Amino Acids (2001) 21: 393–399

Amino
Acids

Conservation of the basic pattern of cellular amino acid composition of archaeobacteria during biological evolution and the putative amino acid composition of primitive life forms

K. Sorimachi[1], **T. Itoh**[2], **Y. Kawarabayasi**[3], **T. Okayasu**[4],
K. Akimoto[5], and **A. Niwa**[1]

[1] Department of Microbiology,
[2] Japan Collection of Microorganisms, Institute of Physical and Chemical Research (RIKEN), Wako-shi, Saitama, Japan
[3] National Institute of Technology and Evaluation, Nishihara, Shibuya, Tokyo, Japan
[4] Center of Medical Informatics, and
[5] Institute of Medical Science, Dokkyo University School of Medicine,
Mibu, Tochigi, Japan

Accepted January 30, 2001

Summary. Previous studies showed that the cellular amino acid composition obtained by amino acid analysis of whole cells, differs such as eubacteria, protozoa, fungi and mammalian cells. These results suggest that the difference in the cellular amino acid composition reflects biological changes as the result of evolution. However, the basic pattern of cellular amino acid composition was relatively constant in all organisms examined. In the present study, we examined archaeobacteria, because they are considered important in understanding the relationship between biological evolution and cellular amino acid composition. The cellular amino acid compositions of *Archaeoglobus fulgidus*, *Pyrococcus horikoshii*, *Methanobacterium thermoautotrophicum* and *Methanococcus jannaschii* differed slightly from each other, but were similar to those determined from codon usage data, based on the complete genomes. Thus, the cellular amino acid composition reflects biological evolution. We suggest that primitive forms of life appearing on earth at the end of prebiotic evolution had a similar-cellular amino acid composition.

Keywords: Amino acids – Cellular amino acid composition – Evolution – Archaea – Codon frequency – Primitive life forms

Introduction

Fossils of many microorganisms have been found in Precambrian rocks (Barghoon and Schope, 1966; Peat and Lloyd, 1974; Nagy and Zumberge, 1976), and it has been suggested that these microorganisms are related to a more primitive form of life. The cellular amino acid composition of eubacteria, primitive eukaryotes and eukaryotic cells (Sorimachi, 1999) and similarly of plant cells (Sorimachi et al., 2000) changes in relation to biological evolution. However, a basic pattern of the cellular amino acid composition was found in all organisms. One may assume that certain rules governing living organisms are applicable to both prebiotic and biological evolution. Therefore primitive forms of life should have a comparable amino acid composition. To challenge this hypothesis, we decided to extent our studies to archaeobacteria, which appear more closely related to eukaryotes than prokaryotes in the evolutionary tree (Iwabe et al., 1987; Gogarten et al., 1989; Brown and Doolittle, 1995). Results of these important groups of living entities may contribute to our understanding of the biological evolution.

Materials and methods

Archaeoglobus flugidus (Stetter et al., 1987; Stetter, 1988), JCM 9628 (medium No. 214), *Pyrococcus horikoshii* (Gonzalez et al., 1998), JCM 9974 (medium No. 151), *Methanobacterium thermoautotrophicum* (Zeikus and Wolfe, 1972), JCM 10044 (medium No. 231), and *Methanococcus jannaschii* (Jones et al., 1983), JCM 10045 (medium No. 232) were cultured as described elsewhere (Nakase, 1999).

Archaeobacteria were washed three times with saline and centrifuged at 7,000 rpm for 10 min. Cells were homogenized using a VP-5S sonicator, TAITEK, (Koshigaya, Japan) at 20 Khz for 30 sec in H_2O. Amino acid analysis of the cell homogenates was carried out by a previously described method (Sorimachi et al., 2000).

