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The amino acid composition of mammalian and bacterial cells

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Summary. High performance liquid chromatography was used to analyze the amino acid composition of cells. A total of 17 amino acids was analyzed. This method was used to compare the amino acid compositions of the following combinations: primary culture and established cells, normal and transformed cells, mammalian and bacterial cells, and *Escherichia coli* and *Staphylococcus aureus*. The amino acid compositions of mammalian cells were similar, but the amino acid compositions of *Escherichia coli* and *Staphylococcus aureus* differed not only from mammalian cells, but also from each other. It was concluded that amino acid composition is almost independent of cell establishment and cell transformation, and that the amino acid compositions of mammalian and bacterial cells differ. Thus, it is likely that changes in amino acid composition due to cell transformation or species differences between mammalian cells are negligible compared with the differences between mammalian and bacterial cells, which are more distantly related.

Keywords: Amino acids – HPLC – Mammalian cells – Bacterial cells

Introduction

Over the past decade, a variety of high performance liquid chromatography (HPLC) methods have been developed for the determination of the amino acid composition of biological materials (Bildingmeyer et al., 1984; Vendrell and Aviles, 1986; Rutledge and Rudy, 1987). We have previously developed a HPLC method for hydroxyproline analysis, and have identified hydroxyproline in rat tail collagen and in conditioned medium (Ikeda et al., 1991; 1993), urine (Ikeda et al., 1995) and cultured rat hepatoma cells (M) (Sorimachi et al., 1995). A suitable HPLC method could be used to identify small differences in the amino acid composition of different cell lines.

In our separate experiments using a HPLC method, we have found that the cellular amino acid composition is almost identical between heparin treated cells and heparin untreated cells, although hydroxyproline concentra-

tion is reduced by heparin in rat hepatoma cells (Akimoto et al., in press). These results have led us to examine whether a suitable HPLC method could be used to identify differences between two cell groups at the cellular level. If these differences could be detected by a HPLC method which is capable of rapid analysis, this would be a convenient method to investigate cellular changes without the purification of certain compounds. A simple and rapid method is required to analyze a large number of samples. Therefore, the present study has been designed to determine whether the amino acid composition differs after the long period of cell culture required to establish a cell line, or after cell transformation. Also, the differences between mammalian and bacterial cells were examined. The amino acid compositions of mammalial cells were found to be similar, but mammalian and bacterial cells had different amino acid compositions.

Materials and methods

Chemicals

A mixture of 17 types of authentic amino acids (H type) was purchased from Wako Pure Chemical Co. (Osaka, Japan). The H type contained 2.5 μmol/ml of asparatic acid (Asp), glutamic acid (Glu), serine (Ser), glycine (Gly), histidine (His), threonine (Thr), alanine (Ala), arginine (Arg), proline (Pro), tyrosine (Tyr), valine (Val), methionine (Met), cysteine (Cys), isoleucine (Ile), leucine (Leu), phenylalanine (Phe), and lysine (Lys). The reagents for the mobile-phases and hydrolysis were obtained from Wako Pure Chemical Co. The reagents used were acetonitorile, methanol, distilled water, sodium acetate, acetic acid, hydrochloric acid and phenol. Triethylamine was purchased from Pierce (Rockford, U.S.A.). Ethanol and phenylisothiocyanate were purchased from Wako Pure Chemical Co. These reagents were of HPLC grade or sequential grade.

Cell culture

Rat hepatocytes were prepared according to the method of Hasegawa et al. (1982). The strains used were as follows: Rat hepatoma cells; R-Y121B (Niwa et al., 1987), M (Katsuta and Takaoka, 1968), monkey hepatocarcinoma cells; NCLP-6E (Dawe et al., 1968), human urinary bladder carcinoma cells; HUB-15 (Kakuya et al., 1983), human osteosarcoma cells; K and I (unpublished), Colon tumor cells; A (unpublished), rat fibroblasts, 3Y1-B1-6 (Kimura et al., 1975) and Ad12-3Y1-Z19 (Zaitsu et al., 1988). The cells were cultured in Eagle's minimum essential medium, modified Eagle's minimum essential medium (Yasumura et al., 1978) or DM-160 (Katsuta and Takaoka, 1976) containing 0.5–10% fetal bovine serum. Cells were harvested in phosphate buffered saline using a silicone-rubber policeman and centrifuged at 2,000 rpm for 10 min. The cells were resuspended in H_2O and homogenized and 50 μl aliquots were used for hydrolysis.

