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Short communication

TLC of some free amino acids from sanguine plasma

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Abstract

This research presents the results obtained from analysis by thin layer chromatography (TLC) of some free amino acids from sanguine plasma samples in the different degree progress in maladies: diabetes, renal syndrome and hepatic cirrhosis. The chromatograms were evaluated with a Shimadzu CS-9000 dual-wavelength flying-spot scanner. Better results were obtained in the case of hepatic cirrhosis.

Keywords: Densitometry; Amino acids; Sanguine plasma; Thin layer chromatography

1. Introduction

The continuos improvement of techniques and apparatus in the chromatography, allows their utilisation for some specific analysis of great sensibility, in clinical and biochemical laboratories [1–3]. Thin layer chromatography (TLC) has a privileged position, due to the simplicity of apparatus, in the biochemical analyses of amino acids, enzymes, steroids etc. These analyses allow on the one hand the solution for some questions in the diagnosis and therapy and on the other hand to point out some biochemical modifications of some diseases at cellular level.

The determination of amino acids from biological tests is very important and up-to-date because it allows to point out some anomalies correlated to different metabolic illnesses [4–6]. All these assimilation disorders of amino acids appear as a diminution of absorption, or as a specific alteration of transport and metabolic systems (absence of some specific enzymes, etc.). Among the diseases caused by anomalies, concentrations of one or more free amino acids to be mentioned: nephritis, hepatic cirrhosis and diabetes, affecting three vital organs: kidney, liver and pancreas. In this context the qualitative and quantitative determinations of content at the level of some organs, tissues or in the sanguine plasma is very important in establishing a correct diagnosis.

This paper presents the results of analysis by TLC of some free amino acids from sanguine plasma samples from subjects: normal and with different degree progress in maladies: diabetes, renal syndrome and hepatic cirrhosis.

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2. Experimental

2.1. Chemicals and apparatus

n-Butanol, acetone and acetic acid, of analytical grade, were obtained from Reactivul (Bucharest, Romania). The elution has been performed in a 'sandwich' chamber on cellulose plates obtained from Merck (20 × 10 cm; 0.1 mm thick) and the densitometry has been achieved with the photodensitometer Schimadzu CS-9000 at 575 nm. The standard solutions of the 13 amino acids (1 mg ml^{-1}) were applied with a micropipette.

The sanguine plasma samples have been collected from (a) normal subjects, (b) diabetic subjects, (c) subjects with renal syndrome, (d) subjects with hepatic cirrhosis.

2.2. Procedure

Samples (5 ml) were collected from healthy persons (three samples), from diabetic suffering (hereditary, II degree, III degree very advanced) (ten samples), renal syndrome (I degree chronic and II/III very advanced chronic) (five samples); hepatic cirrhosis (I degree, II degree advanced, III degree very advanced) (five samples).

The samples were preserved at 4 °C for 15 min. Then a $Na_3(PW_{12}O_{40})$ solution was used for removing the proteins from the samples by precipitation. After centrifugation (30 min with 3000 rotations min^{-1}) the solutions obtained was applied on the chromatographic plates (2 μl). The separation and identification of the free amino acids from sanguine plasma samples was achieved by TLC with double elution. The elution distance was 7.5 cm, in unsaturated N chamber using an *n*-butanol–acetone–acetic acid–water (35:35:7:23 v/v/v), mixture [7] as the mobile phase. After the first elution the plates were dried in hot air, then eluted in the same direction, along the same distance and with the same solvent mixture. The detection was carried out by spraying the plates with a ninhydrine solution in *n*-butanol–acetone (1:1 v/v) mixture, then dried at 105 °C for 15 min. The identification of the amino acids was achieved by comparing the R_f values and the colours.

3. Results and discussions

For identification of amino acids from the analysed samples, the standard chromatogram from Merck [7] and our standard solutions were used. The R_f values of some amino acids from sanguine plasma samples are presented in Table 1. It can be seen that as well as in the case of standard chromatograms in our experiments some amino acids from the plasma were identified as a mixture of two or three, for example: lysine + histidine, glutamine + citruline, glycine + serine + asparagine.

