Bioinformatics Analysis on Ribulose-1,5-bisphosphate Carboxylase/Oxygenase Large Subunits in Different Plants

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Abstract Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCo) is a crucial enzyme in plant photosynthesis. Therefore, to elucidate the characteristics of RuBisCo is important in improving the efficiency of plant photosynthesis, especially the photosynthetic efficiency in staple crops which relates to the biomass and yield directly. In order to reveal the characters of RuBisCos from different higher plants, we analyzed the nucleotide sequences and deduced amino acid sequences of ribulose-1,5-bisphosphate carboxylase/oxygenase large subunits (rbcL) from Zea mays, Arabidopsis thaliana, Pisum sativum, Citrus sinensis, Phalaenopsis aphrodite subsp. formosana, emphasizing Oryza sativa subsp. Japonica, by the tools of bioinformatics. The sequences data were collected from the Genbank of National Center for Biotechnology Information (NCBI). The contents of the analysis cover following aspects: the compositions and the physical and chemical characteristics of nucleotide sequences and deduced amino acid sequences, signal peptide, transmembrane topological structure, hydrophobicity or hydrophilicity, and secondary structure of the polypeptide, nucleotide and amino acid sequences comparisons, and molecular systemic evolution of rbcL DNA sequences. As a result, the amino acid compositions of the rbcLs set out few differentiations. The physical and chemical characteristics are approximately identical among different higher plants. Signal peptide and transmembrane topological structure were not detected in the rbcLs. We classified the rbcLs as hydrophilic protein on account of the distributive features of the amino acid residues in the polypeptides. The rbcLs are mainly composed of α-helix and random coil which are interspersed with extended strand and β-turn elements. The nucleotide sequences and deduced amino acid sequences possess high homologies among different higher plants. The rbcL DNA sequences can reflect the evolutionary relationship among various higher plants clearly.

Keywords Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCo); Plant; Bioinformatics

Background Ribulose-1,5-bisphosphate carboxylase/oxygenase (EC 4.1.1.39, RuBisCo), transforming the carbon dioxide and ribulose-1,5-bisphosphate (RuBP) into two molecular 3-phosphoglyceric acid, catalyzes the first reaction of carbon dioxide fixation in photosynthetic dark reaction. Also, RuBisCo catalyzes the reaction of oxygen and RuBP to phosphoglyceric acid and phosphoglycerolalic acid, which is the first reaction of photorespiration. Therefore, RuBisCo is the key enzyme deciding the photosynthetic efficiency by regulating photosynthesis and photorespiration. Base on the dissimilarity of the primary and quaternary structures, the RuBisCos can be partitioned into three types: I form exists in higher plants and most prokaryotes, consisting of eight large subunits (50~60 kD) and eight small subunits (12~18 kD), presenting square symmetry structure (L8S8) (Andersson et al., 1989); II form was discovered in purple non-sulfur photosynthetic bacteria, and composed of only two large subunits (L2); III form was dug out in Thermococcus kodakaracinsis lately by Kitano (Kitano et al., 2001), likewise formed with only large subunits, and no small subunit, appearing structure of (L2)10. The RuBisCo large subunit (rbcL) gene of higher plants sets in the chloroplast DNA, and it is translated by chloroplast ribosome. On the contrary,
the RuBisCo small subunit (rbcS) is synthesized in cytoplasm 80S ribosome because the gene exists in cell nucleus genome, and then transfers to chloroplast as precursor protein to assemble with large subunit after processed (Ellis, 1987; Roy H, 1989). To date, the rbcL gene has been cloned from a great many plants, such as Oryza sativa subsp. Japonica (Hiratsuka et al., 1989), Zea mays (Maier et al., 1995), Nicotiana tabacum (Shinozaki et al., 1986), Arabidopsis thaliana (Sato et al., 1999), Citrus sinensis (Bausher et al., 2006), Phalaenopsis aphrodite susp. Formosana (Chang et al., 2006), Astragalus mongholicus (Guo et al., 2010), Marchantia polymorpha (Ohyama et al., 1986; 1988), Picea abies (Relle et al., 1995).

