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Monitoring changes over time of amino acids in cell culture supernatants by high-speed amino acid analysis

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1. Introduction

Since amino acids are closely related to energy acquisition and protein synthesis, it is important to measure changes in amino acid concentrations over time in culture media for cell growth and production of useful substances. Generally, amino acids are derivatized by *o*-phthalaldehyde derivatization, ninhydrin derivatization, or other methods, and detected by a UV detector or a fluorescence detector. These detection methods require longer analysis time to sufficiently separate amino acids and insufficient separation from foreign components results in a decrease in the accuracy of quantitation.

To solve this problem, we used the amino acid analysis system "UF-Amino Station". The system combines pre-column derivatization with (3-pyridyl) carbamic acid succinimidyl ester (APDS) reagent and detection by mass spectrometer. This system combines fast separation in reversed-phase mode by pre-column derivatization and detection by mass spectrometer, allowing short analytical time. In addition, since a mass spectrometer is used, amino acids can be selectively detected.

In this study, we used this system to monitor changes over time in the concentration of amino acids in cell culture supernatant.

2. Methods

CHO-K1 cells were cultured in batch cultures and the culture supernatant was collected every 24 hours. The supernatants were diluted 10-fold with ultrapure water. A double volume of acetonitrile was added to the diluted supernatant, mixed thoroughly, and centrifuged at 15,000 rpm for 10 minutes. The supernatant was then collected, followed by the addition of an equal volume of a solution containing the internal standard mixture and another 2-fold volume of acetonitrile to the sample for analysis. The UF-Amino Station (Fig.1) was used for analysis.

Fig.1 Shimadzu UF-Amino Station

The reaction equation of derivatization of amino acids by ADPS is shown in Fig. 2. This derivatization (mixing the sample and derivatization reagent and solvent, heating it for derivatization reaction, and injecting) was performed automatically by using the pretreatment function of the autosampler. In sequential analysis, the derivatization of next sample is automatically performed during the analysis.

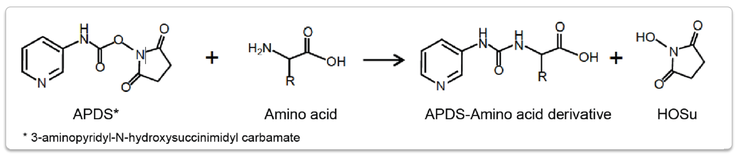

Fig.2 Reaction formula for amino acid derivatization

Table 1. Analytical Conditions

Column	:	Shim-pack UF-Amino (100 mm L. x 2.1 mm I.D., 2 µm)
Mobile phase	:	A: APDSTAG® Wako Eluent, B: Acetonitrile Gradient elution
Flow rate	:	0.3 mL/min
Column temperature	:	40 ℃
Reaction reagent	:	Amino acid analysis reagent (For LC/MS)(APDSTAG®)
Reaction solvent	:	APDSTAG® Wako Borate Buffer
Detector	:	LCMS-2020, ESI Probe
Injection volume	:	5 µL

3. Results

The chromatogram of a standard amino acid sample is shown in Fig.3. The 38 amino acid components are separated in 9 minutes (additionally 3 minutes for column equilibration) and detected by a mass spectrometer.

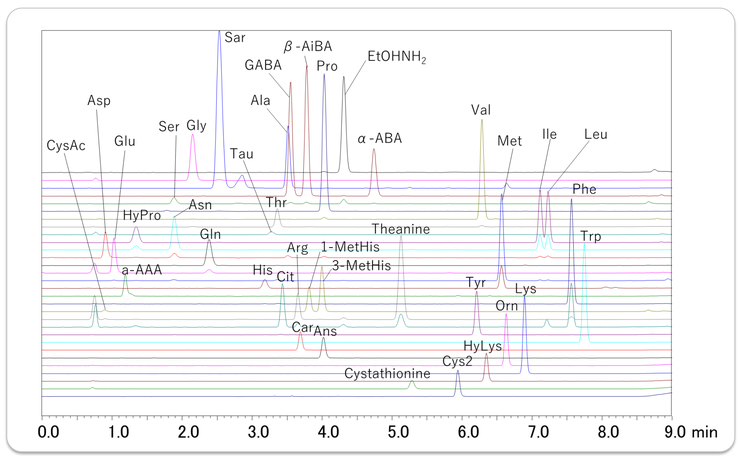

Fig.3 Chromatograms of 38 amino acid component analysis

Table 2 Linearity of 38 amino acids (Concentration : µmol/L)

