Journal of Chromatography, 339 (1985) 366—369
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 2499

Note

Plasma amino acid analysis by gas chromatography

Removal of glucose interference

D. LABADARIOS*, G.S. SHEPHARD, I.M. MOODIE, L. JARDINE and E. BOTHA

Metabolic Unit, Tygerberg Hospital, Department of Internal Medicine, University of Stellenbosch and the M.R.C. National Research Institute for Nutritional Diseases, P.O. Box 63, Tygerberg 7505 (South Africa)

(First received August 29th, 1984; revised manuscript received November 24th, 1984)

Until recently it has been a widely held and accepted view, as judged by the number of publications, that amino acid analysis is best accomplished by the classical ion-exchange technique using an automatic amino acid analyser. Despite the high degree of sophistication attained, however, this method has the inherent disadvantages of requiring high initial capital outlay, high running costs, and of being capable of a very limited application, mainly that of amino acid analysis. Furthermore, the actual time for analysis of one sample is approximately 2—2½ h which, when added to the time required for sample preparation, is a major limiting factor in sample throughput.

Several research groups have adopted the alternative procedure based on gas chromatographic (GC) analysis which, in most cases, and in common with the classical ion-exchange technique, requires treatment of the plasma with a protein precipitant. Subsequently, deproteinised plasma must undergo a purification procedure prior to the synthesis of volatile amino acid derivatives necessary for GC. The method of choice for such purification has over the years been that of cation-exchange chromatography. The latter, however, has been used alone by some workers [1, 2] who avoid protein precipitation and directly clean-up a plasma sample pretreated with acetic acid. The limitations and disadvantages of the cation-exchange clean-up step have recently been reviewed [3]. Of particular relevance in this regard is the non-specific nature of this adopted procedure since it removes all anionic and non-charged species present in the plasma irrespective of whether or not they contribute to interference in the subsequent analysis. Furthermore, a wide diversity of protocols

0378-4347/85/$03.30

has been described for this procedure, some of which involve conditions that have been shown to be not only sub-optimal but also detrimental to accurate analysis. Thus, considerable doubt is thrown on the validity of quantitative results obtained from protocols involving this procedure. In this preliminary communication we demonstrate that an alternative specific approach based on the removal of a positively identified interfering compound [4] has been accomplished.

EXPERIMENTAL

Reagents

Pipecolic acid and hexokinase (as an ammonium sulphate suspension) were obtained from Sigma (St. Louis, MO, U.S.A.). ATP (disodium salt) was obtained from Boehringer Mannheim (F.R.G.).

Procedure

To plasma (500 μl) were added aqueous hexokinase solution (200 U in 200

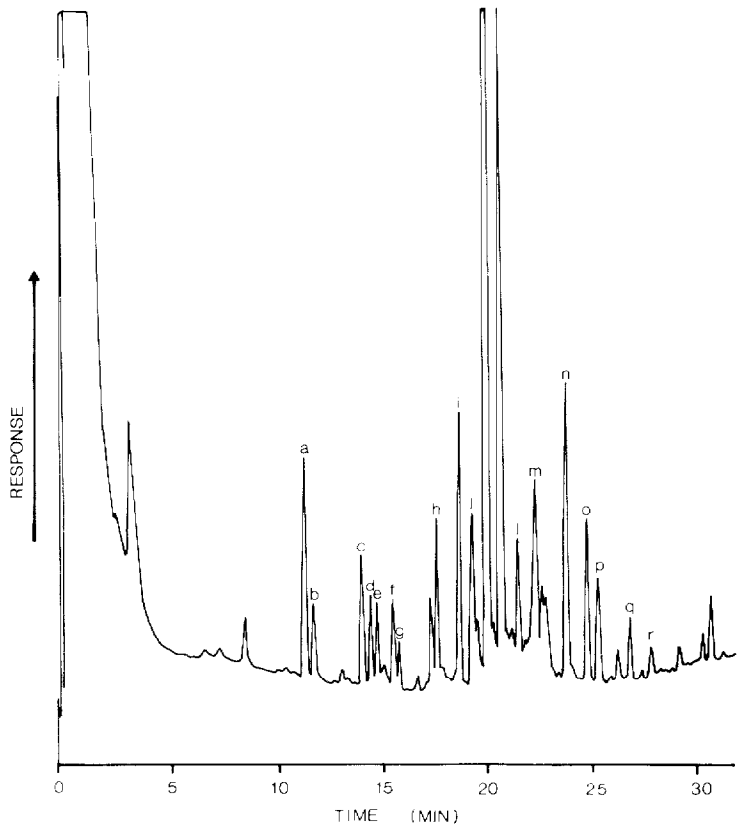

Fig. 1. Chromatogram of plasma amino acid derivatives showing the multiple peaks of glucose interference between hydroxyproline and phenylalanine. Peak identification: a = alanine; b = glycine; c = valine; d = threonine; e = serine; f = leucine; g = isoleucine; h = proline; i = pipecolic acid (internal standard); j = hydroxyproline; k = methionine; l = aspartic acid; m = phenylalanine; n = glutamic acid; o = lysine; p = tyrosine; q = arginine; r = histidine.

μl) and 0.08 *M* ATP solution (40 μl), the pH then being adjusted with 0.5 *M* ammonium hydroxide to 7.4. The mixture was incubated at 30°C for 15 min, deproteinised by addition of 2 ml of methanol—12 *M* hydrochloric acid (4:1) and centrifuged at 1600 *g* for 10 min. The supernatant was taken to dryness in vacuo. Subsequent derivatisation and chromatography of the residual material was carried out as previously described [5].