Fig. 1 shows the densitograms of the amino acids for a normal subject as compared with the diabetic subjects. All the amino acids present in the normal plasma were also noticed in the samples of diabetic subjects but at different concentration as mentioned in the literature [8,9]. The increase of amino acids concentration: lysine, histidine, glutamine, glycine, serine, asparagine, methionine is due to the insulin and the ribonuclease degradation (hydrolysis) in the corresponding amino acids, because these amino acids are placed at the ends and in the connection bridges of these two proteins [9].

Fig. 2 shows the densitograms comparative for the identified amino acids in plasma of the normal adult, respectively, for adult with renal syndrome of I degree and II/III degree. In this case it is also noticed that there is an increase in the concentration of certain amino acids: lysine, histidine, citruline, glutamic acid, threonine, glutamine, alanine, for the adults with renal syndrome, as compared with the normal one. This increase is explained [6,9] by genetic deficiencies of tubular and renal absorption. Due to these genetic deficiencies (e.g. the oxidative decarboxylation of amino acids), these are accumulated in the blood and removed in urine together with their degradation products (ceto acids, hydroxy acids). An other factor determining this increase, is correlated to the genetic deficiencies of insufficiency for the enzymatic systems involved in the proteins synthesis.

At the end, Fig. 3 shows the comparative densitograms of amino acids for a normal adult and an adult affected by hepatic cirrhosis (I degree, respectively, III degree). In this case also, an

Table 1
The R_f values of some amino acids from sanguine plasma

Amino acid[a]	R_f (intensity)[b]							
	Subjects							
	Normal	Diabetes			Renal syndrome		Cirrhosis	
		Hereditary	II degree	III degree	I degree	II/III degree	I degree	III degree
Lys, His	0.07 (++)	0.07 (++)	0.07 (++)	0.07 (++)	0.07 (++)	0.06 (+++)	0.06 (++)	0.07 (+++)
Gln, Cit	0.16 (+++)	0.16 (+++)	0.16 (+++)	0.16 (+++)	0.15 (+++)	0.16 (+++)	0.16 (+++)	0.16 (+++)
Gly, Ser, Asp	0.20 (++)	0.20 (+++)	0.20 (+++)	0.20 (++++)	0.20 (++)	0.20 (++)	0.20 (++)	0.21 (++)
Thr, Glu	0.30 (++)	0.30 (++)	0.30 (++)	0.30 (++)	0.30 (+++)	0.30 (++++)	0.30 (++)	0.30 (++)
Ala	0.33 (++)	0.33 (++)	0.33 (++)	0.33 (++)	0.33 (+++)	0.33 (++++)	0.34 (++)	0.34 (++)
Pro	0.38 (+)	0.38 (+)	0.38 (+)	0.38 (+)	0.38 (+)	0.38 (+)	0.38 (+++)	0.39 (++++)
Tyr	0.43 (+)	0.43 (+)	0.43 (+)	0.43 (+)	0.43 (+)	0.43 (+)	0.43 (+++)	0.43 (++++)
Met	0.50 (++)	0.50 (+++)	0.50 (++++)	0.50 (++++)	0.50 (++)	0.50 (++)	0.50 (++)	0.51 (+++)

[a] Lysine (Lys), Histidine (His), Glutamine (Gln), Citruline (Cit), Glycine (Gly), Serine (Ser), Asparagine (Asp), Threonine (Thr), Glutamic acid (Glu), Alanine (Ala), Proline (Pro), Tyrosine (Tyr), Methionine (Met).

[b] (++++), very high intensity; (+++), high intensity; (++), medium intensity; (+) low intensity.