1 Results

1.1 The compositions and the physical and chemical characteristics analysis of rbcL nucleotide sequences and deduced amino acid sequences from plants

The compositions and the physical and chemical characteristics of rbcL nucleotide sequences and deduced AA sequences were described by ORF Finder, DNAstar, ProtParam and pI/Mw. The analyzed rbcLs data were derived from Oryza sativa subsp. japonica, Zea mays, Arabidopsis thaliana, Pisum sativum, Citrus sinensis, Phalaenopsis aphrodite susp. formosana. All the initiation codons of the rbcLs genes are ATG, and the termination codons are TAG or TAA. The lengths of ORFs are about 1434 bps, and the encoding proteins are approximately 477 AA residues. The molecular weight and theoretical isoelectric point of the polypeptides are similar among different plants. The proportions of acidic AA, alkaline AA, total electric AA, polar AA and hydrophobic AA in the total AA residues of the rbcLs show tiny differences. On the whole, the most abundant AA residues are Gly, Ala, Leu, Glu and Val. The rbcLs of Pisum sativum and Citrus sinensis belong to stable protein, while that of the other three plants are unstable protein, but the instability indexes of all rbcLs are close to 40% (Table 1).

1.2 The signal peptide analysis of plant rbcLs

The rbcL AA sequence signal peptide of Oryza sativa subsp. japonica was predicted by SignalP Server v. 3.0 online program (Nielsen et al., 1997; Bendtsen et al., 2004). The analysis was performed using Neural Networks Model (NN) method. The top values of original shearing site (C score), signal peptide (S score), and synthesized shearing site (Y score) are 0.059, 0.133, and 0.021, which locate at the 24th, 4th, and 8th AA residues, respectively (Figure 1). All the scores are far less than the critical threshold. Moreover, the probability of presence of signal peptide in the analysis of polypeptide applying Hidden Markov Models (HMM) method is zero. Therefore, it indicates that no signal peptide shearing site exists in the rbcL polypeptide. The similar results were observed in the prediction of rbcL AA sequences of Zea mays, Triticum aestivum, Arabidopsis thaliana, Citrus sinensis. Accordingly, it was inferred that the rbcLs polypeptide synthesized in higher plants chloroplast don’t require to be protein transmembrane transferred.

1.3 The transmembrane topological structure analysis of plant rbcLs

The rbcL AA sequence transmembrane topological structure of Oryza sativa subsp. japonica was explored applying TMHMM Server v. 2.0 program (Ikeda et al., 2002). The total rbcL polypeptide locates outside the membrane (Figure 2), namely, it is absent of transmembrane topological structure in the rbcL AA sequence of Oryza sativa subsp. japonica. The same results can be gained from the analysis of rbcL AA sequences of Zea mays, Lolium perenne, Oncidium Gower Ramsey, Calycanthus floridus var. glaucus, Phalaenopsis aphrodite subsp. formosana, and so on.
### Table 1
Comparison of compositions and physical and chemical characteristics of nucleotide sequences and deduced amino acid sequences of RuBisCo large subunits among different higher plants