Bacteria

E. coli and *S. aureus* were cultured on agar plates containing bouillon for 24 h at 37°C. Bacteria were suspended in phophate buffered saline and maintained at 65°C for 30 min for pasteurization. The cells were centerifuged at 10,000 rpm for 10 min and then homogenized in H_2O.

Apparatus

Sample hydrolysis and derivatizations were performed with the Water Pico-Tag workstation (Millipore, Waters Chromatography Division, Milford, MA, U.S.A.). The HPLC system consisted of a program controller (M-600), a solvent system (M-600), an tunable UV/VIS detector (M-486), a data module (M-741), and a column heater (TCM) obtained from Waters. A reverse phase column containing 5 μm beads of ODS (WS-5C18, 250 mm $\times$ 4.6 mm I.D.) was purchased from Wako Pure Chemical Co. The column was maintained at 40°C. An amino acid analyzer (HITACHI L-8500A) was also used to analyze cellular amino acids.

Amino acid standard and sample preparation

The authentic amino acids were dissolved in 0.1 N hydrochloric acid to make a 0.1 μmol/ml solution. m-Bromobenzoic acid (Sigma Chemical Co.) solution was prepared with solvent A (described later) containing 5% methanol and used as the internal standard. Sample hydrolysis and derivatization were performed using a preparation module Pico-Tag workstation.

Derivatization

Phenylisothiocyanate (PITC), which is known as the Edman reagent, was used for the derivatization of amino acids. The phenylisothiocalbamyl (PTC)-amino acids obtained were analyzed by the method described below. Twenty microliters of a mixture of ethanol, water, triethylamine (2:2:1) was added to each tube containing the hydrolyzed sample. The mixtures were dried under reduced pressure, and 20 μl of the derivatizing solution (ethanol: triethylamine: distilled water: phenylisothiocyanate, 7:1:1:1) was then added to each tube and mixed well. After the derivatization at room temperature for 20 min, the derivatized samples were dried completely under reduced pressure. The PTC-authentic amino acids were dissolved in 10 μl of solvent A to make a 2.5 μmol/ml internal standard. The solution was mixed well, and a sample (10 μl) was then applied to the column.

Chromatography

Analysis was performed using three solvents (A, B and C) as the mobile phase. Solvent A was 50 ml of acetonitorile mixed with 950 ml of 50 mM sodium acetate buffer, pH 6.0. Solvent B was a mixture of 575 ml acetonitorile, 400 ml distilled water and 25 ml methanol. Solvent C was a 500 ml methanol. All solvents were passed through a 0.45 μm filter (Millipore Co., Bedford, MA, U.S.A.) before analysis. The elution program for amino acid analysis was described in our previous paper (Ikeda et al., 1991). Briefly, elution was carried out using a linear gradient of three solvents (A, B, and C). Flow rate was 1.0 ml/min starting with 95% A solution and 5% B, then increased to 45% A, 50% B and 5% C for 23 min. The flow rate increased linearly from 1.0 ml/min to 1.23 ml/min over this time.

Results

Analysis of 17 amino acids by HPLC

The phenylisothiocyanate (PITC)-derivatives of 17 amino acids were separated by high performance liquid chromatography using the octa-decyltrichloro-silane (ODS) column shown in Fig. 1. There was complete

