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# Conservation of the basic pattern of cellular amino acid composition during biological evolution in plants

*Short Communication*

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**Summary.** The cellular amino acid composition of plant cells was analyzed. The callus of carrot (*Daucus carota*), leaves of *Torenia fournieri* and protocomb-like body of *Cymbidium*, s.p. were examined as examples of plant cells. The cellular amino acid compositions differed in the plant cells, but their basic patterns were quite similar. It is concluded that the basic pattern of the cellular amino acid composition is conserved in all terrestrial organisms, including plants.

**Keywords:** Amino acid – Cellular amino acid – Evolution – Plant cells

## Introduction

We found in our previous study (Okayasu et al., 1997) that the cellular amino acid composition was almost identical in mammalian cells, but differed markedly between mammalian cells and bacterial cells. These results led us to examine whether the cellular amino acid composition reflects changes in biological evolution. Significant changes in the cellular amino acid composition were observed in eubacteria, blue-green alga, green alga, fungi, protozoa, slime mold and vertebrates, whereas a basic pattern of amino acid composition is maintained, in spite of a long period of evolutional divergence among the various organisms (Sorimachi, 1999). Therefore, it was concluded that the cellular amino acid composition reflects changes in biological evolution, and it is proposed that the primitive life forms that were established on the earth had a similar amino acid composition (Sorimachi, 1999). However, plant cells, which have greatly diverged not only from bacteria but also from mammals, were not examined in our previous study. In the present study, plant cells were examined, because their data are considered important in understanding the relationship between biological evolution and cellular amino acid composition.

## Materials and methods

Callus of carrot (*Daucus carota*), *Torenia fournieri* and the protocomb-like body of *Cymbidium*, s.p., which were cultured on Murashige & Skoog medium, were kindly supplied by ASAHI TECHNO GLASS CORPORATION (Funabashi, Chiba, Japan).

Tissues washed with phosphate buffered saline were homogenized in $H_2O$ with a Physcotron, NITI-ONI Rikagaku Seisakusho (Tokyo, Japan) as described elsewhere (Sorimachi, 1999). Aliquots (100–200 $\mu$l) of cell homogenates were mixed with 0.9 ml of ethanol and centrifuged at 14,000 rpm for 5 min to separate precipitates and supernatants. The precipitates were washed again in 1 ml of ethanol and centrifuged using the same conditions. Samples dried *in vacou* were dissolved in 200 $\mu$l of 6 N HCl containing 0.1% phenol and the mixtures were kept at 110°C for 24 h. After the hydrolyzates were dried *in vacou* at room temperature, they were dissolved in 200 $\mu$l of 0.2 N HCl and passed through a membrane filter of 0.45 $\mu$m pore size before application to the amino acid analyzer, HITACHI L-8500A.

## Results and discussion

Three species of plant, carrot, *Torenia fournieri* and *Cymbidium*, were examined. Their amino acid compositions are shown in Table 1 and Fig. 1. The

**Table 1.** Cellular amino acid composition of plant cells

| Amino acid | Carrot | *Torenia* | *Cymbidium* | *E. coli* |
|---|---|---|---|---|
| Asp | 9.73 ± 0.04 | 9.44 ± 0.06 | 17.47 ± 0.23 | 10.70 ± 0.04 |
| Glu | 11.58 ± 0.07 | 12.56 ± 0.09 | 10.78 ± 0.06 | 10.90 ± 0.16 |
| Ser | 6.22 ± 0.05 | 5.24 ± 0.04 | 6.76 ± 0.04 | 4.61 ± 0.01 |
| Gly | 10.19 ± 0.07 | 10.64 ± 0.10 | 9.47 ± 0.14 | 9.44 ± 0.03 |
| His | 2.26 ± 0.06 | 1.72 ± 0.05 | 1.81 ± 0.01 | 1.81 ± 0.01 |
| Arg | 5.29 ± 0.04 | 5.11 ± 0.07 | 3.79 ± 0.08 | 4.93 ± 0.03 |
| Thr | 5.06 ± 0.05 | 4.94 ± 0.04 | 4.73 ± 0.06 | 5.16 ± 0.02 |
| Ala | 8.50 ± 0.02 | 8.70 ± 0.03 | 7.42 ± 0.09 | 10.75 ± 0.06 |
| Pro | 5.03 ± 0.17 | 5.05 ± 0.17 | 4.69 ± 0.19 | 4.93 ± 0.13 |
| Tyr | 2.56 ± 0.06 | 3.06 ± 0.05 | 3.03 ± 0.14 | 2.92 ± 0.05 |
| Val | 6.72 ± 0.04 | 6.63 ± 0.09 | 5.84 ± 0.05 | 7.46 ± 0.01 |
| Met | 2.04 ± 0.05 | 1.75 ± 0.08 | 1.57 ± 0.01 | 2.34 ± 0.00 |
| Cys | 0.42 ± 0.03 | 0.39 ± 0.09 | 0.31 ± 0.02 | 0.29 ± 0.00 |
| Ile | 4.67 ± 0.04 | 4.85 ± 0.06 | 4.79 ± 0.05 | 5.04 ± 0.01 |
| Leu | 8.19 ± 0.07 | 9.22 ± 0.06 | 8.48 ± 0.09 | 8.78 ± 0.02 |
| Phe | 3.67 ± 0.01 | 4.71 ± 0.02 | 2.95 ± 0.07 | 3.55 ± 0.00 |
| Lys | 7.87 ± 0.11 | 6.00 ± 0.05 | 6.11 ± 0.07 | 6.38 ± 0.02 |