<table>
<thead>
<tr>
<th>Item</th>
<th>Rice</th>
<th>maize</th>
<th>Arabidopsis</th>
<th>Pea</th>
<th>Orange</th>
<th>Moth orchid</th>
</tr>
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<tr>
<td>Length of ORF (bp)</td>
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<td>1431</td>
<td>1440</td>
<td>1428</td>
<td>1428</td>
<td>1464</td>
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<td>ATG</td>
<td>ATG</td>
<td>ATG</td>
<td>ATG</td>
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<tr>
<td>Termination codon</td>
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<td>TAA</td>
<td>TAG</td>
<td>TAA</td>
<td>TAA</td>
<td>TAA</td>
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<tr>
<td>Number of deduced AA</td>
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<td>476</td>
<td>479</td>
<td>475</td>
<td>475</td>
<td>487</td>
</tr>
<tr>
<td>Molecular weight (kDa)</td>
<td>52.88</td>
<td>52.70</td>
<td>52.95</td>
<td>52.76</td>
<td>52.52</td>
<td>54.04</td>
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<tr>
<td>Theoretical isoelectric pI</td>
<td>6.22</td>
<td>6.33</td>
<td>5.87</td>
<td>6.55</td>
<td>6.29</td>
<td>5.96</td>
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<td>Gly (9.6%)</td>
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<td>Gly (9.8%)</td>
<td>Gly (9.7%)</td>
<td>Ala (9.9%)</td>
<td>Gly (9.7%)</td>
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<tr>
<td>Ala (9.4%)</td>
<td>Ala (9.5%)</td>
<td>Ala (9.0%)</td>
<td>Ala (9.3%)</td>
<td>Gly (9.7%)</td>
<td>Ala (9.0%)</td>
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<tr>
<td>Leu (7.8%)</td>
<td>Leu (7.8%)</td>
<td>Leu (8.6%)</td>
<td>Leu (8.6%)</td>
<td>Leu (8.6%)</td>
<td>Leu (7.8%)</td>
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<tr>
<td>Glu (6.9%)</td>
<td>Glu (6.5%)</td>
<td>Glu (7.3%)</td>
<td>Val (6.7%)</td>
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<td>Val (6.5%)</td>
<td>Thr (6.5%)</td>
<td>Val (6.9%)</td>
<td>Val (6.5%)</td>
<td>Glu (6.5%)</td>
<td>Val (7.0%)</td>
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<td>Acidic AA (%)</td>
<td>12.58%</td>
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<td>12.73%</td>
<td>12.21%</td>
<td>12.21%</td>
<td>13.14%</td>
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<tr>
<td>Alkaline AA (%)</td>
<td>11.32%</td>
<td>11.34%</td>
<td>10.65%</td>
<td>11.58%</td>
<td>11.16%</td>
<td>11.29%</td>
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<tr>
<td>Total electric AA (%)</td>
<td>23.90%</td>
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<td>23.38%</td>
<td>23.79%</td>
<td>23.37%</td>
<td>24.44%</td>
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<tr>
<td>Polar AA (%)</td>
<td>21.38%</td>
<td>21.43%</td>
<td>21.92%</td>
<td>21.47%</td>
<td>21.47%</td>
<td>21.15%</td>
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<td>Hydrophobic AA (%)</td>
<td>34.80%</td>
<td>34.87%</td>
<td>35.07%</td>
<td>35.37%</td>
<td>36.00%</td>
<td>34.50%</td>
</tr>
<tr>
<td>Instability index (%)</td>
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<td>unstable</td>
<td>unstable</td>
<td>stable</td>
<td>stable</td>
<td>unstable</td>
</tr>
</tbody>
</table>

**1.4 The hydrophobicity and hydrophilicity analysis of plant rbcLs**

The hydrophobicity and hydrophilicity analysis of the rbcL AA sequence of *Oryza sativa subsp. japonica* was fulfilled with ProtScale program (Kyce and Doolittle, 1982). The most hydrophilic AA residue in the polypeptide is Asn, located at 306th, because of the lowest score of −2.644. And the most hydrophobic AA residue is Ala, situated at 378th, which has the top score of 1.778. As for the whole polypeptide, the hydrophobic and hydrophilic AA residues distribute uniformly, but the number of hydrophilic AA residues is higher than that of hydrophobic AA residues, and any obvious hydrophobic AA residues concentrative region can't be detected (Figure 3). Similar distributive rule of hydrophobic and hydrophilic AA residues was found in other rbcL AA sequences from *Nicotiana tabacum, Lolium perenne, Medicago truncatula, Pisum sativum,* and *Citrus sinensis.* Thus, the results implies that the rbcLs in higher plants are hydrophilic protein, which is in accord with the previous conclusion that transmembrane topological structure is absent in rbcLs of higher plants.
1.5 The rbcL secondary structure analysis of plants

