

Free Amino Acids of *Hevea brasiliensis* Latex

Very little information is available on the free amino acids of *Hevea* latex. Former investigations¹⁻⁴ showed a very limited number of these compounds present in latex. In the more recent works of SOEI⁵ and of D'AUZAC and PUJARNISLE^{6,7}, this number has been extended to include most of the classic amino acids. However, the evaluation of the respective quantities of each compound was based on paper chromatography and could therefore only be considered as approximative. The only quantitative results were those concerning the total amino acid content of latex^{7,8} and the sum of aspartic and glutamic acids⁷. In the present work, the amino acid composition of both cytoplasmic and lutoidic serums were investigated.

Materials and methods. Two batches of trees of clone PR 107 were chosen on experimental plots of the I.R.C.A. (Institut de Recherches sur le Caoutchouc en Afrique), in Bimbresso, Ivory Coast. The trees were tapped, on full spiral, twice a week (d/3, d/4). The latex was allowed to run for 1 min in order to eliminate the fraction containing the organelles which had suffered from the traumatic effects of tapping. A 35 ml subsequent fraction was collected in a tube immersed in ice. Half-an-hour later the latex samples were centrifuged for 30 min at 40,000 rpm (Spinco, rotor 50 Ti) at 0°C. The tubes were pierced, and the clear cytoplasmic serum was withdrawn. The upper part of the tube was cut and discarded with the rubber. The pellet consisting mainly of lutoid particles was recovered and resuspended in water to which ethanol was immediately added. Ethanol was also added to the cytoplasmic serum to a final concentration of 85%. After breaking of the lutoides and precipitation of the proteins of the 2 fractions (lutoidic and cytoplasmic), the ethanol extracts were centrifuged. All operations were carried out at 2-4°C.

Free amino acids of the 2 fractions were determined quantitatively with a Technicon Autoanalyzer using the one-column technique and an elution system composed of 3 buffers: pH 2.875, 3.8 and 5.0 forming a continuous gradient^{9,10}. The temperature of the column was maintained at 60°C. Under these conditions the 2 amides (glutamine and asparagine) and threonine run together. As shown by paper chromatography, glutamine and threonine are the ever present constituents of both cytoplasmic and lutoidic serums of *Hevea* latex, whereas asparagine is virtually absent. In order to evaluate each of these 2 compounds separately, the aliquots were hydrolyzed in 1 N HCl at 40°C for 24 h and then chromatographed. Threonine was measured directly. Glutamine evaluation was based on the increase of glutamic acid or on the decrease of the 'threonine' peak.

Results and discussion. The determinations of the free amino acids have been carried out on 16 latex samples coming from 12 trees, taken during the period from July

to October 1972. The data in Table I represent the average values of these determinations.

The free amino acid content varies, of course, between the samples taken at a given moment from different trees, as well as the samples taken from the same tree at different periods. However, all analyzed samples show the same general features. The major part of the free amino acids of latex - 78 to 83% of the total content which ranges from 16.9 to 23.2 μ moles per ml of fresh latex - is localized in the cytoplasmic serum.

However, when the difference of volume between the lutoidic and cytoplasmic serums is taken into consideration, it appears that the total amino acid concentration in each of the 2 compartments is of the same order: 30.3 μ moles/ml of cytoplasmic serum and 31.3 μ moles/ml of lutoidic serum. The latter value is somewhat too low. In fact, the volume of lutoidic serum has been assimilated to that of the lutoid pellet and, therefore, overestimated. Neither has the interstitial volume been taken into account, nor that of the membranes. The essential differences appear in the distribution of individual amino acids in the two compartments (Table I).

The predominant amino acids of the cytoplasmic serum are glutamic acid and its amide, alanine and aspartic acid. They account for 81% of the total amino acid content. The other amino acids are present in much smaller amounts, sometimes in trace quantities.

The lutoidic serum is distinguished from the cytoplasmic serum by a high proportion of compounds basic in character (Table II). A guanidic derivative, not yet identified (X_6) is the principal constituent of the group. It is followed by lysine and ornithine which also occur in considerable amounts. Their concentrations are, respectively, 12 times (guanidic derivative) and 6 times (ornithine and lysine) superior to those found in the cytoplasmic serum.

An opposite concentration gradient between the 2 compartments exists at the level of the principal constituents of the cytoplasm: alanine, aspartic acid and glutamic acid. Their concentrations in the lutoids is distinctly lower.