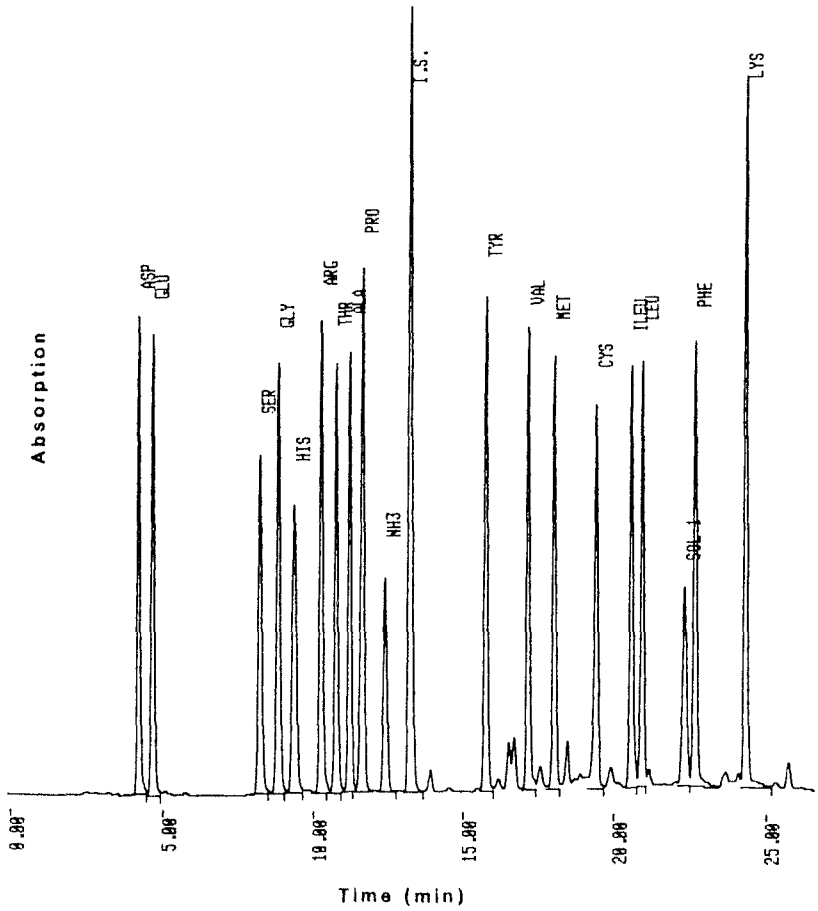

Fig. 1. HPLC elution profile of authentic amino acids derivatized with phenylisothiocyanate. The concentration of each amino acid was 0.2 nmol

separation of the amino acids. This is consistent with our previous result (Ikeda et al., 1991). All amino acids were eluted within 25 min using this column. The total elution time plus washing period was 37 min.

The peak area was determined by the width at the half peak height. The relationship between the peak area and concentration was linear in a range of 50 to 1,000 pmol (Ikeda et al., 1991).

The color yields of the PITC-derived amino acids were similar for all the amino acids. The amino acid composition was expressed by the percentage of total amino acids in the sample.

Amino acid composition of M cells

The elution profile of M cell amino acids is shown in Fig. 2. The elution profile was simple and large unknown peaks were not observed. This shows that the present HPLC method is applicable to the analysis of cellular amino acid composition.

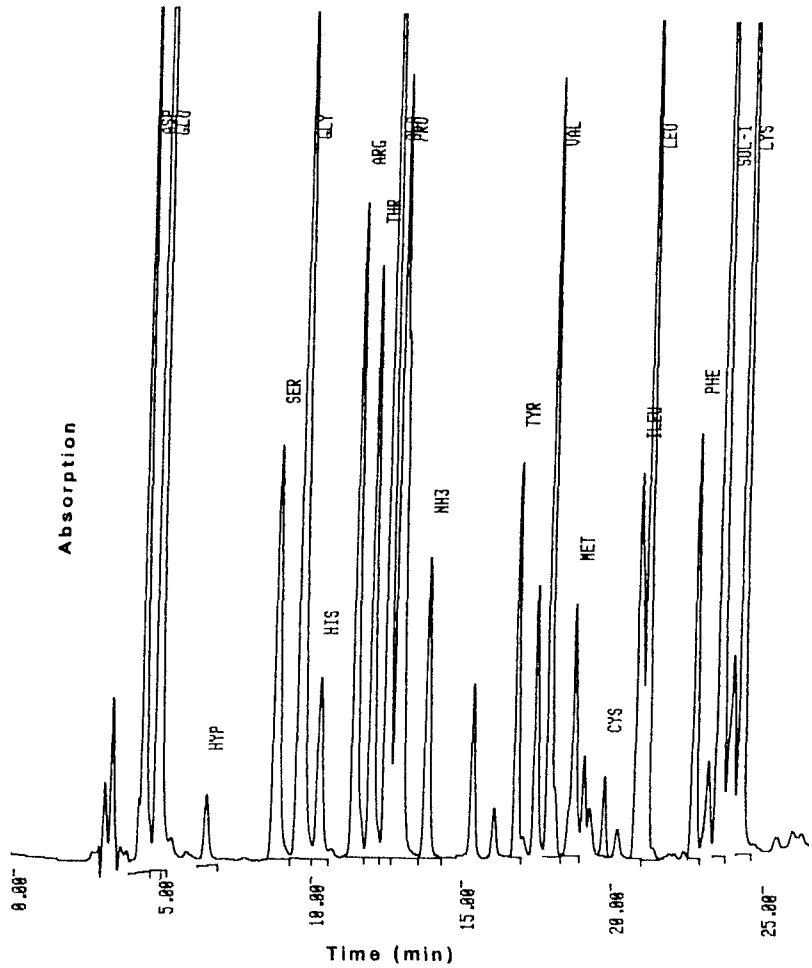

Fig. 2. HPLC elution profile of M cell lysates derivatized with phenylisothiocyanate

Nine different cell preparations were hydrolyzed, derivatized and applied to the HPLC column. The amino acid compositions are shown in Table 1. The mean values and standard deviations (S.D.) obtained from a total of 9 experiments were almost identical to those obtained from 3 experiments chosen at random from among the 9 experiments. Therefore, the values obtained from at least 3 experiments were used in subsequent experiments.

