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Note

Use of a low-salt, alkaline buffer in the elution of basic amino acids from a single-column amino acid analyzer

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Automated column chromatography of amino acids has usually been performed on a two-column system, a short column used to separate basic amino acids and a longer one to separate neutral and acidic amino acids[1,2]. Later, the one-column method was developed[3,4]. Several modifications of the analyzer have been made by variation in column size, elution temperature, buffer composition, and detection system[5–12]. A major advance has been the development of an ion-exchange resin with resolution power and permitting use of low elution pressure. Using the improved resins and the single-column method, analysis time can be reduced if a proper buffer is used to elute basic amino acids. Since all basic amino acids are eluted by the last buffer, the column need not be regenerated with an alkaline solution before re-equilibrating with the first buffer for the next run.

In this study we present the elution patterns of basic amino acids with a high pH buffer that is relatively low in salt concentration as compared to other buffers currently being used in the elution of basic amino acids from the single-column system[13–15]. By use of this buffer, the resolution of His, Orn, Trp, Lys, NH_3 and Arg is complete, no detectable volume change of the resin occurs during the third buffer elution, and re-equilibration with little more than one column volume of the first buffer is sufficient prior to starting a second run.

EXPERIMENTAL

Two Beckman/Spinco 120 B amino acid analyzers were converted to the one-column system with the incorporation of a four-way buffer change valve and a three stepdown timer (purchased from MER Chromatographic). One analyzer was equipped with a 48 × 0.9 cm column packed with Durrum DC-1A resin and the other analyzer was equipped with a 48 × 0.9 cm column packed with Beckman UR-30 resin. A JEOL JLC-6AH amino acid analyzer with a two-column system, a 15 × 0.8 cm and a 50 × 0.8 cm column, was also converted to a system with two single 25 × 0.8 cm columns. Both columns were packed with JEOL LC-R-2 resin.

Three buffers were used in the elution of amino acids: The first buffer consisted of 0.2 *N* Na citrate (pH 3.25) and the second buffer consisted of 0.2 *N* Na citrate (pH 4.25). The third buffer consisted of 0.35 *N* sodium citrate and 0.1 *N* sodium

borate; the pH of the latter was adjusted with concentrated HCl to 9.35 ± 0.02. 0.3% Brij, 0.5% thiodiglycol and 0.01% caprylic acid were added to all three buffers[2]. The ninhydrin solution used in the modified Beckman-Spinco 120 B amino acid analyzer was prepared according to Spackman *et al.*[2].

In the modified Beckman/Spinco 120 B amino acid analyzer the column was re-equilibrated with the first buffer for 50 min after the elution of the basic amino acids with the third buffer. Usually no alkaline solution was used to regenerate the column after each run. However, regeneration with 0.2 *M* NaOH for 20 min was performed after every 15–20 total amino acid analyses and following the regeneration, re-equilibration with the first buffer was prolonged to 55 min. Six ninhydrin-reactive materials were used in the study of their elution pattern by the third buffer, *i.e.*, histidine, ornithine, tryptophan, lysine, ammonia and arginine. Alkaline buffers (0.45 *N* Na) of different pH were used to achieve the separation of these materials. The pH 9.35 buffer with different sodium concentrations was tried in order to test for the best separation.

RESULTS AND DISCUSSION

The third buffer used in the single-column amino acid analysis system was designed for the fast analysis and better separation of basic amino acids, especially Orn, Trp, and Lys. The major application of this system is to the quantitative analysis of a sequenator sample after alkaline hydrolysis[16,17]. After alkaline hydrolysis in 0.1 *N*

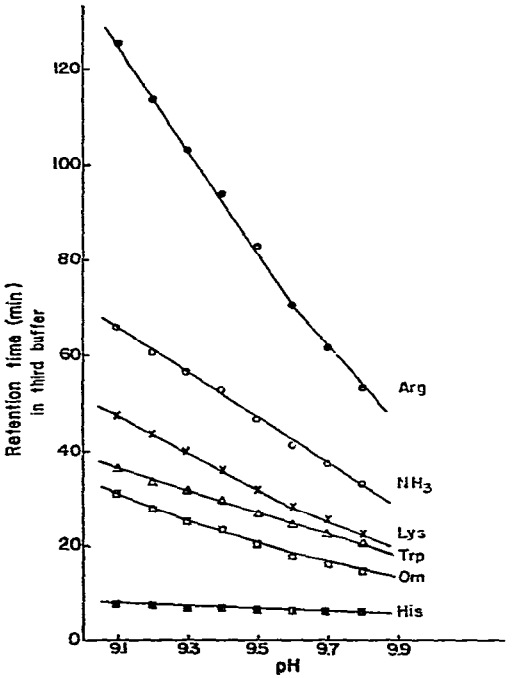

Fig. 1. Resolution of basic amino acids as a function of the pH of the third buffer on a 48 × 0.9 cm column packed with Durrum DC-1A resin. The buffer was made up of 0.1 *N* Na borate and 0.35 *N* Na citrate titrated to pH 9.1–9.8 with either HCl or NaOH solution.