RESULTS AND DISCUSSION

Our investigations into GC analysis of amino acids in deproteinised plasma have shown that, although most amino acids can be successfully resolved, there is a major component responsible for a number of chromatographic peaks (Fig. 1) which elute over a broad area precluding the resolution of hydroxyproline, methionine, aspartic acid and phenylalanine. We have further demonstrated [4] that this component is glucose. There are two possible mechanisms which may explain the interference caused by glucose, a reducing sugar, during amino acid derivatisation. Firstly, the hydroxyl groups of glucose may react with the acylating reagent (heptafluorobutyric anhydride) [6] forming derivatives which co-elute with hydroxyproline, methionine and aspartic acid, making the quantitation of these amino acids unattainable. Secondly, and in view of the high

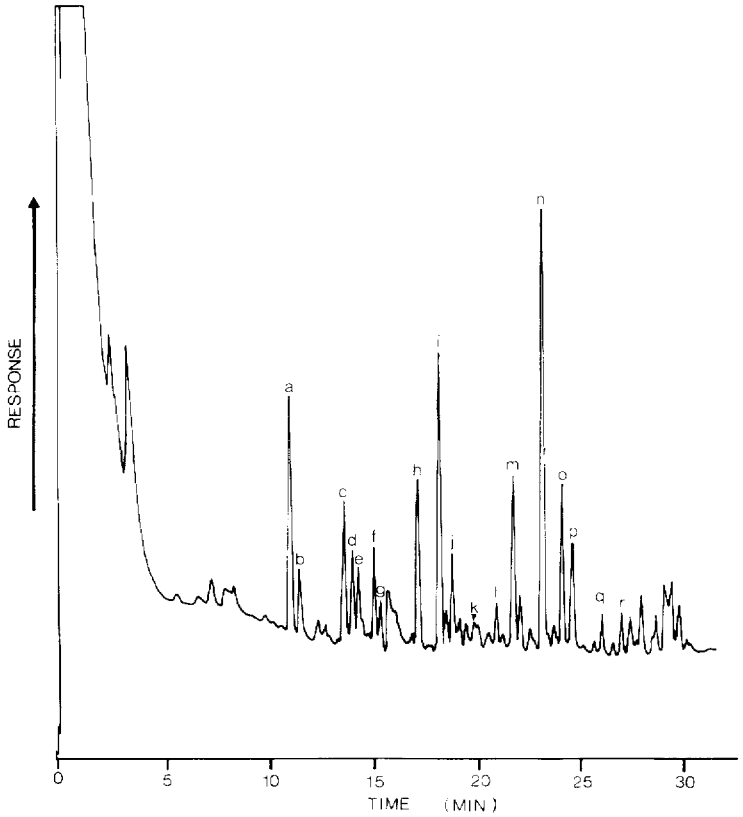

Fig. 2. Chromatogram of plasma amino acid derivatives showing the absence of glucose interference. For peak identification, see legend to Fig. 1.

temperature employed during derivatisation, glucose may react with free amino acids forming the so-called Maillard compounds. These latter compounds may themselves contribute directly to the spurious peaks observed or indirectly, following their esterification and acylation during derivatisation. Under mild conditions of temperature (37°C) lysine will be the main contributor to the Maillard reactions. However, with the increasing severity of temperature conditions (> 90°C), as employed in the derivatisation of amino acids, glycine, arginine, aspartic acid and glutamic acid will also participate in the Maillard reactions [7].

We have successfully overcome the glucose-induced interference by the enzymic conversion of glucose to glucose-6-phosphate. The elimination of glucose interference is shown in Fig. 2. Resolution of peaks is good and identification of amino acids is readily achieved, permitting quantitative results to be obtained. In addition, this novel procedure requires only 15 min in incubation time and obviates the use of the cation-exchange clean-up step. Furthermore, the brevity of our sample preparative procedure compares favourably with that employed in the amino acid analyser technique and thus lends GC to routine laboratory plasma amino acid analysis.

Preliminary data, obtained from a plasma sample spiked with a known quantity of amino acid standards, compared with an unspiked sample of the same plasma, indicate that recovery of amino acids, following deproteinisation, derivatisation and GC, is in general better than previously published data. These results will be reported elsewhere in due course.

CONCLUSION

In seeking a means of removing glucose from plasma, a novel alternative approach to the existing clean-up process has been adopted. By using a technique designed to remove a specific compound (viz. glucose), a more controlled process has been achieved together with the avoidance of a sample purification step which, though widely used, is far less than optimal.

ACKNOWLEDGEMENT

We thank SABAX S.A. for the donation of the gas chromatograph.

REFERENCES

1 R.F. Adams, J. Chromatogr., 95 (1974) 189.
2 P. Husek, G. Herzogová and V. Felt, J. Chromatogr., 236 (1982) 493.
3 D. Labadarios, I.M. Moodie and G.S. Shephard, J. Chromatogr., 310 (1984) 223.
4 D. Labadarios, G.S. Shephard, I.M. Moodie and E. Botha, J. Chromatogr., 294 (1984) 419.
5 D. Labadarios, G.S. Shephard, I.M. Moodie and E. Botha, S. Afr. J. Sci., 80 (1984) 240.
6 A.I. Vogel, Practical Organic Chemistry, Longmans, London, 3rd ed., 1966, Ch. 3, p. 451.
7 H. Erbersdobler, Z. Tierphysiol. Tierernähr. Futtermittelkd., 25 (1969) 119.