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 mammalian 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 acetonitrile, 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 (Zaitso 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 H2O 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 phosphate buffered saline and maintained at 65°C for 30 min for pasteurization. The cells were centrifuged at 10,000 rpm for 10 min and then homogenized in H2O.