Computational Screening of microalgae and cyanobacteria RuBisCO as poteinal precursor for bioactive peptides

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ABSTRACT

Ribulose-1,5-bisphosphate carboxylase/oxygense (RuBisCO) is present in plants and autotrophic organisms like microalgae. The aim of this study was to perform an *in silico* evaluation of RuBisCo protein in microalgae and cyanobacteria as potential precursors of bioactive peptides, as well as to determine whether such peptides can be released by selected proteolytic enzymes. Fourteen RuBioCo sequences of microalgae and cyanobacteria were analysed by using the BIOPEP server amd database. The biological activity, enzyme action and calculation of active peptide tools were used to determine the frequency of occurrence of fragments, proteolysis, and the frequency of release of fragments with given activity by selected enzymes. The physio-chemical parameters of the selected sequences were performed with Protpram tool. Amongst the RuBisCo proteins of selected algae, *Chaetoceros. calcitrans* exhibits the best prospect as a source of DPP-IV inhibiting peptides, *Chlorella pyrenoidosa* for ACE inhibitor and *Aphanizomenon flos-aquae* for antioxidative, activating ubiquitin, and antiamnestic activities. High number of bioactive fragments in *Aphanizomenon flos-aquae*,

Dunaliella salina, *Chlorella pyrenoidosa*, and *Chlorella vulgaris* are associated with a high content of glycine and proline amino acids that are most rich in biologically active fragments. Papain and Proteinase K, an enzyme with wide specificity, can release considerably more biologically active fragments than bromealin and chymotrpsin. These findings will contribute towards consumption of microalgal and cyanobacterial RuBisCO as alternative sources of bioactive peptide fragments based nutraceuticals for human.

Keywords: BIOPEP, *Dunaliella salina*, microalgae, peptide, Protparam, RuBisCO

INTRODUCTION

Ribulose-1,5-bisphosphate carboxylase/oxygense (RuBisCO) is heterohexadecamer structured protein (L8S8, 540 kDa) made up of eight large subunits and present in land biota including plants and autotrophic organisms like microalgae (Losh et al., 2013). It is actively involved in conversion of atmospheric carbon dioxide into organic carbon through calvin cycle (Beardall and Raven, 2013). Both large and small subunits of RuBisCO possess functionally bioactive peptides for treatment of cardiovascular diseases, diabetes, neurodegenerative disorders, and oxidative stress (Udenigwe et al., 2013). Large subunit of RuBisCO is homologically diversed in many photoshynthetic organisms like cyanophyta, chemolithoautotrophs and phototropic bacteria (Minic and Thongbam, 2011). Especially, the carboxylase enzyme is actively involved in atmospheric carbon accumulation and the oxygenase responsible for biosphere fixed carbon loss by photorespiratory pathway. However the ratio of catalytic reaction of carboxylation and oxygenation depend on the nature of the species. The activation of RuBisCO protein is maintained by RuBisCO activase a soluble protein in chloroplast (McKay et al., 1991). In particular bacillariophyceae, chloropyceae, cryptophyceae, dinophyceae, euglenophyceae and rhodophyceae exhibited RuBisCO localization in pyrenoid complex containing species *viz*., *Chlamydomonas reinhardtii*, *Chlorella pyrenoidosa*, *Coleochate scutata*, *Dunaliella salina* and *Phorphyridium cruentum*

(Mckay and Gibbs, 1989; Vladimirova et al. 1982; Borkhsenious et al., 1998; Lin and Carpenter, 1997). However, in non pyrenoid organisms, RuBisCO is distributed throughout the chloroplast region (Jordan et al., 1981). Under nitrogen deprived condition there was decreases in the RuBisCO content of green microalgae *Chlorella emersonii* and *Gloeomonas sp* (Beardall and Raven, 1990).