Results and discussion

The cellular amino acid compositions of *Archaeoglobus fulgidus*, *Pyrococcus horikoshii*, *Methanobacterium thermoautotrophicum* and *Methanococcus jannaschii* (and *E. coli*) are given in Table 1 and Fig. 1. Data represent the percentage of each amino acid in relation to total amino acid content of whole cell proteins, as free amino acids were separated from proteins by ethanol precipitation of homogenates (Okayasu et al., 1997). Although the amino acid distribution differs slightly between the four species of archaeobacteria, the basic pattern is quite similar (Fig. 1A–D). The complete genome sequences (about 2,000 genes) of *Archaeoglobus fulgidus*, (Klenk et al., 1997) *Pyrococcus horikoshii* (Kawarabayasi et al., 1998), *Methanobacterium thermoautotrophicum* (Smith et al., 1997) and *Methanococcus jannaschii* (Bult et al., 1996) have been determined during the last 4 years. Figure 1a–d shows the radar graphs of the cellular amino acid composition based on codon usage data. The pattern of protein amino acid composition of whole cells (Fig. 1, A–D on the left hand side) is very similar to that obtained from the codon usages of the complete genomes (Fig. 1, a–d on the right hand side), although it was mathematically assumed that all genes are expressed equally. These results indicate that the basic pattern of cellular amino acid composition is conserved in the genome, which has also been shown for *Bacillus subtilis* (Sorimachi, unpublished data).

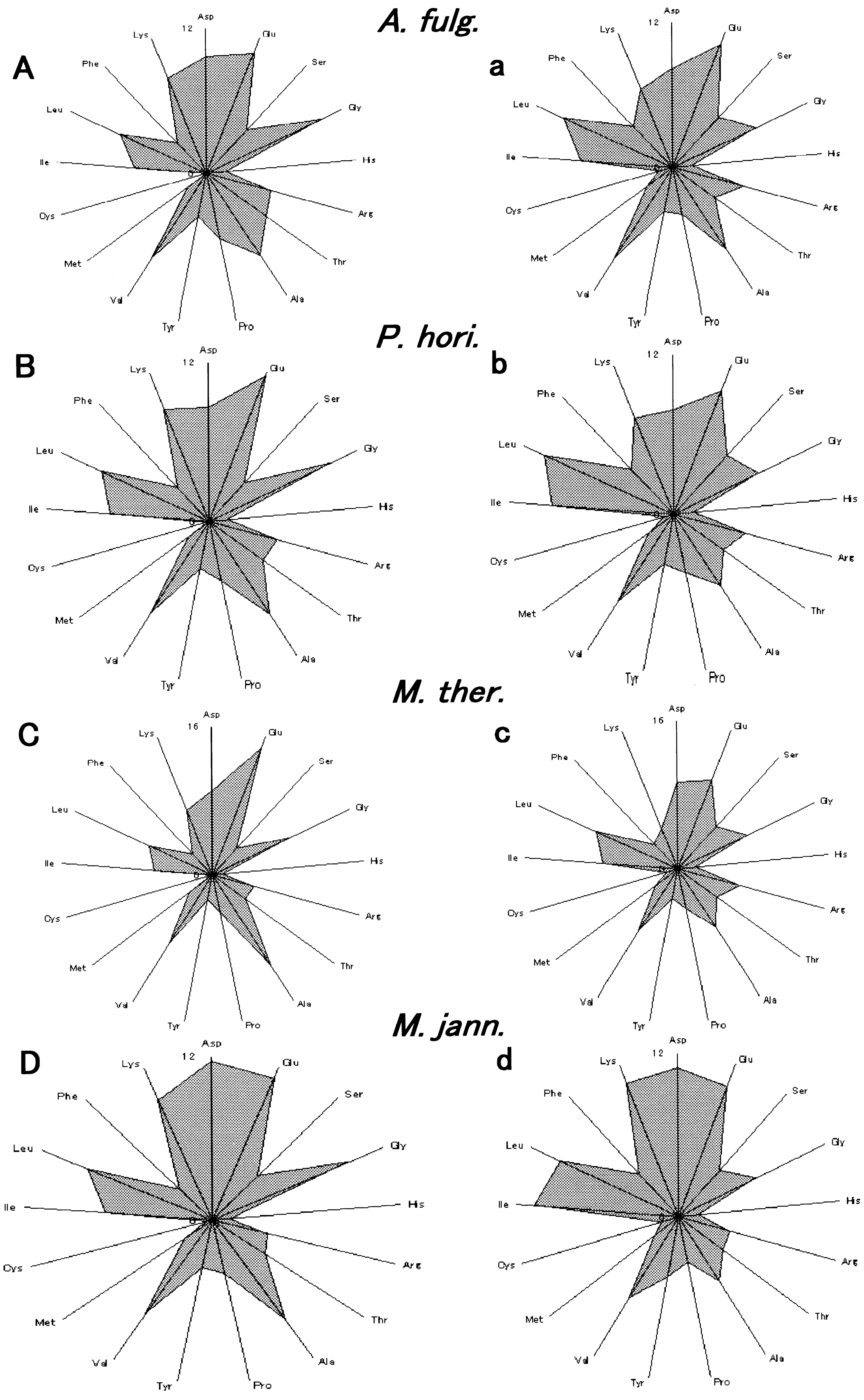
Table 1. Cellular amino acid composition of bacteria