The concentration of hydroxyproline of M cells, which are known to be very active in collagen synthesis (Sorimachi, 1992), was less than 1% of the total amino acids (data not shown). Therefore, hydroxyproline was neglected in the present study.

Comparison of two different methods

The amino acid composition of M cells was analyzed by HPLC and an amino acid analyzer. Both methods gave similar results (Table 2). However, the

Table 1. Amino acid compositions of M cells and comparison of the values based on small experimental groups

	Total (n = 9)	Group I	Group II	Group III
Asp	8.61 ± 1.20	8.81 ± 1.15	9.19 ± 1.57	8.49 ± 1.14
Glu	11.79 ± 0.58	12.22 ± 0.39	11.70 ± 0.37	11.71 ± 1.06
Ser	6.19 ± 0.40	6.16 ± 0.39	5.99 ± 0.57	6.12 ± 0.09
Gly	9.12 ± 0.70	9.32 ± 0.84	8.46 ± 0.24	9.70 ± 0.45
His	2.23 ± 0.18	2.33 ± 0.09	2.23 ± 0.16	2.30 ± 0.13
Arg	4.76 ± 0.27	4.84 ± 0.11	4.80 ± 0.37	4.72 ± 0.33
Thr	5.35 ± 0.21	5.39 ± 0.23	5.28 ± 0.28	5.26 ± 0.13
Ala	9.20 ± 0.43	8.93 ± 0.30	9.02 ± 0.38	9.35 ± 0.69
Pro	5.36 ± 0.76	5.69 ± 0.70	4.72 ± 0.28	6.05 ± 0.35
Tyr	2.74 ± 0.09	2.70 ± 0.07	2.77 ± 0.14	2.73 ± 0.05
Val	6.74 ± 0.39	6.49 ± 0.11	6.66 ± 0.22	6.87 ± 0.73
Met	2.44 ± 0.20	2.24 ± 0.17	2.49 ± 0.16	2.45 ± 0.18
Cys	0.25 ± 0.12	0.18 ± 0.04	0.32 ± 0.16	0.19 ± 0.02
Ile	5.78 ± 0.45	5.38 ± 0.22	6.01 ± 0.46	5.59 ± 0.58
Leu	9.58 ± 0.68	8.94 ± 0.37	9.86 ± 0.44	9.26 ± 0.87
Phe	3.74 ± 0.12	3.64 ± 0.15	3.78 ± 0.06	3.64 ± 0.15
Lys	7.64 ± 0.83	7.65 ± 0.99	8.42 ± 0.48	6.93 ± 0.74

The values are expressed by percentages of total amino acids, and are the means ± S.D. "Total" is based on the values obtained for 9 samples. Groups I, II, and III are based on the values for 3 samples chosen at random from among 9 samples. The differences are not statistically significant.

Table 2. Comparison of amino acid compositions of M cells measured with HPLC and an amino acid analyzer

	HPLC	A.A.A.
Asp	8.61 ± 1.69	8.35 ± 0.28
Glu	13.93 ± 1.32	12.24 ± 0.29
Ser	4.96 ± 1.18	6.64 ± 0.25
Gly	9.10 ± 1.15	10.86 ± 0.49
His	2.07 ± 0.10	1.85 ± 0.03
Arg	5.34 ± 0.17	5.83 ± 0.01
Thr	5.30 ± 0.18	5.70 ± 0.54
Ala	11.04 ± 1.34	9.10 ± 0.20
Pro	6.33 ± 1.26	5.19 ± 0.07
Tyr	2.66 ± 0.18	2.39 ± 0.22
Val	5.46 ± 0.42	5.88 ± 0.09
Met	2.14 ± 0.34	0.73 ± 0.86
Cys	0.54 ± 0.38	0.19 ± 0.31
Ile	4.52 ± 0.32	4.52 ± 0.11
Leu	8.24 ± 0.55	9.33 ± 0.31
Phe	3.45 ± 0.10	3.75 ± 0.19
Lys	6.31 ± 0.62	7.44 ± 0.07