Bioactive peptides contain 5 to 20 amino acid residues which are active at intestinal sites and inactive within the parent protein sequences. During physiological digestion, these can be liberated by endogenous and exogenous proteases and peptidases (Bhat et al., 2015). Recently, researchers have been focused on unicellular and multicellular marine algae for bioactive peptides production due to their considerable amount of protein content ~15 to 47 % of dry weight (Ibanez and Cifuentes, 2013). Noteably, the algal biomass possess a large number of essential amino acids and non-protein coding amino acid residues. Therefore efforts have been made to isolate definite protein without any change in their molecular structure to enhance their application in pharma and fermentation process (Becker et al., 2011). Nevertheless, most microalgae posses rigid cell wall which unfacilitate the isolation of intracellular proteins. Therefore, various physical and chemical methods have been followed such as alkali agent mediated cell dissolution, extraction using various organic solvents, high pressure homogenization etc. However, there are still many challenges raised over the application of bioactive peptides before getting into clinical trials. Elaborative computational modeling is required to explore structure and function relationships of peptide fragments form microalgae. Therefore the present study aimed to identify bioactive peptides from large subunit of RuBisCO protein of *Chaetoceros calcitrans* (Paulsen) Takano, *Chlamydomonas rheinhardii* P.A.Dang., *Chlorella pyrenoidosa* H.Chick, *Chlorella vulgaris* Beyerinck (Beijerinck), *Dunaliella salina* (Dunal) Teodoresco, *Euglena gracilis* Klebs, *Haematococcus pluvialis* Flotow, *Isochrysis galbana* Parke, *Porphyridium cruentum* (S.F.Gray) Nägeli,

Spirogyra porticalis (O.F.Müller) Dumortier, *Spirulina maxima* (Setchell & N.L.Gardner) Geitler, *Spirulina platensis* (Gomont) Geitler, *Synechococcus sp.* Nägeli and *Aphanizomenon flos-aquae* Ralfs ex Bornet & Flahault through computational methods.

MATERIALS AND METHODS

Tools and softwares

The following computational tools were used in the study UniProtKB databases (http://www.uniprot.org), ExPASy portal (http://www.expasy.org/proteomics), BIOPEP (http://www.uwm.edu.pl/biochemia/index.php/en/biopep), Protparam (http://web.expasy.org/protparam/) and algal database (http://www.algaebase.org/).

RuBisCO sequences of differnt microalgae

The primary sequences of RuBisCo protein of *C. calcitrans* (K4ES21), *C. rheinhardii* (P00877), *C. pyrenoidosa* (A8TKR2), *C. vulgaris* (P12466), *D. salina* (D0FXZ7), *E. gracilis* (P00878), *H. pluvialis* (B7U6F7), *I. galbana* (Q9GFX7), *P.cruentum* (W0RYV8), *S. porticalis* (Q85X88), *S. maxima* (B5VXI0), *S. platensis* (D4ZVW7), Synechococcus sp. (P00880), *A. flos-aquae* (Q934E6) and for reference *O. sativa* (P0C510), *T. aestivum* (P08823), *Z. mays* (P00874), *G.max* (P13917), *G.gallus* (P01012), *B. taurus* (P02666) were obtained from UniProtKB database and used for further analysis.

Determination of frequcency of bioactive peptides in RuBisco

The frequency of occurrence of bioactive peptide fragments in the sequence (A) of microalgal and cyanobacterial RuBisco were calculated using the BIOPEP server. A = a/N where “a” indicates the number of bioactive peptide fragments within a protein sequence and N is the total number of amino acid residues in the protein (Dziuba et al., 1999; Dziuba et al., 2003; Udenigwe et al., 2013). Bioactive peptide fragments of each protein were determined by their features as ACE inhibitor, ubiquitin activation, antiamnestic, antioxidative, antithrombotic and DPP IV inhibitor. Bioactivity, values of A for different microalgae

RuBisCo proteins were compared to values for commonly consumed food protein sources of *Oryza sativa*, *Triticum aestivum*, *Zea mays*, *Glycine max*, *Gallus gallus* and *Bos taurus*.

***In silico* Proteolysis**

The proteolysis of RuBisCo protein of selected microalgae species was conducted by using the BIOPEP web server on enzyme action tool (Dziuba et al., 1999; Minkiewicz et al., 2011). There are more than fifteen proteolytic enzymes available in this server. Among them, food processing enzymes such as bromelain, chymotrypsin, Proteinase K, and papain enzymes were selected for this study, because of their unique cleavage patterns. Two parameters were determined: 1) the frequency of release of fragments with selected activity by specific enzyme (A_E), which was calculated as follows $A_E = d/N$

Where d is the number of fragments with given activity in the protein sequence, that could be released by enzyme and N is the number of amino acid residuces in the protein chain. 2) the relative frequency of release of fragments with given activity by the selected enzyme (W), which was calculated as follows $W = A_E/A$

Where, A_E is the frequency of release of fragments with selected activity by specific enzyme and A is the frequency of occourance of bioactive fragments in the protein chain.

