP surrounding Ser-221, RFFpSNP surrounding Ser-470) of SPSA are the active sites to interact with T14-3d and T14-3 g in tobacco. Comparison of SPSA and SPS of tomato showed that there are two motifs as same as those of SPS of tobacco, one is RQVSSP located 218-223, the other is RFFSNP located 467-470. These facts imply that SPS of tomato have the similar active sites, which to interact with 14-3-3s.

Therefore, it was easy to deduce that TFT1 and TFT10 interact with SPS. Furthermore, tomato 14-3-3 proteins belonged to non-ε group and ε-like group on the phylogenetic tree, this is consistent with the results reported

Fig. 1: Phylogenetic tree of 14-3-3 proteins, T14-3d and T14-3 g, in tomato

The tree was generated using MEGA4.0 with neighbor-joining method. Branch numbers represent percentages of bootstrap values in 1000 re-sampling replicates



by (Roberts & De Bruxelles, 2002) and (Xu & Shi, 2006). Additionally, TFT1, T14- 3d, TFT10 and T14-3 g all belonged to the non-ε-like group, TFT1 and T14-3d, TFT10 and T14-3 g were on the same branch, respectively and they were 2 groups of orthologous genes and would have similar functions, which supports the above mentioned speculation.

However, the interaction between tobacco T14-3d\T14-3 g and SPS is only confirmed by *in vitro* studies and no *in vivo* have been reported so far. Further research is required to verify whether TFT1 and TFT10 interact with SPS, the difference of interactions and the mechanism of such interactions.

Based on the absence of auto-inhibitory domain and similar 3D structure between TFT1\TFT10 and T14-3d\T14-3 g, we could concluded that TFT1\TFT10 are most likely to interact with SPS in tomato, thus supporting the above mentioned assumption.

Fig. 2: The predicted three-dimensional structure of Candidate protein sequences

These three-dimensional structures of proteins (A-TFT1; B-T14-3d; C-TFT10; D-T14-3 g; E-SPSA; F-SPS) were simulated using Swiss-Model with homology modeling method.Grooves is a conserved structure to identify the phosphorylated target proteins for 14-3-3 proteins. The spacefill structures represent the active sites of SPSA\SPS,of those the phosphorylation site is the serine residue (ser)

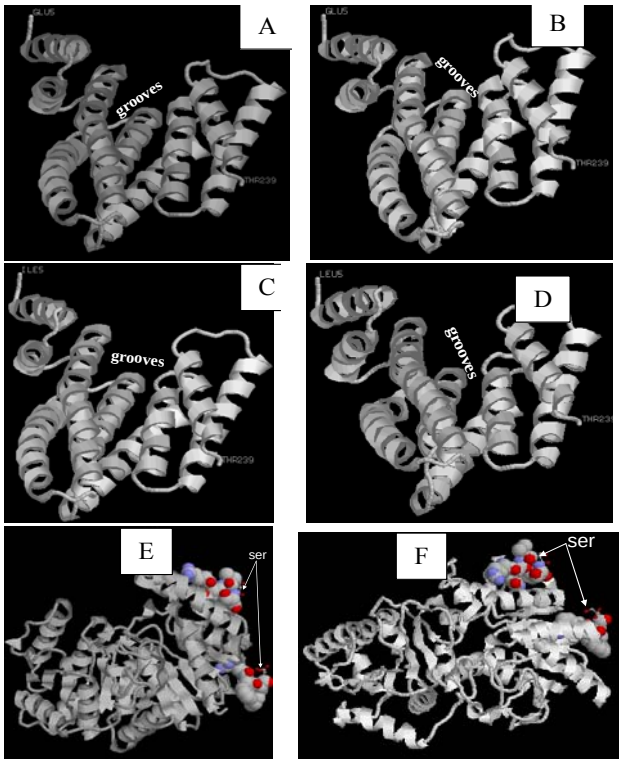


Fig. 3: Amino acid sequence of the variable C-terminus of different 14-3-3 isoforms

CONCLUSION

TFT1 and TFT10 are most likely to interact with SPS in tomato.

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