The rbcL polypeptide secondary structure of *Oryza sativa subsp. japonica* was detected with SOPMA (Geourjon and Déjéan, 1995). Alpha-helix and random coil are the principal structural elements in rbcL polypeptide of *Oryza sativa subsp. japonica*, and extended strand and β-turn occupy a little scale, which intersperse among the whole protein (Figure 4). According to the statistic assay consequence, the proportions of α-helix, extended strand, β-turn and random coil in the rbcL secondary structural components of *Oryza sativa subsp. japonica* are 40.25%, 16.56%, 9.85% and 33.33%, respectively. Likewise, similar distributive regularity of the four kinds of secondary structural units in rbcL has been detected in many other higher plants, such as *Zea mays*, *Triticum aestivum*, *Nicotiana tabacum*, *Medicago truncatula*, *Oncidium Gower Ramsey*, *Calycanthus floridus var. glaucus*, *Podocarpus macrophyllus*. The identities are 96%, 88%, 96%, 87%, 91%, 88%, 85%, respectively. The identities of further deduced AA sequence comparisons using BLASTp are 97%, 93%, 97%, 93%, 96%, 94%, 94%, respectively, while the similarities of that are separately 99%, 97%, 99%, 97%, 98%, 97% and 98%. Relative to the results of nucleotide sequence comparisons by BLASTn, higher similarities can be discovered in the AA sequence comparisons between *Oryza sativa subsp. japonica* and other six plants. Also, analogous results were obtained in the comparisons of rbcL nucleotide sequences and deduced AA sequences between *Oryza sativa subsp. japonica* and a great number of other higher plants, such as *Zea mays*, *Arabidopsis thaliana*, *Pisum sativum*, *Citrus sinensis*, *Phalaenopsis aphrodite subsp. formosana*. The regularity, which exhibited higher and broader similarities in rbcL AA sequences than in nucleotide sequences among various plants, is supported by above-mentioned results.

The multiple alignment analysis of rbcL AA sequences among *Cathaya argyrophylla*, *Calycanthus floridus var. glaucus*, *Nicotiana tabacum*, *Solanum lycopersicum*, *Citrus sinensis*, *Arabidopsis thaliana*, *Allium cepa*, *Phalaenopsis aphrodite subsp. formosana*, *Oryza sativa subsp. japonica* and *Zea mays* was carried out using Clustalx (Higgins and Sharp, 1988; 1989; Thompson et al., 1997; Jeanmougin et al., 1998) and DNAMAN software. Super identities of rbcL AA sequences were illustrated among higher plants, regardless of between gymnosperm and angiosperm, or between dicotyledon and monocotyledon (Figure 5). It was demonstrated that there are exceeding conservatism and homologies among higher plant rbcLs.

1.6 Comparisons among the rbcL nucleotide sequences and deduced amino acid sequences from different plants

The homologous comparisons of rbcL nucleotide sequences and deduced AA sequences were accomplished by online tool BLAST in NCBI (Alcschul et al., 1997). The results indicate high homologies of rbcL nucleotide sequences between *Oryza sativa subsp. japonica* and other higher plants which are comprised of *Triticum aestivum*, *Nicotiana tabacum*, *Lolium perenne*, *Medicago truncatula*, *Oncidium Gower Ramsey*, *Calycanthus floridus var. glaucus*, *Podocarpus macrophyllus*. The identities are 96%, 88%, 96%, 87%, 91%, 88%, 85%, respectively. The identities of further deduced AA sequence comparisons using BLASTp are 97%, 93%, 97%, 93%, 96%, 94%, 94%, respectively, while the similarities of that are separately 99%, 97%, 99%, 97%, 98%, 97% and 98%. Relative to the results of nucleotide sequence comparisons by BLASTn, higher similarities can be discovered in the AA sequence comparisons between *Oryza sativa subsp. japonica* and other six plants. Also, analogous results were obtained in the comparisons of rbcL nucleotide sequences and deduced AA sequences between *Oryza sativa subsp. japonica* and a great number of other higher plants, such as *Zea mays*, *Arabidopsis thaliana*, *Pisum sativum*, *Citrus sinensis*, *Phalaenopsis aphrodite subsp. formosana*. The regularity, which exhibited higher and broader similarities in rbcL AA sequences than in nucleotide sequences among various plants, is supported by above-mentioned results.

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1.7 The molecular systemic evolution analysis on rbcL DNA sequences from plants