	Range	Coefficient r^2		Range	Coefficient r^2		Range	Coefficient r^2		Range	Coefficient r^2
CysAc	25-500	0.9870	His	5.0-500	0.9981	Ans	5.0-500	0.9986	HyLys	2.5-300	0.9941
Asp	2.5-500	0.9986	Tau	5.0-250	0.9989	b-AiBA	2.5-500	0.9994	Met	2.5-500	0.9990
Glu	2.5-500	0.9998	Thr	2.5-500	0.9986	Pro	2.5-500	0.9984	Orn	2.5-500	0.9997
a-AAA	2.5-250	0.9992	Cit	5.0-500	0.9970	EtOHNH2	2.5-200	0.9981	Lys	5.0-500	0.9999
HyPro	2.5-500	0.9979	Ala	5.0-500	0.9999	a-ABA	2.5-200	0.9979	Ile	5.0-500	0.9997
Asn	2.5-500	0.9998	1-MetHis	2.5-500	0.9989	Theanine	5.0-200	0.9982	Leu	5.0-500	0.9985
Ser	5.0-500	0.9993	Car	2.5-500	0.9986	Cysthi	2.5-200	0.9990	Phe	5.0-500	0.9999
Gly	2.5-500	0.9998	Arg	2.5-500	0.9995	Cys2	2.5-200	0.9882	Trp	5.0-500	0.9997
Gln	5.0-500	0.9998	GABA	2.5-500	0.9994	Tyr	5.0-500	0.9999			
Sar	10-500	0.9919	3-MetHis	2.5-500	0.9996	Val	2.5-500	0.9993			

The amino acid concentrations monitoring every 24 hours under each of the two cell culture condition (medium A, B) are shown in Fig.4. As a result of measuring changes in the concentration of amino acids in the culture medium using this method, consumption and secretion associated with culture were observed.

