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Note

Improved single-column procedure for the amino acid analysis of collagen-like proteins

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The preparation of collagen-like proteins that contain a high level of cysteine[1–4] involves several stages, including reduction and alkylation by iodoacetamide[5], which converts the cysteine residues into carboxymethylcysteine. The difficulty arises that when the classical technique is used for amino acid analysis the derivative is eluted at the same time as 4-hydroxyproline. The buffer solution proposed here for a good separation of these two components is easily adapted to the first-generation amino acid analyser using cation-exchange resins, and permits the determination of amino acids present in the hydrolysate of basement membrane collagen.

EXPERIMENTAL

The material used was the same as that described previously[6]. The 54 × 0.9 cm column contained M 82 resin (Beckman, Fullerton, CA, U.S.A.). A 1-ml volume of the sample was loaded on to the column, which was eluted at a rate of 65 ml/h with 0.2 *M* citrate buffer (pH 3.08) for 170 min, then with 0.2 *M* citrate buffer (pH 4.10) for 80 min, followed by 0.2 *M* citrate buffer (pH 5.00) containing 1.0 *M* sodium chloride for 95 min. The column was regenerated with 0.2 *N* sodium hydroxide solution for 15 min and then re-equilibrated with the first buffer solution for 30 min. A complete cycle of analysis required 6.5 h. The initial chromatography temperature was 39°C, and was increased to 67°C after 55 min and reduced to the initial temperature at the end of the chromatography. The detection of amino acids took place after treatment for 9 min with ninhydrin. Absorbances were recorded at 440 and 570 nm and the areas of the peaks at these two wavelengths were used to determine the ninhydrin colour index of each amino acid.

RESULTS AND DISCUSSION

The retention times of the various amino acids and ninhydrin colour index obtained for each component are presented in Table I. The conditions described above permitted the clear separation and determination of carboxymethylcysteine and 4-hydroxyproline. These components are important as they permit the charac-

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TABLE I

RESULTS AUTOMATICALLY COMPUTED BY THE SYSTEM AA INTEGRATOR

Amino acid	*Retention time (min)*	*Ninhydrin colour index*	*Amino acid*	*Retention time (min)*	*Ninhydrin colour index*
3-Hydroxyproline	47.7	881	Methionine	202.5	2156
4-Hydroxyproline	56.2	460	Isoleucine	210.8	4167
Carboxymethylcysteine	59.8	3111	Leucine	214.9	4400
Aspartic acid	64.8	4015	Norleucine	219.7	4443
Threonine	68.5	3947	Tyrosine	228.3	4552
Serine	72.2	3999	Phenylalanine	237.8	4531
Glutamic acid	96.9	4680	Hydroxylysine	290.3	4407
Proline	101.2	1080	Ammonia	296.7	3577
Glycine	117.4	4251	Lysine	299.5	6199
Alanine	128.2	3924	Histidine	311.5	4946
Valine	168.5	3761	Arginine	345.3	4709
Cystine	186.6	2747			

terization of the collagen proteins recently described by several workers[1–4]. These molecules contain relatively few 4-hydroxyproline residues (80/1000) and are particularly rich in 1/2Cys (18 residues per 1000 residues). Hence it is important to determine precisely these two amino acids. Recently this problem was well resolved by Vergnes and Freeman[7]. However, they used more sophisticated techniques and we have obtained a separation of similar quality using more conventional apparatus; hence this technique may be of use to laboratories using wide columns (0.9 cm) of M 72 or M 82 cation-exchange resin. The procedure is applicable to the amino acid analysis of procollagens and basement membrane collagens.

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REFERENCES

1 R. Timpl, J. Risteli and H. P. Bachinger, *FEBS Lett.*, 101 (1979) 265.
2 D. K. Furuto and E. J. Miller, *J. Biol. Chem.*, 255 (1980) 290.
3 R. Jander, J. Rauterberg, B. Voss and D. B. von Bassevitz, *VII Eur. Symp. Connect. Tissue Res., Prague, 1980.*
4 G. Laurain, T. Delvincourt and A. G. Szymanowicz, *FEBS Lett.*, 120 (1980) 44.
5 E. Chung, E. M. Keele and E. J. Miller, *Biochemistry*, 13 (1974) 3459.
6 A. G. Szymanowicz, G. Poulin, N. Fontaine, J. P. Werquin and J. P. Borel, *J. Chromatogr.*, 190 (1980) 457.
7 J. P. Vergnes and I. L. Freeman, *Anal. Biochem.*, 99 (1979) 427.