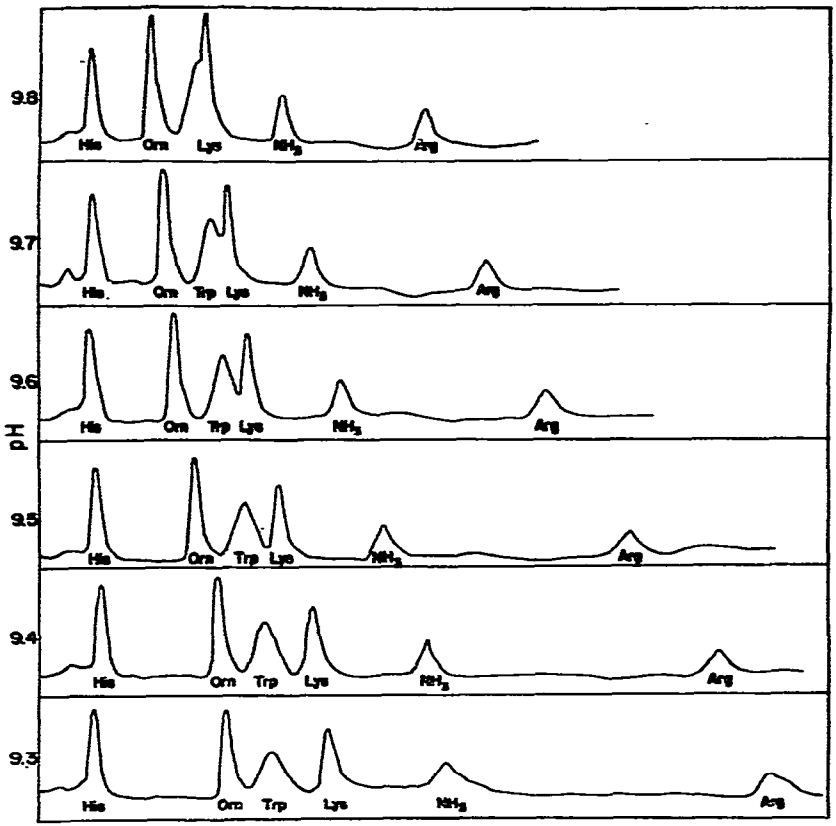

Fig. 2. Chromatograms of the elution of basic amino acids from a 48 × 0.9 cm column packed with Durrum DC-1A resin at different pH values. Monitored at 570 nm absorbance.

NaOH + 0.2 *N* $Na_2S_2O_4$ at 126° for 3.5 h, PTH-tryptophan is usually converted to tryptophan and PTH-arginine recovered as ornithine and arginine[16]. The system described here is also suitable for a complete amino acid analysis of protein hydrolysate within 3.5 h.

Fig. 1 shows the retention time of His, Orn, Trp, Lys, NH_3 and Arg on columns packed with Durrum DC-1A resin. The columns were eluted with 0.2 *N* Na citrate (pH 3.25) for 75 min, then with 0.2 *N* Na citrate (pH 4.25) buffer for 52 min, and finally with the third buffer, containing 0.45 *N* in Na at pH values varying between 9.1 and 9.8. The retention time of each peak was calculated from the appearance of the third buffer change to the position of individual peaks on the chromatogram. This figure shows that the retention time of each peak is inversely proportional to the pH of the third buffer. The retention time of arginine is doubled when the pH of the buffer changes from 9.8 to 9.1. The actual chromatographic separation is shown in Fig. 2. Incomplete separation of Trp and Lys is observed above pH 9.5. The best separation of Orn, Trp, and Lys is around pH 9.40. As soon as the sample of protein hydrolysate is analyzed, the pH of the third buffer can be raised to 9.8 to speed up the elution of arginine, since Trp and Orn are not present in acid hydrolysates. The

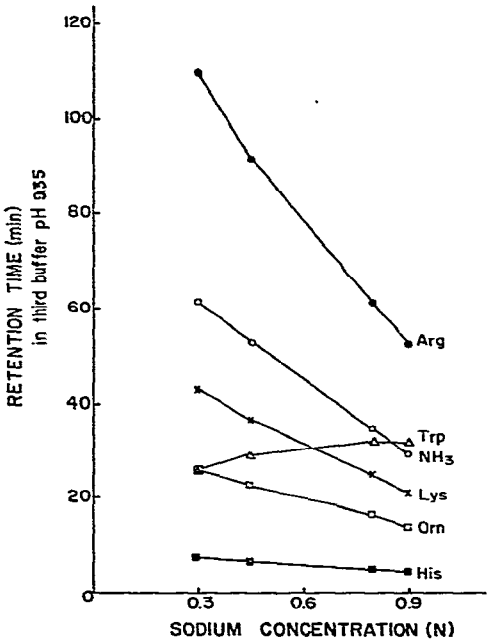

Fig. 3. Resolution of basic amino acids as a function of the sodium concentration of the third buffer at pH 9.35, on a 48 × 0.9 cm column packed with Durrum DC-1A resin. The 0.3 *N* buffer contains 0.1 *N* Na borate and 0.2 *N* Na citrate, the 0.45 *N* buffer contains 0.1 *N* Na borate and 0.35 *N* Na citrate, the 0.8 *N* buffer contains 0.1 *N* Na borate and 0.7 *N* Na citrate, and the 0.9 *N* buffer contains 0.2 *N* Na borate and 0.7 *N* Na citrate.

elution patterns of basic amino acids are quite similar in columns packed with three different resins (Durrum DC-1A, Beckman UR-30, and JEOL LC-R-2).

The effect of the sodium concentration of a pH 9.35 buffer on the retention times of different amino acids is shown in Fig. 3. The retention time of each amino acid is inversely related to the sodium concentration, except for Trp, which shows a longer retention time in higher sodium concentrations. The Trp peak is eluted between Orn and Lys at 0.45 *N* sodium concentration and between Lys and NH_3 at 0.75 *N* sodium concentration.

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