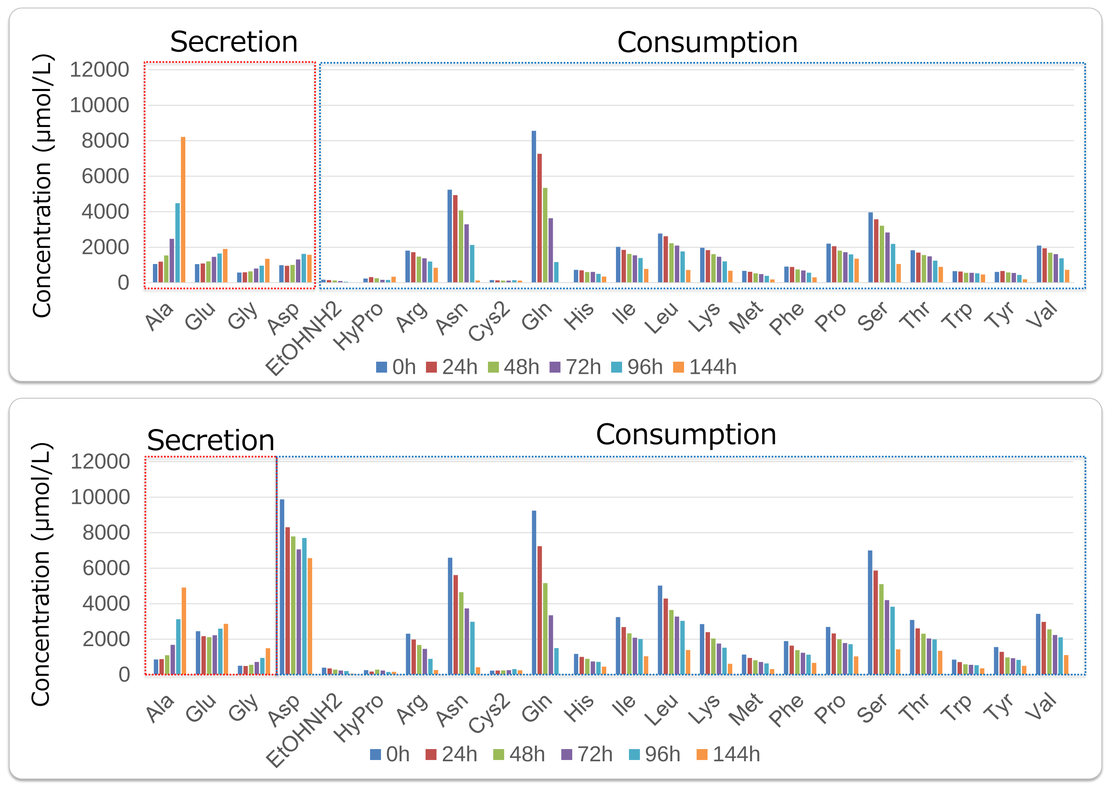

Fig.4 Amino acid concentration in cell culture medium (upper: medium A, lower: medium B)
* Not detected amino acids are not shown. *At 120h without sampling

It is known that each component in the medium is correlated and that changing one element will affect the others. So, rather than analyzing each measurement independently, all measurements were comprehensively analyzed using principal component analysis (PCA) to visualize their differences and identify each sample (Fig.5).

In the score plot, the medium A and B were categorized in each cluster, and the plots of both media tended to shift negatively in the X-axis direction and positively in the y-axis direction over time. The loading plot indicated that the medium A and B are characterized by the concentrations of Ala (alanine), Gln (glutamine), and Asp (aspartic acid). This suggested that the concentrations of these four components may be related to the cell concentration approximately 1.5 times higher in medium A than in medium B.

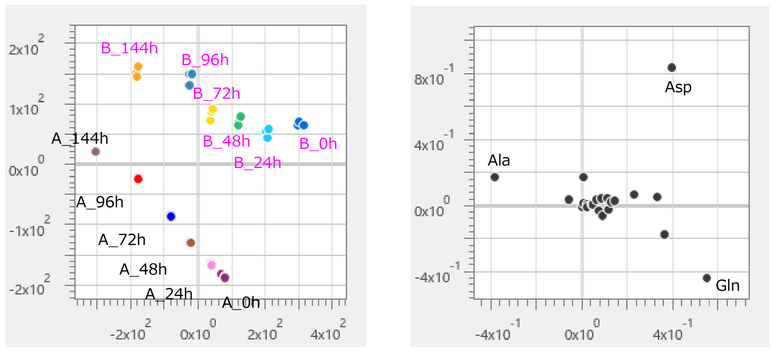

Fig.5 The results of PCA of amino acid concentration of medium A and B.
（left）Score plot、（right）Loading plot

The recovery rates including amino acid derivatization efficiency of UF-Amino Station are shown in Fig.6. The samples were prepared by adding the amino acid standards to the supernatant after deproteination. This results indicated that the recovery rates are approximately 90~110%.

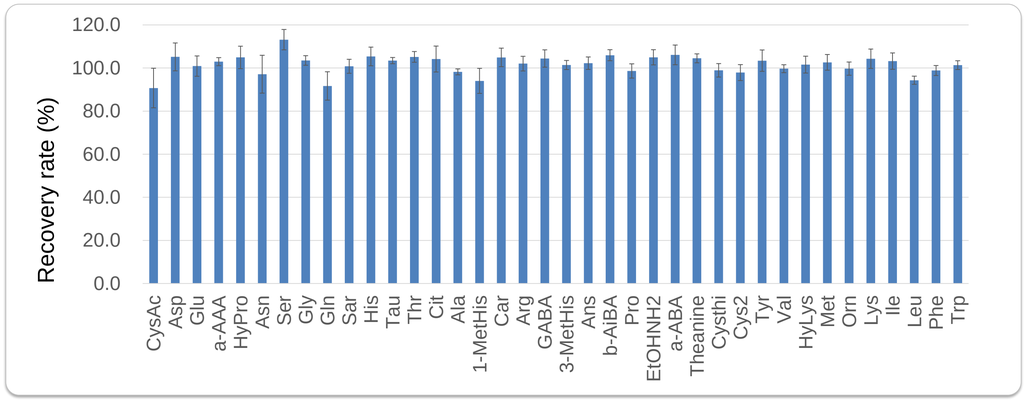

Fig.6 Recovery rate of 38 amino acids at UF-Amino Station

4. Conclusion

- ✓ Using the UF-Amino Station, 38 amino acid components were analyzed simultaneously in 9 minutes.
- ✓ This system can be used to measure amino acid concentrations in cell culture supernatants in a short time and the PCA can be used for data analysis. They provide information that supports to the optimization of culture conditions, including feed conditions.

Thanks

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