A. A.	*A. flugidus*	*P. horikoshii*	*M. thermoaut.*	*M. jannashii*	*E. coli*
Asp	9.66 ± 0.03	8.55 ± 0.03	9.01 ± 0.07	11.63 ± 0.04	10.70 ± 0.04
Glu	10.57 ± 0.16	11.65 ± 0.16	14.60 ± 0.25	11.02 ± 0.17	10.90 ± 0.16
Ser	4.85 ± 0.04	3.80 ± 0.02	3.78 ± 0.06	4.33 ± 0.01	4.61 ± 0.01
Gly	10.06 ± 0.06	9.69 ± 0.04	8.89 ± 0.04	9.47 ± 0.03	9.44 ± 0.03
His	1.55 ± 0.01	1.34 ± 0.01	1.10 ± 0.01	1.11 ± 0.01	1.81 ± 0.01
Arg	5.32 ± 0.04	5.13 ± 0.02	4.54 ± 0.01	3.61 ± 0.02	4.93 ± 0.03
Thr	5.86 ± 0.04	4.84 ± 0.05	4.32 ± 0.13	4.15 ± 0.05	5.16 ± 0.02
Ala	7.92 ± 0.05	8.26 ± 0.05	11.45 ± 0.16	8.63 ± 0.03	10.75 ± 0.06
Pro	5.42 ± 0.19	4.57 ± 0.15	4.17 ± 0.17	3.98 ± 0.12	4.93 ± 0.13
Tyr	3.59 ± 0.08	3.61 ± 0.06	2.61 ± 0.06	3.56 ± 0.06	2.92 ± 0.05
Val	8.18 ± 0.07	8.11 ± 0.01	8.51 ± 0.02	8.04 ± 0.02	7.46 ± 0.01
Met	1.91 ± 0.13	1.89 ± 0.01	3.00 ± 0.06	2.23 ± 0.01	2.34 ± 0.00
Cys	0.00 ± 0.00	0.12 ± 0.02	0.13 ± 0.02	0.35 ± 0.01	0.29 ± 0.00
Ile	5.70 ± 0.01	7.24 ± 0.03	6.11 ± 0.02	6.74 ± 0.03	5.04 ± 0.01
Leu	7.51 ± 0.01	8.71 ± 0.02	7.30 ± 0.01	8.74 ± 0.02	8.78 ± 0.02
Phe	3.45 ± 0.05	3.50 ± 0.01	3.00 ± 0.01	3.08 ± 0.02	3.55 ± 0.00
Lys	8.43 ± 0.04	9.00 ± 0.02	7.48 ± 0.04	9.35 ± 0.03	6.38 ± 0.02

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

The amino acid composition based on codon usage data differs between the four archaeobacteria examined (Fig. 1, a–d), whereas the relative amount of these certain amino acid is almost identical in the cellular (protein based) amino acid composition. These differences might provide information on codon formation as the genome numbers are nearly identical in these four species of archaeobacteria. The content of leucine and valine is almost the same whether obtained by amino acid analysis of whole cells or from genome analysis of the archaeobacteria (Fig. 1). Figure 2 presents the codon usage patterns for leucine and valine calculated from already published data (Klenk et al., 1997; Kawarabayasi et al., 1998; Smith et al., 1997; Bult et al., 1996). These codon usage patterns for leucine and valine differ markedly between the four species, although the total coding number of each amino acid is identical. We conclude that there is no preferential usage of certain nucleotides for codon formation because of the great differences in codon usage among the archaeobacteria. During prebiotic evolution, codon usage was selected by the character of a particular protein, like a feedback mechanism. Overcoming various selection forces resulted in the appearance of a basic pattern of cellular amino acid composition. The “Star shape” representing the cellular amino acid composition symbolizes terrestrial organisms.