The homologous evolutionary relationship among
rbcL DNA sequences from *Nicotiana tabacum*, *Solanum lycopersicum*, *Spinacia oleracea*, *Arabidopsis thaliana*, *Citrus sinensis*, *Calycanthus floridus* var. *glauca*, *Phalaenopsis aphrodite* subsp. *formosana*, *Oryza sativa* subsp. *japonica*, *Saccharum officinarum*, Zea mays, *Cycas taitungensis*, Cathaya argyrophylla, and *Pinus thunbergii*, was performed by Clustalx and MEGA5 software (Saitou and Nei, 1987; Tamura et al., 2004), using Neighbor-Joining (NJ) method. The rbcL DNA sequences, derived from thirteen higher plants, were assembled into two big clusters. One group is comprised of *Cycas taitungensis*, Cathaya argyrophylla, *Pinus thunbergii*, and other ten plants constitute the other group. All the thirteen plants originate from a common ancestor. In the phylogenetic tree, the connection of the branches reflects the rbcLs evolutionary relationship of different plants clearly. The three Gymnospermous plants, containing *Cycas taitungensis*, Cathaya argyrophylla and *Pinus thunbergii*, constitute a branch, which is distinguished from the other branch that include other ten plants, belonging to angiosperm. And the ten angiospermous plants can be further divided into two branches of dicotyledon and monocotyledon. Also, the *Solanaceae* plant, containing *Nicotiana tabacum* and *Solanum lycopersicum*, and the *Poaceae* plant, including *Oryza sativa* subsp. *japonica*, Zea mays, and *Saccharum*...
officinarum, constitute two small branches, respectively, on account of their close relationship (Figure 6).

![Figure 6 Molecular evolutionary analysis of rbcL DNA sequences among different higher plants](image)


The molecular level evolutionary relationship was applied in biological systemic taxology widespreadly, after the advance of "molecular evolutionary clock" and "neutral theory" in 1960s. A few divergences are present in the application of molecular evolution to biological taxonomy, due to the dispute of "constant speed of sequence evolution" and "darwinian positive selection" in academic world. However, it is acknowledged that the evolutionary units above family can be differentiated exactly with the phylogenetic analysis of DNA and AA sequence, which was proved adequately in this study. The *Zea mays* and *Saccharum officinarum* are separated from *Oryza sativa subsp. Japonica* correctly (Figure 6), in virtue of their closer relationship, even though all the three plants belong to *Gramineae.*

2 Discussion

In this study, we demonstrated that the rbcLs from different higher plants don't possess signal peptide, transmembrane topological structure and the traits of hydrophobic protein. The principal secondary structural elements are α-helix and random coil. The compositions and the physical and chemical characteristics are similar, and extremely high homologies were exhibited among different higher plants. The evolutionary relationship reflected by DNA sequences corresponds with traditional botanical taxonomy.

It is known that the sequences and structures of rbcLs from different higher plants get high homologies, and the similarities of that are above 80%, while the similarities of rbcSs are much smaller and less than 50%. All the analyzed rbcL ORFs from higher plants are about 1434bp, and translate into polypeptides that consist of nearly 477 AA residues (Table 1). The similarities of the rbcL AA residues from different higher plants are more than 97%, and the inferior homologous region in the rbcL polypeptide mainly locates at the C-terminal (Figure 5). The high homology of rbcLs indicates the importance of structural stability in maintaining high catalytic efficiency. Also, it implies that the overwhelming majority of rbcL AA residues play a crucial role in keeping the structural stability, as the report that the RuBisCo catalytic efficiency can be altered obviously when some AA residues of rbcL were substituted (Chen et al., 1988; 1993; Seokjoo and Robert., 1997; Bainbridge et al., 1998; Pippa et al., 1998).

As a double functional enzyme, RuBisCo catalyzes the oxygenation reaction of RuBP when it is catalyzing the carboxylation reaction of that. Because of the characteristics of RuBisCo, the plant will suffer a great loss of about 20-50% of the organic carbon, fixed by the carboxylation reaction, no merely energy (Li et al., 2001). So in theoretically, the improvement of crop RuBisCo is a breakthrough point in crop variety improvement using modern biotechnology, and has a tempting perspective (Mann, 1999; Parry et al., 2007). Up to now, rapid progress has been making in studies on RuBisCo structures, biological functions and regulations, and enzymatic characters, but it is
still theoretical in improving crop photosynthetic efficiency and increasing yield via the modification of RuBisCo. Therefore, further exploration of RuBisCo natures and molecular characteristics are indispensable to lay a solid foundation of enhancing crop RuBisCo catalytic efficiency and increasing the photosynthetic output, for instance, the diversity of RuBisCo structures and functions among different plants, environmental regulations and active mechanisms, and the relationship of protein structures and functions.

Author Contributions

BIZ and LGL have finished the paper, XXZ, SY, RLL, DWL, YYN, YBZ, QGL and YHW also read the manuscript and revised it. All authors had read and consented the final text.

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