Determination of physio-chemical parameters of the selected sequences

Amino acid composition of proteins were determined by using the ProtParam program based on the RuBisCO sequence of selected microalgae and cyanobacteria (Gasteiger et al., 2005).

RESULTS

Frequency of occurrence (A) of bioactive peptide fragments in selected RuBisCO protein

RuBisCO subunits are estimated rich in microalgae and cyanobacteria that are not frequently consumed as primary human food. Cultivation of microalgae in developing countries is a considerable policy to support rural economies. There is increasing growth of underutilized algal biomass that can readily be used in human food system as a source of RuBisCO.

Sequence alignment of the microalgal RuBisCO subunits in UniProt indicated that they have highly homologous regions in their sequences with 80 % similarity among the selected algal species. However, the cyanobacteria *A. flosaquae* protein has less conserved regions in its sequences with 6.57 % similarity among the selected algae. This difference can direct to prominent variations in their respective peptide profiles depending on location of occurrence. Table 1 shows the frequency of occurrence of the bioactive peptides within microalgal and cyanobacteril RuBisCO subunits for the ACE inhibitor, activating ubiquitin, antiamnestic, antioxidative, and antithrombotic and DPP IV inhibitory activities. Most bioactive peptides in the RuBisCo subunits were defined as DPP-IV inhibitors, and had DPP-IV inhibiting value of A which is similar to that of cereal plants but lower than bovine milk protein, which has the highest value of A compared to other proteins of microalgae and cereal plants. In addition, the frequencies of occurrence of DPP-IV inhibiting peptides in the microalgae and cyanobacteria were predominantly same, which was unusual considering their highly homologous primary structures. Conversely, RuBisCO subunits had remarkable variations in their values of A with *C. calcitrans* exhibiting the best prospects as sources of DPP-IV inhibiting peptides (Supplementary Information 1), and *Chlorella pyrenoidosa* for ACE inhibitor (Supplementary Information 2) whereas *A. flosaquae* showed the lowest value of A, while it is comparable with A values for other microalgae and cereal plant proteins. The RuBisCO sequences of *A. flos-aquae* showed highest A value on antioxidative, activating ubiquitin, and antiamnestic frequencies of peptide fragements when compared to other microalgae and cereal crops. The antioxidant peptide sequences of *A. flos-aquae* was shown in Supplementary Information 3.

Proteolysis of RuBisCo for producing ACE and DPP-IV inhibitory peptides

Table 2 and 3 demonstrate the values of factor describing the predicted efficiency of release of DPP-IV inhibitor and ACE inhibitor peptide fragments from microalgal and cyanobacterial RuBisCo proteins. These two activities were selected as models since fragments showing

these activity peptides are most rich in the sequences of all proteins. The possibility for the release of bioactive peptide fragments from protein depends on two factors: the number of bioactive peptide fragments in the protein sequence and the precision of the enzyme used. A high number of peptide fragment does not mean that they will be liberated. Factor A_E estimates the predicted potential for the release of bioactive peptides with given activity from a single protein. It may be used for comparing the potential of various enzymes for the hydrolysis of the same substrate or of a single enzyme for the hydrolysis of various proteins. Factor W estimates the ratio of fragments with given activity released from a single protein by a single enzyme. Mapping of *Aphanizomenon flos-aquae* RuBisCO showed the presence of more than 20 bioactive peptide fragments in its sequences stimulated by the selective proteolytic enzymes (Fig. 1).