The differences systematically noticed in the distribution of the free amino acids between the two compartments, cytoplasmic and lutoidic, become even more important when the inevitable contamination of one fraction by the other is taken into consideration. Thus, it is very likely that certain compounds (for example α - γ -diaminobutyric acid, unknowns: X_4 , X_5 and X_7)

Table II. Distribution of acidic, neutral and basic free amino acids in the cytoplasmic and lutoidic serums

| Amino acid group | Free amino acid pool (%) | |
|------------------|--------------------------|---------|
| | Serum C | Serum L |
| Acidic | 56.9 | 22.9 |
| Neutral | 36.4 | 21.1 |
| Basic | 6.6 | 56.0 |

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Table 1. Free amino acids and related ninhydrin-reactive compounds in *Hevea brasiliensis* latex, clone PR 107 (Means of 16 analysis)

| Compounds | μmoles per 100 ml latex | | Serum C ^a | | Serum L ^b | | Serum L ^c |
|--------------------------------------|-------------------------|---------|----------------------|------|----------------------|------|----------------------|
| | Serum C | Serum L | μmoles per ml | % | μmoles per ml | % | Serum C |
| Cysteic acid | 5.2 | 1.0 | 0.10 | 0.3 | 0.08 | 0.3 | 0.8 |
| Taurine | 10.7 | 1.7 | 0.20 | 0.7 | 0.14 | 0.4 | 0.7 |
| N ₁ | trace | 1.9 | trace | — | 0.16 | 0.5 | — |
| N ₂ | 1.5 | 1.8 | 0.03 | 0.1 | 0.15 | 0.5 | 5.0 |
| N ₃ | 2.0 | 1.7 | 0.04 | 0.1 | 0.14 | 0.4 | 3.5 |
| Aspartic acid | 298.3 | 22.4 | 5.74 | 19.0 | 1.87 | 6.0 | 0.3 |
| Threonine | 34.0 | 6.3 | 0.65 | 2.1 | 0.52 | 1.7 | 0.8 |
| Serine | 26.5 | 4.2 | 0.51 | 1.7 | 0.35 | 1.1 | 0.7 |
| Glutamine | 279.5 | 33.8 | 5.38 | 17.8 | 2.82 | 9.0 | 0.5 |
| Glutamic acid | 297.5 | 21.6 | 5.72 | 18.9 | 1.80 | 5.8 | 0.3 |
| Proline | trace | trace | trace | — | trace | — | — |
| Glycine | 32.1 | 4.1 | 0.62 | 2.0 | 0.34 | 1.1 | 0.5 |
| Alanine | 404.0 | 34.6 | 7.77 | 25.7 | 2.88 | 9.2 | 0.4 |
| Valine | 32.8 | 10.2 | 0.63 | 2.1 | 0.85 | 2.7 | 1.3 |
| Isoleucine | 6.8 | 2.5 | 0.13 | 0.4 | 0.21 | 0.7 | 1.6 |
| Leucine | 3.1 | 3.0 | 0.06 | 0.2 | 0.25 | 0.8 | 4.2 |
| Tyrosine | 28.4 | 12.5 | 0.55 | 1.8 | 1.04 | 3.3 | 1.9 |
| Phenylalanine | 5.6 | 2.0 | 0.11 | 0.4 | 0.17 | 0.5 | 1.5 |
| γ-Aminobutyric acid | trace | trace | trace | — | trace | — | — |
| Ethanolamine | 9.3 | 27.5 | 0.18 | 0.6 | 2.29 | 7.3 | 12.7 |
| N ₄ | 1.6 | 5.9 | 0.03 | 0.1 | 0.49 | 1.6 | 16.3 |
| α,γ-Diaminobutyric acid | 2.3 | 9.5 | 0.04 | 0.1 | 0.79 | 2.5 | 19.8 |
| Ornithine | 20.2 | 30.6 | 0.39 | 1.3 | 2.55 | 8.2 | 6.5 |
| Lysine | 31.7 | 40.8 | 0.61 | 2.0 | 3.40 | 10.9 | 5.6 |
| Tryptophane | 7.3 | 15.2 | 0.14 | 0.5 | 1.27 | 4.1 | 9.1 |
| Histidine | 2.0 | 1.8 | 0.04 | 0.1 | 0.15 | 0.5 | 3.8 |
| N ₅ | trace | 2.3 | trace | — | 0.19 | 0.6 | — |
| N ₆ (Guanidic derivative) | 19.2 | 53.7 | 0.37 | 1.2 | 4.48 | 14.3 | 12.1 |
| N ₇ | trace | 5.6 | trace | — | 0.47 | 1.5 | — |
| Arginine | 11.3 | 17.0 | 0.22 | 0.7 | 1.42 | 4.5 | 6.5 |
| Total | 1572.9 | 375.2 | 30.26 | 100 | 31.27 | 100 | 1.0 |

^a Serum C, cytoplasmic serum; ^b Serum L, lutoidic serum; ^c Ratio of the 2 concentrations.

found in the cytoplasmic serum in trace quantities, or in very small amounts, come from the degradation of the lutoids during the manipulations. If this is so, the differences between the 2 fractions would not only be quantitative but also qualitative.

The above results set the problem of distinct metabolic pathways in each of 2 compartments, as well as that of the selective permeability of the lutoidic membranes to the amino acids.

Résumé. Les études des acides aminés libres ont été effectuées sur deux compartiments du latex d'*Hevea brasiliensis*: les serums cytoplasmique et lutoidique. La majeure partie des acides aminés est localisée dans le cytoplasme dans lequel dominant Glu et son amide, Asp et Ala. La fraction particulière se distingue par une dominante des composés basiques.

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