The cellular amino acid composition differs not only between archaeobacteria, as shown in the present study, but also among the organisms examined in a previous study (Sorimachi, 1999). This difference must be due to divergencies in biological evolution. On the other hand, the basic pattern of the cellular amino acid composition is similar in all organisms examined in our


A. fulg.
A
a
P. hori.
B
b
M. ther.
C
c
M. jann.
D
d
12
16
0
Asp
Glu
Ser
Gly
His
Arg
Thr
Ala
Pro
Tyr
Val
Met
Cys
Ile
Leu
Phe
Lys

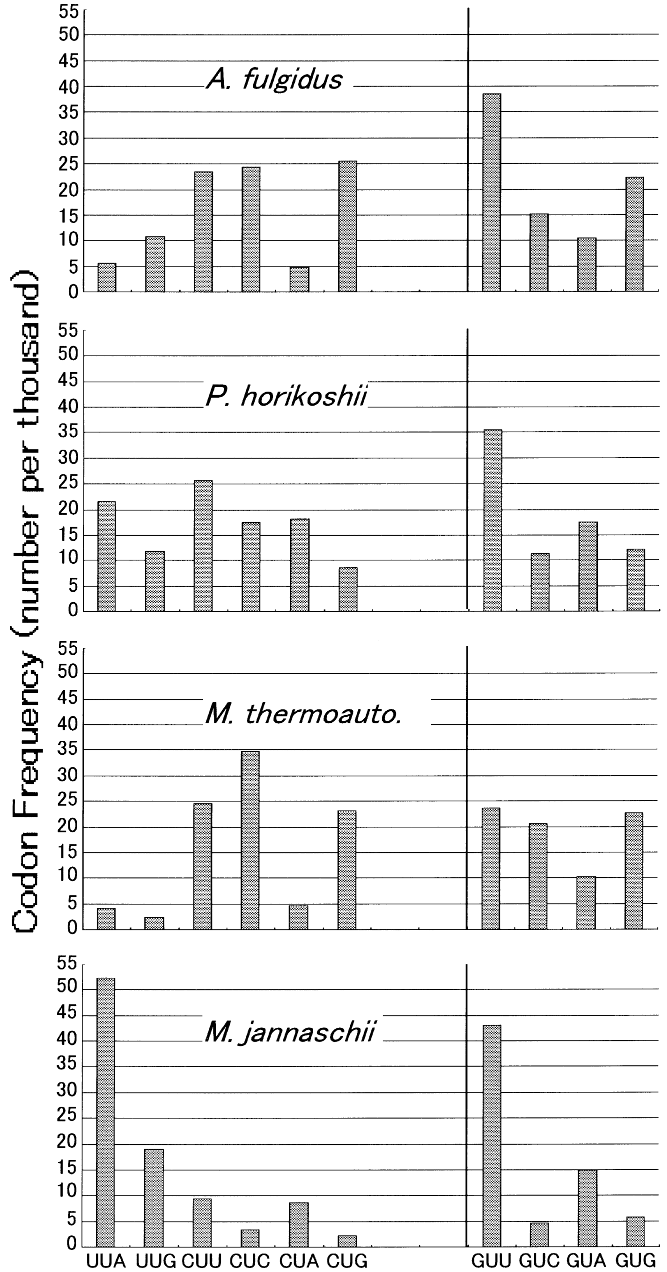


Fig. 2. Codon usages for leucine and valine in the archaeobacteria. The left panel represents the codons for leucine and the right panel represents the codons for valine

◀

Fig. 1. Amino acid analysis of bacteria. The value represents the mean of three analyses and S.D. was less than 1%. Radar graphs of the cellular amino acid composition of archaeobacteria: **A** and **a** *Archaeoglobus flugidus*, **B** and **b** *Pyrococcus horikoshii.*, **C** and **c** *Metanobacterium thermoautotrophicum*, **D** and **d** *Methanococcus jannashii*. **A–D** protein amino acid composition, and **a–d** genomic amino acid composition

studies. In addition, also plant cells exhibited a similar basic pattern of cellular amino acid composition (Sorimachi et al., 2000). Therefore, it can be concluded that primitive life formed at the end of prebiotic evolution had a similar-cellular amino acid composition. Based on this assumption, it seems the basic pattern of cellular amino acid composition has been conserved during biological evolution for a period of 3.5 or 4 billion years. The comparison of the cellular amino acid composition between terrestrial and extraterrestrial organisms will provide a key to understand the origin of life, not only on earth, but within the universe as a whole, if extraterrestrial organisms are found in the future.