The value is presented as the percentage of total amino acids and is the mean ± S.D. of 3 analyses.

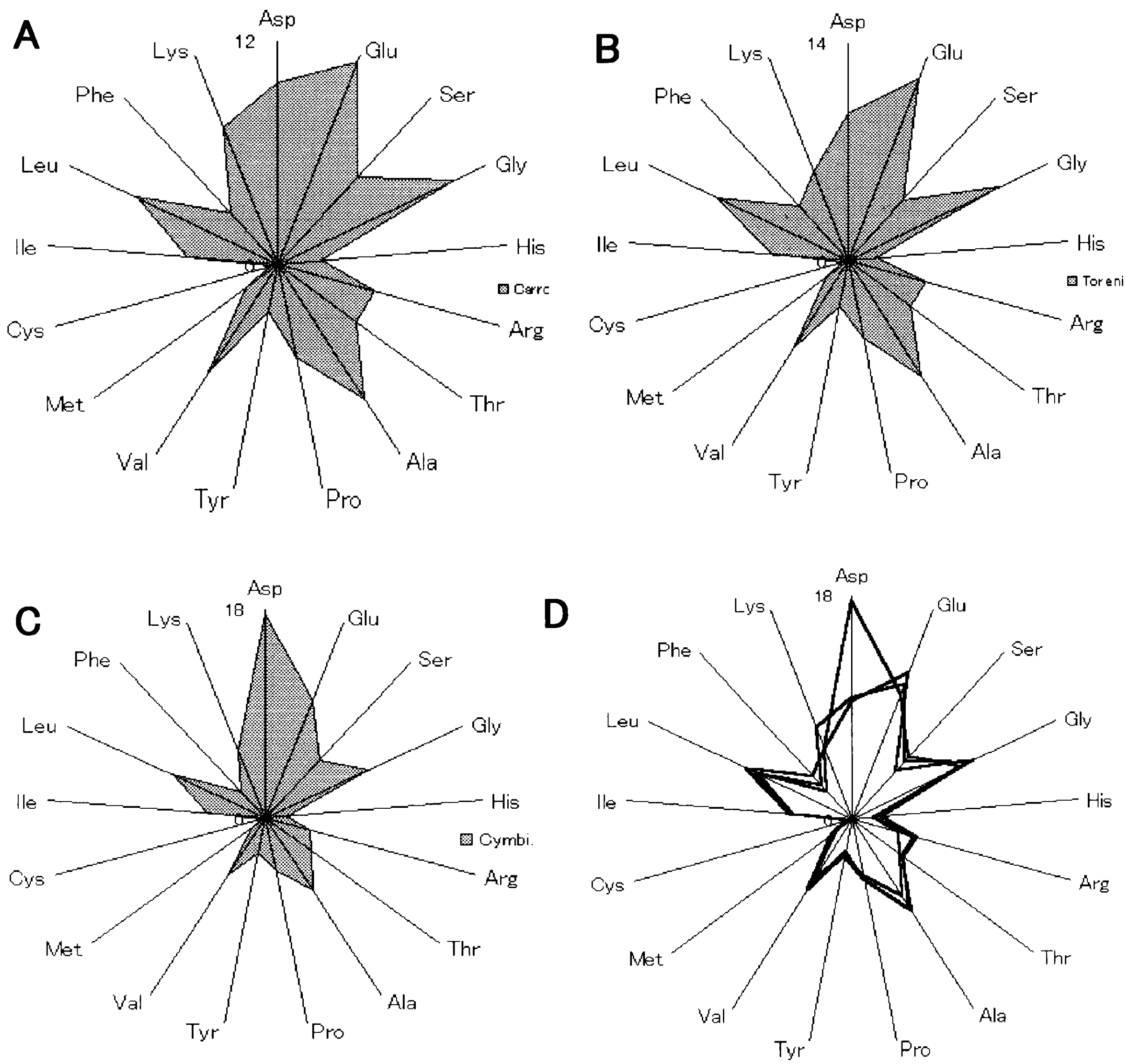


**Fig. 1.** Radar graphs of the cellular amino acid compositions of plant cells. (**A**) Carrot, (**B**) *Torenia fournieri*, (**C**) *Cymbidium* and (**D**) cellular amino acid compositions of three species of plant cells were overlaid

