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Characteristic Patterns of Free Amino Acid Content in Plasma, Erythrocytes, Lymphocytes, and Granulocytes in Man*)

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ABSTRACT

The concurrent concentrations of free amino acids in plasma, erythrocytes, lymphosytes, and granulocytes are investigated in healthy men. The concentrations of most amino acids, except for aspartic acid which is much higher in erythrocytes, are more or less similar in plasma and erythrocytes. In lymphocytes the levels of taurine and aspartic acid are 100 and 200 times higher than the plasma levels, respectively, while in granulocytes the levels of taurine and aspartic acid are 300 and 100 times higher than the plasma levels, respectively. Aspartic acid in blood cells may play an important role common to the formed compartments of the blood, while taurine may play important roles in functions peculiar to leukocytes, especially in granulocytes.

INTRODUCTION

An understanding of the roles of free amino acids in blood cells requires information not only about their concentrations in cells but also about their distribution between the plasma and the cells. No simultaneous measurement of free amino acids in plasma, erythrocytes, lymphocytes, and granulocytes has yet been reported. The methods for the measurement of free amino acids in lymphocytes and granulocytes have been reported in our previous communication[2].

In this paper we describe methods for the measurement of concurrent concentrations of free amino acids in the four blood compartments, and report the results obtained from the study of 18 healthy men. The amino acid distributions between the plasma and the three formed compartments of the blood are also compared and the meanings of the characteristic predominances of some amino acids in the different cell types are discussed.

MATERIALS AND METHODS

Preparation of plasma, erythrocyte, lymphocyte, and granulocyte samples

Heparinzed venous blood was donated from 18 fasting healthy men. After centrifuagation at 150×g for 10 min, the platelet-rich plasma was removed and centrifuged at 1,500×g for 10 min. The supernatant was ragarded as platelet-free plasma and used for the measurment of plasma amino acid concentrations.

After the platelet-rich plasma was removed, we isolated mononuclear cells (MNC) by centrifugation with a Ficoll-diatrizoate sodium gradient at 400×g for 30 min. Polymorphonuclear cells (PMN) were obtained by sedimentation in saline solution with 3% dextran. Contaminating erythrocytes were lysed by treatment with Tris-buffered isotonic ammonium chloride. This solution was prepared by adding nine volumes of 0.83% NH_4Cl to one volume of Tris buffer (20.594 g of tris base per liter, adjusted to ph 7.65 with HCl). Leukocytes were washed three times and suspended in phosphate-buffered saline (PBS) that contained, per liter, 8.0 g of NaCl, 0.2 g of KCl, 2.9 g of $Na_2HPO_4 \cdot 12H_2O$, and 0.2 g of KH_2PO_4. Purity of these cell preparation was checked

*) 福田清貴，臼井朋包：成人男性の血漿，赤血球，リンパ球，顆粒球における遊離アミノ酸の特徴的パターン

microscopically. MNC preparations contained 80–85% lymphocytes, 15–20% monocytes, and less than 2% granulocytes, PMN preparations contained more than 98% granulocytes. Platelet contamination was less than one platelet for three MNC or less than one for 50 PMN, which little modified the quantitative determination of amino acids in either MNC or PMN[3].

After MNC and PMN were obtained, the remaining erythrocytes were washed twice with PBS by centrifugation at 1,500 × g for 10 min. These packed erythrocytes were used for amino acid analysis.

Chromatographic analysis

Platelet-free plasma was deproteinized with 5% trichloroacetic acid (TCA) solution containing 10^{-5}M norleucine, which served as the internal standard for the chromatographic analysis, and the supernatant was used for amino acid analysis. In order to determine the amino acid content of erythrocytes, the packed cells were hemolyzed and deproteinized with 5% TCA solution with 10^{-5}M norlecine in one step. For the extraction of free amino acids from MNC and PMN, we used the ultrasonication method, the details of which were reported in our previous communication[2].

The specimens were kept frozen at −80°C until analysis. After precipitated protein had been removed by centrifugation at 1,500 × g for 5 min, we quantitated the amino acids in the supernatant. Free amino acids were analyzed by ion-exchange chromatography, separating the compounds on TSK GEL LS-215 (Toyo Soda Co. Ltd., Tokyo, Japan) with the stepwise elution method, followed by fluorometric determination with *o*-phthalaldehyde[10].