Physio-chemical parameters of the selected sequences

ProtParam program was used to calculate the physio-chemical properties such as the absorbance coefficient, aliphatic index, Grand average hydropathy (GRAVY) index, isoelectric point, *in vivo* half-life, instability index, and molecular weight of selected RuBisCo sequences. As shown in Table 4, the RuBisCo sequences of *C. reinhardtii*, *C. pyrenoidosa*, *C. vulgaris*, *D. salina*, *E. gracilis*, *H. pluvialis*, *Spirogyra* sp. and *A. flos-aquae* have exceptionally high content of the amino acids, glycine and proline. The incidence of bioactive fragments in RuBisCo sequences is related with the content of glycine and proline. The aliphatic index indicates the amount of alanine, valine, isoleucine, and leucine occupied in the RuBisCo sequences of selected microalgae. All the selected sequences represent thermo-stability of RuBisCo proteins. The GRAVY value for a peptide of RuBisCo protein indicates the sum of hydropathy values of all the amino acids, divided by the number of residues in the sequence.

DISCUSSION

Cultivation of microalgae is a major strategy for strengthening rural economies and achieving growth in the global economy. There is an increasing accumulation of biomass that can readily be utilized in the production of food products and therapeutic agents from the source of metabolic proteins. DPP-IV (E.C. 3.4.14.5) is responsible for conversion of glucose tolerance (GLP-1), into inactive form. The inhibition of DPP-IV would be beneficial in the treatment of diabetes mellitus. DPP-IV inhibiting five different peptides Trp-Lys, Trp-Arg, Trp-Leu and Ile-Pro-Ile-Gln-Tyr was isolated from *Amaranthus hypochondriacus*, *Hordeum vulgare*, *Zea mays*, *Chenopodium quinoa*, *Avena sativa*, *Oryza sativa* subsp. Japonica, *Sorghum vulgare*, *Triticum aestivum* large subunit of RuBisCO (Nongonierma and 2015). Results from *in silico* analysis specified that some other RuBisCO sequences of microalgae can inhibit DPP-IV activity. Thus, utilization of microalgae and cyanobacterial RuBisCO will offer alternative resources of producing DPP-IV inhibitory peptides, thereby reducing serious stress on costly protein precursors, e.g. dairy milk, which leftovers a popular raw material for the production of the DPP-IV inhibitory peptides. Angiotensin-converting enzyme (EC 3.4.15.1) indirectly increases blood pressure by converting angiotensin I to angiotensin II, which tighten the blood vessels. It is secreted in the lungs and kidneys by cells in the endothelium of blood vessels (Kierszenbaum, 2007). ACE inhibiting four different peptides MRWRD, MRW, LRIPVA, and IAYKPAG were isolated from spinach RuBisCO (Yang et al., 2003). High frequency of occurance of ACE inhibiting peptides in microalgal and cyanobacterial RuBisCo sequences provide a promising platform for sustainable production of potent ACE inhibiory peptide based anti-hypersenstive drugs.

Reactive oxygen species (ROS) are obvious metabolic derivatives that can attack macromolecules such as nucleic acids, proteins and lipid membrane leading to many health disorders (Moskovitz et al., 2002). Antioxidants may have a constructive result on human diseases as they can reduce metabolic damages caused by ROS. Proteins can inhibit lipid

oxidation by the mechanism of antioxidant enzymes and iron-binding proteins. Proteins also have excellent potential as antioxidant additives in foods. The overall antioxidant activity of a protein can be increased by disruption of its tertiary structure to increase the accessibility of amino acid residues that can chelate pro-oxidative metals and free radicals (Elias et al., 2008). The production of antioxidant peptides through hydrolytic reactions seem to be the most promising method, because peptides have significantly higher antioxidant activity than intact proteins. In recent years, peptides from many sources such as meat, food crops, and milk protein have been found to possess antioxidant activity (Kim et al., 2007; Qin et al., 2011).

Metabolic enzymes in human body, that regulate all the metabolites from liver function to the immune system, are mostly proteolytic enzymes. Proteolytic enzymes are facilitating the chemical breakdown of proteins by the hydrolysis. There are six different types of proteolytic enzymes (aspartate protease, cysteine protease, glutamic acid protease, metalloproteases, serine protease and threonine protease) whch are classified according to sites in which they catalyze the cleavage of proteins (Thomas, 1993). Serine proteases (chymotrypsin and Proteinase K) are responsible for managing different physiological roles including blood coagulation, digestion, immune response, inflammation, and reproduction (Hedstrom, 2002). In humans, cysteine proteases (bromelain and papain) are responsible for cell aging and cell death by attacking collagen and elastin at sites of inflammation (Chapman et al., 1997). Thereby, in the present study four different proteases; bromelain, chymotrypsin, papain and Proteinase K were chosen for proteolytic cleavage. These four enzymes hydrolyse bonds formed by the carboxyl groups of arginin, alanine, glycine, isoleucin, leucin, methionine, phenylalanine, proline tyrosine, tryptophan and valine (Barrett et al., 2012). The A_E value in the proteolytic cleavage indicates the specificity of the enzyme suitable for the release of peptides with C-terminal proline in *D. salina*, *E. gracilis*, *P. cruentum*, *C. vulgaris* and *Synechococcus sp.* RuBisCo protein. Conversely, W indicates that the consistency with the