Acknowledgements

We are greatly indebted to Dr. Masashi Ikeda of Dokkyo University School of Medicine for analyzing the cellular amino acid composition, and to Dr. Arno Mullbacher of the Australian National University and to Dr. Elizabeth Anne Kamei of Gunma University for revising this manuscript.

References

Barghoon ES, Schope JW (1966) Microorganisms three billion years old from the Precambrian of South Africa. Science 152: 758–763

Brown JR, Doolittle WF (1995) Root of the universal tree of life based on ancient aminoacyl-tRNA synthetase gene duplications. Proc Natl Acad Sci USA 92: 2441–2445

Bult CJ et al (1996) Complete genome sequence of the methanogenic archaon, *Methanococcus jannashii*. Science 273: 1058–1073

Gogarten JP et al (1989) Evolution of the vacuolar H^+-ATPase: implications for the origin of eukaryotes. Proc Natl Acad Sci USA 86: 6661–6665

Gonzalez JM et al (1998) *Pyrococcus horokoshii* sp. nov., a hyperthermophilic archaeon isolated from a hydrothermal vent at the Okinawa Trough. Extremophiles 2: 123–130

Iwabe N, Kuma K, Hasegawa M, Osawa S, Miyata T (1989) Evolutionary relationship of archaeobacteria, eubacteria, and eukaryotes inferred from phylogenetic trees of duplicated genes. Proc Natl Acad Sci USA 81: 9355–9359

Jones WJ, Leigh JA, Mayer F, Woese CR, Wolfe RS (1983) *Methanococcus jannashii* sp. nov., an extremely thermophilic methanogen from a submarine hydrothermal vent. Arch Microbiol 136: 254–261

Kawarabayasi Y et al (1998) Complete sequence and gene organization of the genome of a hyper-theromophilic archaebacterium, *Pyrococcus horikoshii* OT3. DNA Res 5: 55–76

Klenk H-P et al (1997) The complete genome sequence of the hyperthermophilic, sulphate-reducing archaeon *Archaeoglobus fulugidus*. Nature 390: 364–370

MacGregor IM, Truswell JF, Eriksson KA (1974) Filamentous algae from the 2,300 m.y. old Transvaal Dolomite. Nature 247: 538–540

Nagy LA, Zumberge JE (1976) Fossil microorganisms from the approximately 2,800 to 2,500 million-year-old Bulawayan stromatolite: application of ultramicrochemical analyses. Proc Natl Acad Sci USA 13: 379–391

Nakase T (1999) Japan Collection of Microorganisms. In: JCM Catalogue of Strains, 7th edn. Japan Collection of Microorganisms, Wako, Japan

Okayasu T, Ikeda M, Akimoto K, Sorimachi K (1997) The amino acid composition of mammalian and bacterial cells. Amino Acids 13: 379–391

Peat CJ, Lloyd BJ (1974) Direct observation of rock macerates. Nature 251: 294–295

Smith DR et al (1997) Complete genome sequence of *Methanobacterium thermoautotrophicum* ΔH: functional analysis and comparative genomics. J Bacteriol 179: 7135–7155

Sorimachi K (1999) Evolutionary changes reflected by the cellular amino acid composition. Amino Acids 17: 207–226

Sorimachi K, Okayasu T, Akimoto K, Niwa A (2000) Conservation of the basic pattern of cellular amino acid composition during biological evolution in plants. Amino Acids 18: 193–197

Stetter KO (1988) *Archaeoglobus fulgidus* gen. nov., sp. nov.: a new taxon of extremely thermophilic archaebacteria. System Appl Microbiol 10: 172–173

Stetter KO, Lauerer G, Thomm M, Neuner A (1987) Isolation of extremely thermophilic sulfate reducers: evidence for a novel brach of archaebacteria. Science 236: 822–824

Zeikus JG, Wolfe RS (1972) *Methanobacterium thermoautotrophicus* sp. n., an anaerobic, autotrophic, extreme thremophile. J Bacteriol 109: 707–713

Authors' address: Dr. Kenji Sorimachi, Department of Microbiology, Dokkyo University School of Medicine, Mibu, Tochigi 321-0293, Japan,
Fax: 81-282-86-5616, E-mail: kenjis@dokkyomed.ac.jp

Received November 28, 2000