RESULTS

The concentrations of free amino acids found in plasma are reported in Table 1. The threonine plus glutamine value was largest and glycine, alanine, valine, and lysine were also abundant. The value for aspartic acid was smallest.

The concentrations of free amino acids in blood cells are expressed in nmol per milliliter erythrocytes, milliliter MNC, ahd milliliter PMN (Table 1). They are obtained by taking the mean erythrocyte volume as 87 fl, the mean granulocyte volume as 1,022 fl, the mean lymphocyte volume as 1,221 fl and the mean monocyte volume as 2,145 fl[5].

In erythrocytes the value for threonine plus

Table 1. Concentrations (nmol/ml) of free amino acids in plasma, erythrocytes, MNC and PMN

Amino acid	Plasma	Erythrocytes	MNC	PMN
Taurine	39.11± 5.79*	38.40± 4.68	3637.02±482.28	12053.82±1813.11
Aspartic acid	6.22± 1.05	294.40±124.38	1347.79±249.46	591.98± 103.72
Threonine+glutamine	820.84±142.11	572.72±146.48	149.67± 28.92	636.01± 136.99
Serine+asparagine	155.86± 25.32	191.22± 27.34	122.20± 32.54	289.63± 60.67
Glutamic acid	86.48± 12.04	244.89± 54.46	2024.58±227.77	902.15± 156.56
Glycine	267.27± 64.30	423.07± 75.34	214.75± 52.78	378.67± 73.39
Alanine	411.90± 89.68	333.60± 88.66	188.00± 37.60	266.14± 38.16
Valine	262.61± 59.50	32.44± 11.75	45.56± 7.95	64.58± 13.70
Methionine	27.67± 10.23	8.82± 2.91	36.88± 33.98	80.23± 73.39
Isoleucine	72.71± 13.83	5.97± 2.55	20.25± 4.63	41.10± 9.10
Leucine	131.72± 22.59	35.13± 7.14	36.88± 7.09	66.54± 14.68
Tyrosine	67.37± 14.73	37.76± 11.22	24.58± 4.92	58.71± 9.20
Phenylalanine	62.45± 6.35	6.59± 5.38	17.35± 3.76	31.31± 7.24
Histidine	89.18± 15.14	81.34± 12.26	41.21± 7.95	109.59± 22.50
Ornithine	60.36± 16.20	129.70± 39.13	38.32± 10.85	126.22± 40.12
Lysine	208.66± 47.05	130.36± 33.28	32.54± 7.95	120.35± 32.29
Tryptophan	66.90± 15.66	n. d.†	n. d.	n. d.
Arginine	104.65± 21.06	28.24± 8.08	30.37± 6.94	69.47± 18.59

* Mean±1SD

† n. d., not detected

glutamine was largest as in the case of plasma. Aspartic acid, glutamic acid, glycine, and alanine were also abundant in erythrocytes. Tryptophan was not detected in erythrocytes.

In MNC taurine was the most abundant free amino acid, followed by glutamic acid and aspartic acid. In PMN taurine was the only abundant free amino acid and accounted for a great part of the pool. As in the case of erythrocytes tryptophan was not detected in either MNC or PMN.

Erythrocyte/plasma, MNC/plasma, and PMN /plasma ratios were obtained from the values of Table 1, in order to compare the free amino acid levels in blood cells with plasma levels (Table 2). Although taurine levels were identical in plasma and erythrocytes, in MNC and PMN they were 100 and 300 times higher than the plasma level, respectively. Aspartic acid was abuntant in all three cell types of the blood and its levels in erythrocytes, MNC, and PMN were 50, 200, and 100 times higher than in plasma, respectively. Glutamic acid levels in MNC and PMN were 10 and 20 times higher than in plasma, while its level in erythrocytes was three times higher than in plasma.