The values are expressed by percentages of total amino acids, and are the means ± S.D. for 5 independent experiments. The differences are not statistically significant.

amino acid analyzer indicated a lower concentration of Met than HPLC, and there was greater variation in the Met concentrations determined for different samples. In addition, the amino acid analyzer took almost 2.5 h to analyze the 17 amino acids of one sample. Therefore, the HPLC method was used to analyze the amino acid compositions of the various samples used in this study. These results indicate that the present HPLC method has a good reproducibility.

Effect of ethanol precipitation on amino acid composition

In order to determine the concentration of free amino acids contained in the cells, cell homogenates were mixed with ethanol to precipitate the proteins, which were then hydrolyzed for amino acid analysis. However, as ethanol precipitation produced no significant effect, as shown in Table 3, whole cells were directly hydrolyzed in subsequent experiments.

Table 3. Amino acid compositions of the cultured cells with and without ethanol precipitation

	R-Y121B	R-Y121B(A)
Asp	9.87 ± 0.93	10.51 ± 0.37
Glu	12.92 ± 0.58	13.31 ± 1.11
Ser	6.59 ± 0.20	6.71 ± 0.15
Gly	10.75 ± 1.63	11.10 ± 0.63
His	2.27 ± 0.17	2.49 ± 0.15
Arg	5.46 ± 0.51	6.36 ± 0.55
Thr	5.47 ± 0.10	5.60 ± 0.07
Ala	9.34 ± 0.97	8.64 ± 0.42
Pro	5.01 ± 1.30	5.21 ± 0.16
Tyr	2.59 ± 0.08	2.70 ± 0.10
Val	6.41 ± 0.21	6.54 ± 0.37
Met	2.00 ± 0.19	1.42 ± 0.23
Cys	0.30 ± 0.04	0.35 ± 0.23
Ile	5.47 ± 0.44	4.84 ± 0.50
Leu	8.24 ± 1.73	7.53 ± 0.91
Phe	3.39 ± 0.15	3.32 ± 0.00
Lys	6.65 ± 1.07	5.89 ± 0.00

Rat hepatoma cells (R-Y121B) were homogenized and used for amino acid analyses. R-Y121B(A); aliquots of cell homogenates were mixed with ethanol to make a final concentration of 70% ethanol. The solution was centrifuged at 10,000 rpm for 10 min to separate the precipitates. The precipitates were used for amino acid analyses. The values are expressed by percentages of total amino acids, and are the means ± S.D. of 3 samples. The differences are not statistically significant.