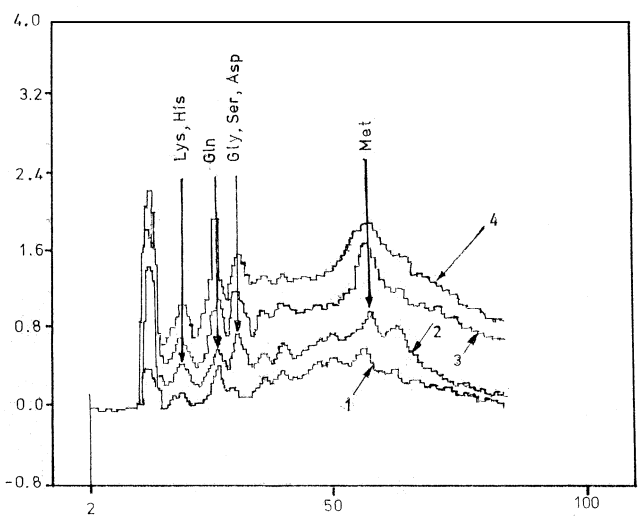

Fig. 1. Densitograms of the studied plasma samples from subjects: one normal; with diabetes: two hereditary, three II degree, four III degree.

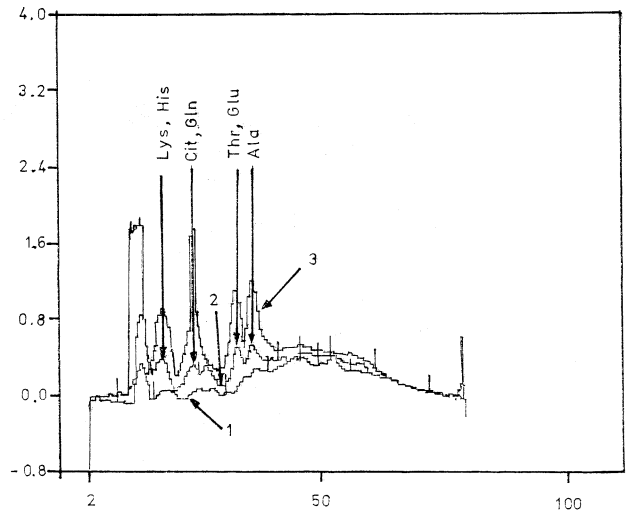

Fig. 2. Densitograms of the plasma samples from subjects: one normal; with renal syndrome: two I degree, three II/III degree.

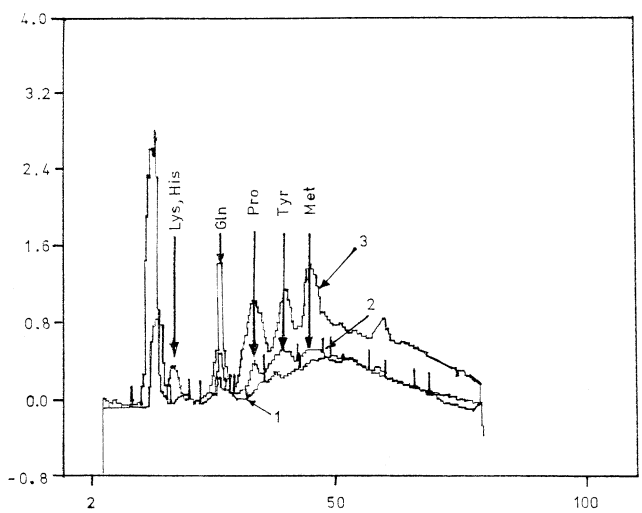

Fig. 3. Densitograms of the plasma samples from subjects: one normal; with hepatic cirrhosis: two I degree, three III degree.

increase in concentration of certain amino acids was observed, these include: lysine, histidine, proline, tyrosine, methionine, for the cirrhosis affected subjects. A serious hepatic or hepato–renale insufficiency, lead to the disturbance of amino acids utilisation for proteins synthesis. This is more obvious in the concentration increase of proline, tyrosine and methionine, which in very critical cases, can reach concentrations 10 times higher than the normal values.

4. Conclusions

Thin layer chromatography allow the analysis of free amino acids from sanguine plasma in

maladies: diabetes, renal syndrome and hepatic cirrhosis. Better results were obtained in the case of hepatic cirrhosis, characterised by a considerable increase in concentration of proline, tyrosine and methionine.

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