DISCUSSION

In this study we measured concurrently concentrations of free amino acids in plasma, erythrocytes, MNC, and PMN, and compared them. The values of plasma agree reasonably well with those reported by Armstrong and Stave except for taurine, our level of which was lower than theirs since they used platelet-rich plasma[1]. Our value for taurine is comparable to that reported by Owen et al.[7] The values of erythrocytes are comparable with those reported by Marescau et al.[5], except for valine, isoleucine, phenylalanine, and arginine. Our value for arginine is higher than theirs, while our values for the other three amino acids are lower. In granulocytes the concentrations of amino acids, except for taurine, are lower than those reported by Houpert et al.[3] who expressed the values per cell count. Since they used the freezing and thawing method for amino acid extraction, their elevated values may be due to the partial proteolysis and peptidolysis as pointed out in our previous communication[2]. No quantitative determination of free amino acids in lymphocytes has yet been reported other than ours[1].

Before interpreting the amino acid concentrations we had to take into account the influence of washing the blood cells with PBS. As observed by Soupart[9], some amino acids are lost into the saline during washing the

Table 2. Erythrocyte/plasma, MNC/plasma and PMN/plasma ratios of free amino acids

Amino acid	Erythrocyte/Plasma	MNC/Plasma	PMN/Plasma
Taurine	1.00± 0.16*	94.26±14.84	313.88±61.95
Aspartic acid	50.52±21.31	221.06±47.69	96.93±20.58
Threonine+glutamine	0.70± 0.13	0.19± 0.03	0.79± 0.18
Serine+asparagine	1.24± 0.15	0.81± 0.25	1.90± 0.45
Glutamic acid	2.92± 0.81	23.64± 2.75	10.49± 1.60
Glycine	1.65± 0.39	0.81± 0.32	1.53± 0.55
Alanine	0.81± 0.13	0.47± 0.09	0.67± 0.15
Valine	0.12± 0.03	0.18± 0.04	0.26± 0.07
Methionine	0.35± 0.13	0.81± 0.74	1.98± 1.76
Icoleucine	0.08± 0.03	0.29± 0.08	0.58± 0.14
Leucine	0.27± 0.05	0.28± 0.08	0.53± 0.13
Tyrosine	0.55± 0.08	0.38± 0.09	0.89± 0.19
Phenylalanine	0.11± 0.08	0.28± 0.05	0.50± 0.13
Histidine	0.90± 0.17	0.47± 0.10	1.24± 0.24
Ornithine	2.11± 0.41	0.69± 0.34	2.23± 0.97
Lysine	0.63± 0.12	0.16± 0.04	0.63± 0.24
Arginine	0.27± 0.08	0.30± 0.07	0.69± 0.21

* Mean±1SD

cells. This loss could explain the deviations of the erythrocyte/plasma ratios from unity for some essential amino acids, such as valine, methionine, isoleucine, leucine, and phenylalanine, as pointed out by Marescau et al.[5] If this fact is taken into account, the concentrations of most amino acids should be fairly similar in plasma and erythrocytes. However, the concentration of aspartic acid was much higher in erythrocytes.

Aspartic acid levels in leukocytes were also high, especially in MNC in which it accounted for 17% of the pool. The high concentrations of aspartic acid in blood cells could be explained by intracellular trapping, by intracellular deamination of asparagine, or by glutamic-oxalacetic transaminase system[5], since the plasma level of this amino acid was quite low. Glutamic acid was also abundant in leukocytes, especially in MNC in which it accounted for 25% of the pool. These results led us to speculate that these amino acids, aspartic acid and glutamic acid, play important roles in blood cells, especially in MNC.

Taurine was abundant in leukocytes, while its level in erythrocytes was low and same as in plasma. This was to be expected, since leukocytes are living cells. The high concentration of this amino acid in leukocytes is explained by active transport from plasma or intracellular biosynthesis from cysteine[4]. Since taurine made up 76% of the intragranulocytic amino acid pool, it is possible that taurine plays an important role in functions peculiar to granulocytes, such as phagocytosis[8].

Thus, there was no correlation between the concentrations of free amino acids in the plasma and in the leukocytes.

The low concentrations of arginine and the elevated levels of ornithine in erythrocytes and PMN are of interest in view of the presence of arginase in these cells[6].

Further studies are necessary to explain the significance of the predominaces of taurine, aspartic acid, and glutamic acid in blood cells. Discoveries of disorders in which the levels of these amino acids in blood cells are affected may provide the clue to elucidate the roles of these amino acids in blood cells.

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