type of amino acid residues forming C-terminal and flanking fragments with DPP-IV and ACE inhibitor activity. The potential and impediments of *in silico* examination in predicting the chance of flow of bioactive peptides were reported by Vercruysse et al. (2009). The competence of *in silico* studies may be reduced by following factors such as deficient peptide databases and complex specificity of the creating molecule, could not possible to perform computational analysis. The association of hydrolysates from identical substrates utilizing identical chemical may differ when response conditions are modified (Cheung et al., 2009). In perspective of the over, a procedure study may be used effectively for fast screening of protein sequences to discover the potential impact of chosen fragments and release them.

CONCLUSIONS

In silico analysis of microalgal and cyanobacterial RuBisCO provides strong projection as pioneer of bioactive peptide fragments when compared to commonly consumed food proteins of cereal crops, except for milk proteins. The prospects square measure is fairly sturdy considering that the property and natural wealth of RuBisCO in algae can scale back the serious dependence on expensive characteristic food proteins and promote future food security. Moreover, the frequency of bioactive peptide fragments increases in the free form during the chemical action compared to frequency in the protein precursor. Papain and proteinase K exhibited the simplest potential for emotional bioactive peptides from RuBisCO. These results can promote new insights into the employment of underutilized biomass from microalge and cyanobacteria as sources of RuBisCO for production of peptide-based nutraceuticals for human consumption.

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Table 1. The frequency of occurrence (A) of bioactive peptide fragments in microalgae and cyanobacterial RuBisCO protein

Name of the species	ACE inhibitor	ubiquitin-med.prot.	antiamnestic	antioxidative	antithrombotic dipeptidyl peptidase IV inhibitor	hypotensive	Immuno modulating	Immuno stimulating	Neuropeptide
Chaetoceros calcitrans	0.3909	0.0151	0.0086	0.0756	0.0086	0.6523	***	0.0065	***	0.0065
Chlamydomonas rheinhardii	0.4403	0.0189	0.0084	0.0776	0.0084	0.6247	0.0063	0.0042	0.0021	0.0042
Chlorella pyrenoidosa	0.4423	0.0231	0.0105	0.0776	0.0105	0.6394	0.0063	0.0042	***	0.0042
Chlorella vulgaris	0.4382	0.0189	0.0105	0.0776	0.0105	0.6289	0.0063	0.0042	***	0.0042
Dunaliella salina	0.4214	0.0189	0.0105	0.0776	0.0105	0.6268	0.0063	0.0042	0.0021	0.0042
Euglena gracilis	0.4319	0.0168	0.0084	0.0797	0.0084	0.6415	0.0063	0.0042	***	0.0042
Haematococcus pluvialis	0.4163	0.0209	0.0105	0.0795	0.0105	0.6423	0.0021	0.0042	0.0021	0.0042
Isochrysis galbana	0.3666	0.0174	0.0108	0.0694	0.0108	0.6269	0.0022	0.0022	0.0043	0.0022
Porphyridium cruentum	0.3694	0.0143	0.0082	0.0633	0.0082	0.6449	0.002	0.002	0.0041	0.002
Spirogyra sp.	0.4349	0.0177	0.0088	0.0684	0.0088	0.6291	***	0.0044	***	0.0044
Spirulina maxima	0.4111	0.0167	0.0105	0.0753	0.0105	0.6234	0.0084	0.0042	0.0021	0.0042
Spirulina platensis	0.4121	0.0167	0.0105	0.0753	0.0105	0.6213	0.0084	0.0042	0.0021	0.0042
Synechococcus sp.	0.4114	0.0169	0.0084	0.0738	0.0084	0.6203	0.0105	0.0042	***	0.0021
Aphanizomenon flos-aquae	0.4118	0.0235	0.0118	0.0941	0.0118	0.5529	0.0353	***	***	***
Oryza sativa	0.4322	0.0209	0.0084	0.0711	0.0084	0.6211	0.0063	0.0042	***	0.0042
Triticum aestivum	0.3891	0.0147	0.0055	0.0367	0.0092	0.6477	0.0037	0.0018	0.0055	0.0018
Zea mays	0.4331	0.0167	0.0084	0.0607	0.0084	0.6131	0.0021	0.0042	***	0.0042
Glycine max	0.3521	0.0186	0.0093	0.0443	0.0093	0.6551	0.0023	0.0023	0.0023	***
Gallus gallus	0.3582	0.0077	0.0026	0.0696	0.0026	0.6134	0.0077	***	***	0.0077
Bos taurus	0.6018	0.0133	0.0442	0.0841	0.0265	0.8053	0.0044	0.0177	0.0044	0.0133