Table 4. Amino acid compositions of cultured cells

	Rat-hep	NCLP-6E	3Y1-B1-6	Ad12-3Y1-Z19	HUB-15	K	I	A
Asp	10.66 ± 1.76	10.13 ± 1.21	9.79 ± 0.11	9.70 ± 0.27	10.13 ± 0.85	10.46 ± 0.60	10.17 ± 1.12	9.92 ± 1.07
Glu	11.80 ± 1.42	13.87 ± 0.40	12.14 ± 0.22	12.06 ± 0.28	12.86 ± 0.81	12.77 ± 0.65	12.38 ± 0.69	12.41 ± 0.32
Ser	6.10 ± 0.79	6.34 ± 0.16	6.08 ± 0.18	5.63 ± 0.22	6.19 ± 0.38	6.20 ± 0.36	5.86 ± 0.30	6.43 ± 0.40
Gly	9.10 ± 0.58	9.78 ± 0.72	8.44 ± 0.44	8.82 ± 0.31	9.44 ± 1.45	8.79 ± 0.73	9.30 ± 1.12	7.80 ± 0.30
His	2.27 ± 0.21	2.26 ± 0.10	2.00 ± 0.10	2.10 ± 0.13	2.26 ± 0.13	2.14 ± 0.08	2.20 ± 0.05	2.06 ± 0.04
Arg	4.58 ± 0.80	5.17 ± 0.42	4.53 ± 0.49	5.02 ± 0.42	5.04 ± 0.59	4.54 ± 0.23	4.62 ± 0.24	4.09 ± 0.56
Thr	5.46 ± 0.59	5.85 ± 0.27	5.23 ± 0.20	4.92 ± 0.07	5.18 ± 0.13	5.23 ± 0.21	5.17 ± 0.29	5.47 ± 0.40
Ala	9.20 ± 0.26	9.13 ± 1.32	8.45 ± 0.57	8.60 ± 0.53	9.38 ± 1.50	8.91 ± 0.36	8.53 ± 0.16	8.50 ± 0.23
Pro	5.85 ± 2.06	6.51 ± 1.87	4.87 ± 0.11	4.60 ± 0.23	5.06 ± 1.80	5.11 ± 0.39	5.12 ± 0.16	4.72 ± 0.19
Tyr	2.93 ± 0.43	2.57 ± 0.29	2.87 ± 0.17	2.77 ± 0.27	2.63 ± 0.11	2.56 ± 0.15	2.72 ± 0.23	2.96 ± 0.16
Val	6.98 ± 0.88	6.03 ± 0.28	6.79 ± 0.41	6.52 ± 0.38	6.70 ± 0.29	6.50 ± 0.50	6.31 ± 0.49	6.52 ± 0.37
Met	2.48 ± 0.29	2.12 ± 0.54	2.40 ± 0.06	2.34 ± 0.07	2.04 ± 1.24	2.35 ± 0.09	2.20 ± 0.20	2.53 ± 0.07
Cys	0.26 ± 0.14	0.26 ± 0.01	0.45 ± 0.10	0.38 ± 0.08	0.24 ± 0.12	0.24 ± 0.06	0.37 ± 0.21	0.22 ± 0.08
Ile	6.06 ± 0.90	5.08 ± 0.54	5.88 ± 0.50	5.61 ± 0.38	5.88 ± 0.82	5.57 ± 0.74	5.63 ± 0.37	6.33 ± 0.47
Leu	8.94 ± 1.66	7.77 ± 1.10	9.64 ± 0.58	9.03 ± 0.50	9.29 ± 1.38	9.16 ± 0.77	8.97 ± 0.77	10.13 ± 0.52
Phe	4.36 ± 0.19	3.17 ± 0.27	4.03 ± 0.11	3.82 ± 0.03	3.73 ± 0.21	3.60 ± 0.24	3.62 ± 0.32	3.83 ± 0.20
Lys	6.37 ± 2.04	6.37 ± 0.84	9.00 ± 1.44	10.07 ± 1.08	7.43 ± 0.33	7.00 ± 0.52	6.42 ± 0.62	7.21 ± 0.15

The values are the means ± S.D. of 3 or 4 samples.

Amino acid composition of mammalian cell lines

M cells were derived from rat hepatocytes (Katsuta and Takaoka, 1968). To determine whether amino acid compositions differ between primary culture and established culture cells, the primary culture cells of rat hepatocytes were hydrolyzed and the amino acids were analyzed by the HPLC method. No significant difference in amino acid composition was observed between M cells and rat hepatocytes, as shown in Table 4. Monkey hepatocarcinoma cells (NCLP-6E) (Dawe et al., 1968) were used to investigate the effect of species differences. However, our results indicated that there is no significant difference in amino acid composition betwee M cells and NCLP-6E cells.

Rat fibroblasts (3Y1-B1-6) were derived from rat fetus (Kimura et al., 1975) and Ad12-3Y1-Z19 cells were obtained by the transformation of 3Y1-B1-6 cells with adenovirus containing the E1A gene (Zaitsu et al., 1988). The amino acid compositions of these two cell lines were similar and no significant difference was observed, as shown in Table 4.

To determine whether amino acid composition is altered in tumor cells, the following tumor cell lines were used; human urinary bladder carcinoma (HUB-15), osteosarcoma; (K) and (I), colon tumor; (A). The amino acid compositions were similar in these cell lines, as shown in Table 4.

Amino acid composition of bacterial cells

As described above, the amino acid compositions were similar in mammalian cells. To determine whether this rule is applicable to bacteria, *E. coli* and