cellular amino acid compositions differ significantly from each other in the three species of plants, and *Cymbidium* differs significantly from the other two species (Fig. 1C). These results suggest that *Cymbidium* has greatly diverged from the other two species. It is interesting that the pattern of the cellular amino acid composition for carrot (Fig. 1A) is very similar to that of the mammalian cells (Sorimachi, 1999).

Figure 2 shows the differences in the cellular amino acid composition of plant cells compared to that of *E. coli*. The concentration of aspartic acid

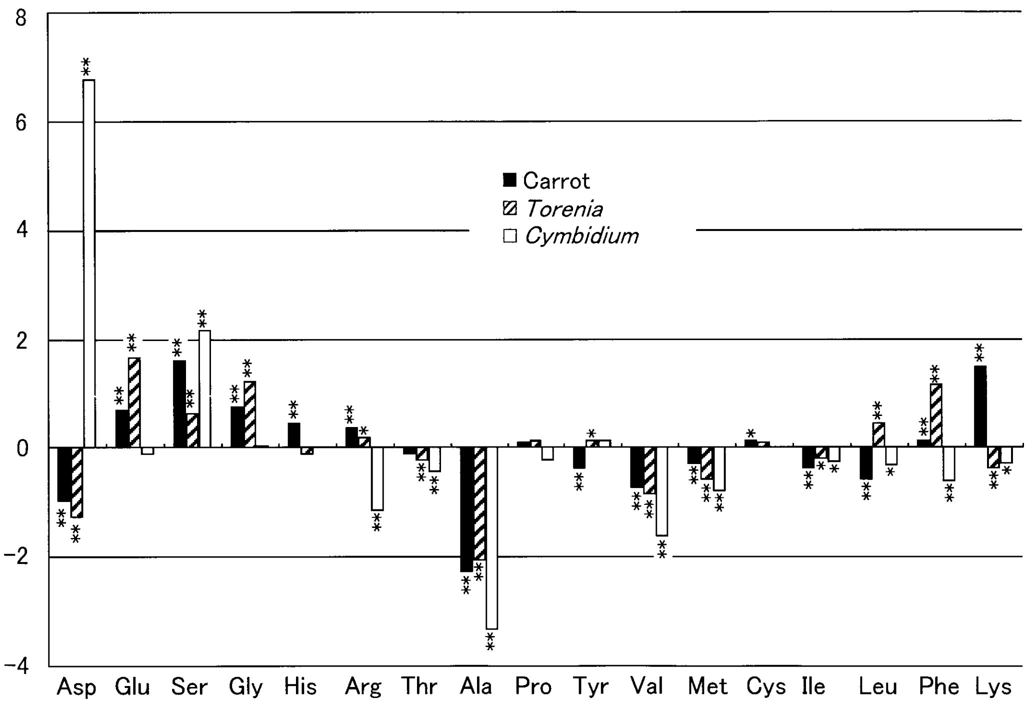


**Fig. 2.** Differences in amino acid composition of the plants compared with *E. coli*. Statistical differences between *E. coli* and other samples were evaluated using Student's *t*-test (**P < 0.01, *P < 0.05)

greatly increased in *Cymbidium*, but decreased slightly in carrot and *Torenia fournieri*. The concentrations of alanine, valine and methionine decreased in three species of plants compared with *E. coli*, but the concentration of serine increased in all three samples (Fig. 2). The decreases in alanine and valine concentrations and the increase in serine concentration were observed in *Tetrahymena*, slime mold, fungi, fish, rat and human, but were not observed in Gram-positive bacteria, cyanobacteria and *Chlorella* (Sorimachi, 1999). Thus, the plant appears to have greatly diverged from *E. coli*.

The basic patterns of the cellular amino acid composition for plants are similar, as shown in Fig. 1D. This basic pattern is quite similar to that obtained from the various organisms (Sorimachi, 1999). Thus, the basic pattern is almost constant for every organism on the earth.

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