Note: *** indicates there is no database information

Table 2. The values of parameters describing the predicted efficiency of release of bioactive fragments from microalgae and cyanobacterial RuBisCO protein by four proteinases for angiotensin-converting-enzyme (ACE) inhibitor

Name of the species	Bromelain		Proteinase K		Chymotrypsin		Papain	
	A_E	W	A_E	W	A_E	W	A_E	W
C. calcitrans	0.0252	0.0679	0.0377	0.1016	0.0314	0.0846	0.0314	0.0846
C. reinhardtii	0.0304	0.0731	0.0365	0.0828	0.0345	0.0830	0.0426	0.1025
C. pyrenoidosa	0.0325	0.0774	0.0346	0.0821	0.0365	0.0869	0.0385	0.0917
C.vulgaris	0.0305	0.0731	0.0367	0.0879	0.0345	0.0830	0.0385	0.0926
D. salina	0.0326	0.0817	0.0346	0.0867	0.0346	0.0867	0.0387	0.0969
E. gracilis	0.0325	0.0793	0.0326	0.0792	0.0401	0.0989	0.0448	0.1089
H. pluvialis	0.0283	0.0721	0.0283	0.0721	0.0305	0.0774	0.0407	0.1032
I.galbana	0.0294	0.0840	0.0335	0.0957	0.0252	0.0720	0.0294	0.0840
P. cruentum	0.0256	0.0739	0.0374	0.1079	0.0257	0.0739	0.0296	0.0851
Spirogyra sp.	0.0299	0.0734	0.0385	0.0941	0.0299	0.0734	0.0384	0.0943
S.maxima	0.0203	0.0526	0.0344	0.0894	0.0263	0.0684	0.0325	0.0842
S. platensis	0.0205	0.0524	0.0367	0.0942	0.0265	0.0680	0.0367	0.0942
Synechococcus sp.	0.0230	0.0606	0.0115	0.0303	0.0113	0.0302	0.0345	0.0910
A. flos-aquae	0.0242	0.0590	0.0343	0.0836	0.0263	0.0664	0.0304	0.0738
O. sativa	0.0284	0.0780	0.0265	0.0730	0.0336	0.0926	0.0460	0.1268
T. aestivum	0.0202	0.0492	0.0344	0.0837	0.0324	0.0789	0.0304	0.0740
Z. mays	0.0113	0.0336	0.0315	0.0941	0.0180	0.0538	0.0180	0.0538
G. max	0.0199	0.0611	0.0250	0..0763	0.0174	0.0534	0.0299	0.0593
G.gallus	0.0256	0.0440	0.0342	0.0568	0.0726	0.1249	0.0214	0.0368
B. taurus	0.0211	0.0370	0.0338	0.0593	0.0169	0.0295	0.0212	0.0371

Note: A_E – frequency of release of fragments with given activity by selected enzymes; W – relative frequency of release of fragments with given activity by selected enzymes

Table 3. The values of parameters describing the predicted efficiency of release of bioactive fragments from microalgae and cyanobacterial RuBisCO protein by four proteinases for DPP-IV inhibitor