Table 5. Amino acid compositions of mammalian and bacterial cells

	S. aureus	*E. coli*	Mammalian cells
Asp	9.90 ± 1.08	11.00 ± 1.12	10.08 ± 0.59
Glu	11.37 ± 0.79*	11.30 ± 0.64*	12.62 ± 0.63
Ser	4.47 ± 0.66*	4.87 ± 0.36*	6.19 ± 0.16
Gly	16.43 ± 0.74*	9.07 ± 0.22	9.14 ± 0.57
His	1.53 ± 0.17*	1.96 ± 0.15*	2.22 ± 0.07
Arg	2.80 ± 0.63*	4.40 ± 0.60	4.74 ± 0.32
Thr	4.23 ± 0.24*	5.58 ± 0.25	5.42 ± 0.22
Ala	14.95 ± 0.64*	11.97 ± 0.68*	9.03 ± 0.32
Pro	3.42 ± 1.07*	4.37 ± 1.07**	5.51 ± 0.58
Tyr	2.22 ± 0.38*	2.67 ± 0.26	2.73 ± 0.14
Val	5.88 ± 1.00	8.23 ± 1.04*	6.54 ± 0.27
Met	1.88 ± 0.15*	2.92 ± 0.15*	2.27 ± 0.14
Cys	0.18 ± 0.12	0.19 ± 0.06**	0.26 ± 0.04
Ile	5.94 ± 1.08	7.00 ± 0.98*	5.73 ± 0.35
Leu	6.22 ± 1.49*	9.46 ± 1.21	9.00 ± 0.68
Phe	3.32 ± 0.32**	4.04 ± 0.44	3.73 ± 0.31
Lys	7.82 ± 1.37	5.52 ± 1.61	6.85 ± 0.49

The values are the means ± S.D. of 8 samples. Statistical differences between mammalian cells and bacterial cells were evaluated using Student's *t*-test (*$P < 0.01$, **$P < 0.05$).

S. aureus which are well known examples of Gram-negative and -positive bacteria respectively, were used. The amino acid compositions are shown in Table 5. The concentrations of glycine, alanine and lysine were much higher in *S. aureus* than those in *E. coli*, while the concentrations of arginine, threonine, valine and leucine were lower in *S. aureus*.

Discussion

Transformed rat fibroblasts (Ad12-3Y1-Z19) respond to lignin derivatives (Sorimachi et al., 1997) or liposomes (Shimura et al., 1988), and the cells die after cell treatment with lignin derivatives or liposomes. However, the parent cells (3Y1-B1-6) are unaffected by lignin derivatives or liposome. These results suggest that the transformation of cells (3Y1-B1-6) with adenovirus induces changes in the cellular characteristics, and that there are certain differences between the parent and transformed cells. The effects of polyanions such as acetyl lignin, sulfonyl lignin and dextran sulfate on cell growth differs among various cell lines (Sorimachi et al., 1997). On the other hand, the amino acid compositions of both 3Y1-B1-6 and Ad12-3Y1-Z19 cell lines were found to be almost the same (Table 4). This means that the transformation of 3Y1-B1-6 cells by adenovirus or carcinogenesis does not induce any change in amino acid composition that can be detected by the present HPLC method.

There is no absolute method of comparison for two groups consisting of many factors. However, a graph may be used to show the quantitative differences due to numerous factors. Thus, amino acid compositions were graphically expressed as shown in Fig. 3. The amino acid composition of M cells is shown in Table 2. When other hepatocyte graphs were overlaid on the M cell

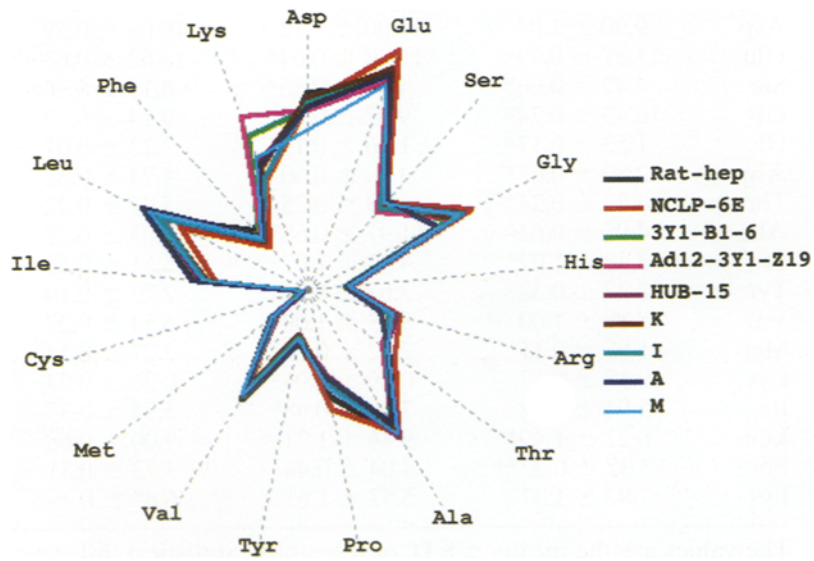

Fig. 3. Radar graphs of amino acid composition of various mammalian cells

graph, significant differences were not observed. Furthermore, no significant differences were observed in the overlaying of tumor cell graphs with the other graphs. These results are consistent with comparisons of each individual value, as described in the results section.