Name of the species	Bromelain		Proteinase K		Chymotrypsin		Papain	
	A_E	W	A_E	W	A_E	W	A_E	W
C. calcitrans	0.0314	0.0503	0.713	0.1141	0.0335	0.0536	0.0335	0.0536
C. reinhardtii	0.0325	0.0547	0.0669	0.1126	0.0446	0.0750	0.0609	0.1025
C. pyrenoidosa	0.0365	0.0600	0.0692	0.1133	0.0467	0.0767	0.0609	0.1001
C.vulgaris	0.0305	0.0508	0.0713	0.1187	0.0467	0.0780	0.0548	0.0916
D. salina	0.0407	0.0680	0.0692	0.1156	0.0448	0.0748	0.6312	0.1054
E. gracilis	0.0345	0.0565	0.0652	0.1064	0.0509	0.0830	0.0692	0.1129
H. pluvialis	0.0364	0.0595	0.0648	0.1060	0.0386	0.0629	0.0610	0.0994
I.galbana	0.0335	0.0559	0.0524	0.0874	0.0356	0.0594	0.0440	0.0734
P. cruentum	0.0276	0.0452	0.0559	0.0903	0.4354	0.0710	0.0514	0.0839
Spirogyra sp.	0.0320	0.0538	0.0664	0.1111	0.0362	0.0609	0.0554	0.0931
S.maxima	0.0305	0.0512	0.0648	0.1093	0.0344	0.0580	0.0528	0.0887
S. platensis	0.0225	0.0379	0.0571	0.0965	0.0367	0.0620	0.0551	0.0931
Synechococcus sp.	0.0460	0.0870	0.0460	0.0870	0.0112	0.0217	0.0920	0.1740
A. flos-aquae	0.0283	0.0480	0.0687	0.1165	0.0364	0.0617	0.0568	0.0959
O. sativa	0.0391	0.0634	0.0478	0.0778	0.0354	0.0576	0.0602	0.0980
T. aestivum	0.0263	0.0451	0.0628	0.1077	0.0385	0.0660	0.0567	0.0973
Z. mays	0.0226	0.0363	0.0652	0.1051	0.0472	0.0761	0.0360	0.0580
G. max	0.0149	0.0254	0.0475	0.0805	0.0348	0.0593	0.0348	0.0593
G.gallus	0.0256	0.00331	0.0726	0.0939	0.1325	0.1713	0.0342	0.0442
B. taurus	0.0211	0.0276	0.0717	0.0939	0.0381	0.0497	0.0297	0.0387

Note: A_E – frequency of release of fragments with given activity by selected enzymes; W – relative frequency of release of fragments with given activity by selected enzymes

Table 4. Physiochemical properties of microalgae and cyanobacterial RuBisCO proteins; results obtained using the ProtParam program

Name of the species	No. of amino acids in residues	% of Glycine	% Proline	Isoelectric point (Pi)	GRAVY index	Aliphatic index
C. calcitrans	461	8.9	3.9	28.27	-0.065	85.73
C. reinhardtii	475	10.3	4.4	38.32	-0.226	79.28
C. pyrenoidosa	475	9.9	4.8	40.07	0.317	78.93
C.vulgaris	475	9.9	4.8	40.32	0.310	78.72
D. salina	475	10.5	4.4	39.38	0.243	78.76
E. gracilis	475	10.5	4.2	40.81	0.313	73.98
H. pluvialis	476	10.3	4.8	42.38	0.212	79.31
I.galbana	459	9.4	3.9	28.78	0.128	85.69
P. cruentum	488	8.8	3.9	31.71	0.162	85.59
Spirogyra sp.	451	10.4	4.7	41.56	0.291	82.00
S.maxima	476	9.5	4.8	46.09	0.322	74.41
S. platensis	476	9.5	4.8	45.65	0.308	74.62
Synechococcus sp.	472	9.5	4.7	42.36	0.270	79.62
A. flos-aquae	83	10.8	3.6	32.37	0.209	82.41
O. sativa	477	9.6	4.8	43.50	0.283	77.34
T. aestivum	543	7.7	3.3	27.40	0.034	104.86
Z. mays	476	9.9	4.6	42.57	0.258	77.92
G. max	427	7.3	6.3	36.64	0.030	84.92
G.gallus	386	4.9	3.6	37.11	0.001	89.65
B. taurus	224	2.2	15.6	94.12	0.154	97.37