The *E. coli* and *S. aureus* amino acid compositions were also shown as graphs (Fig. 4). There is a clear difference between the *E. coli* and *S. aureus* graphs. When these two graphs were overlaid with the mammalian cell graphs obtained from all the mammalian cells used, significant differences in amino acid compositions were observed among these three graphs. However, the mammalian cell graph has greater similarity with the *E. coli* graph than with the *S. aureus* graph.

In order to express the difference in amino acid composition quantitatively, the differences in the paired percentage for each amino acid in the two samples was summed and the total differences were compared among the various samples. This may be used to express the degree of difference in

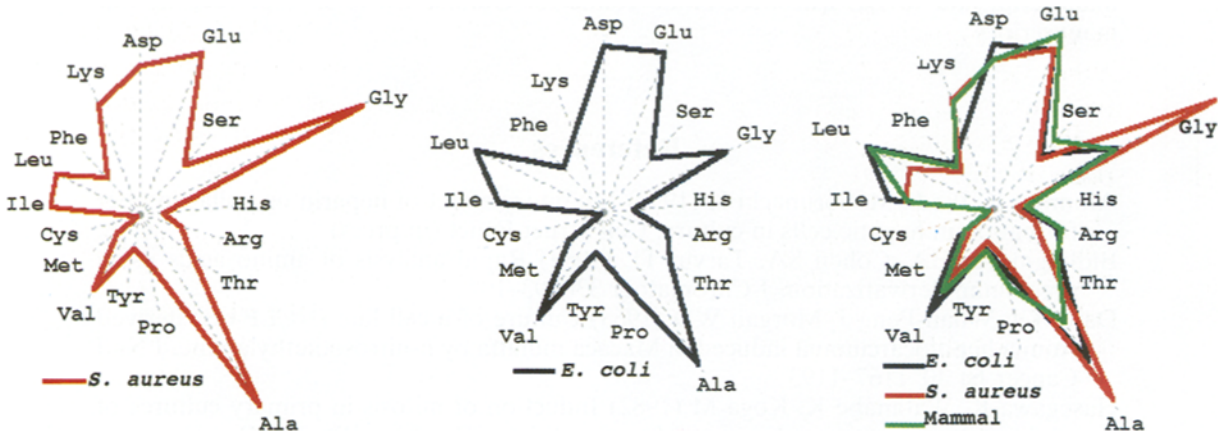

Fig. 4. Radar graphs of amino acid composition of bacterial and mammalian cells. The mammalian cell graph represents the average of 9 different cultured cells

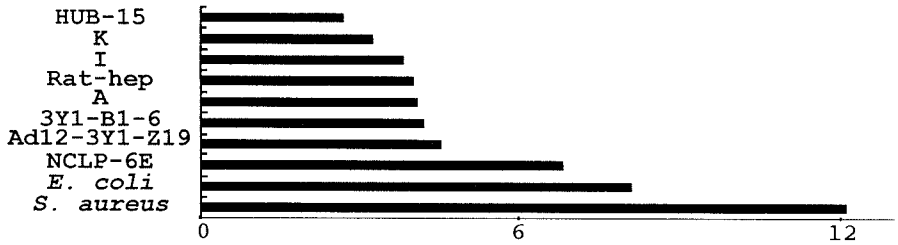

Fig. 5. Degrees of difference in amino acid composition between M cells and the other cell samples. To quantify the differences in amino acid composition among the various samples, the absolute value of the difference in the amino acid percentage between a standard and another sample of the same amino acid was divided by the largest absolute value of the difference of this group of samples. Then, the quotients of the 17 amino acids in each cell sample were summed up. The summations were compared for the various samples

amino acid composition (Fig. 5). The difference in amino acid composition between mammalian cells and *S. aureus* was the greatest, and the difference between M cells and *E. coli* was greater than those betwee M cells and other mammalian cells.

However, the present HPLC method was not able to detect any differences in amino acid composition between normal and transformed cells, or among the various mammalian cell llines. However, this indicates that cellular amino acid compositions are almost constant in mammalian cells, including tumor cells and transformed cells.

The present results suggest that mammalian cells are closer to *E. coli* than *S. aureus* in the evolutionary tree, and that *E. coli* and *S. aureus* are highly diverged.

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