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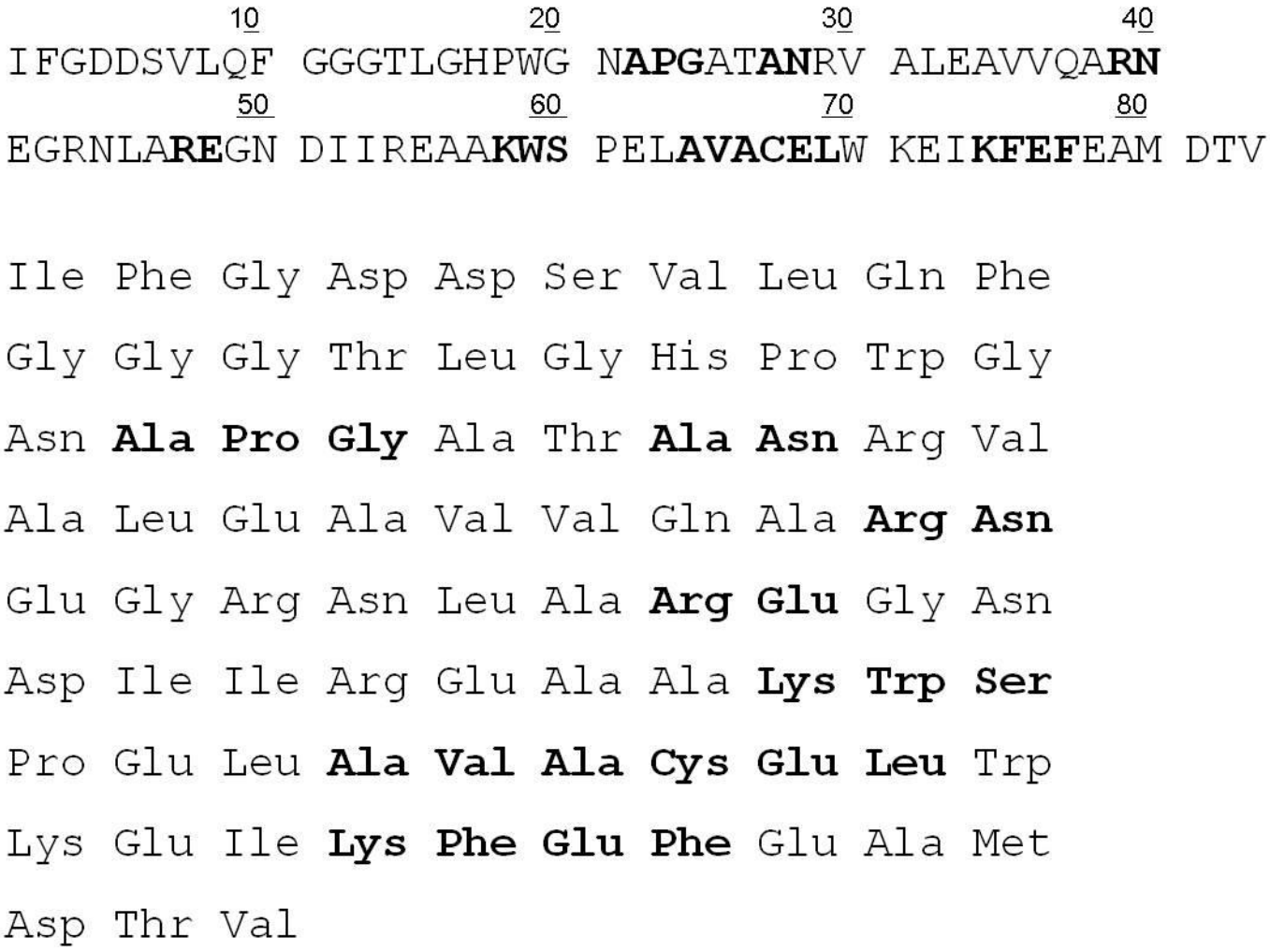

Figure.1. Mapping of *Aphanizomenon flos-aquae* RuBisCO showed the presence of bioactive peptide fragments in its sequences stimulated by the selective proteolytic enzymes.

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