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Mutual Separation of Amino Acids in Aqueous Solutions with Silica-Containing Mixed-Oxide Gels

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Mixed amino acids in aqueous solutions have successfully been separated mutually using silica-containing mixed-oxide gels as adsorbents: L-glutamic acid, glycine, and L-lysine have solely separated by a batch operation with silica-magnesia; L-glutamic acid and L-aspartic acid by a column operation with silica-alumina; L-histidine and L-lysine also by a batch operation with silica-titania.

As biotechnological production develops, an efficient technique for separation and purification of objective bioproducts will be required. A chromatographic technique based on the interaction between an objective material and the solid surface of adsorbent may well become the most feasible technique for this purpose among various advanced methods. The study on the adsorption of amino acid on metal oxide has not been attempted so much except for titania,[1,2] iron hydroxide,[3] and aluminum hydroxide.[4] We have been interested in the preparation of characteristic silica-containing mixed-oxide gels through wet processes.[5-9] The adsorption behaviors of typical amino acids in aqueous solutions on silica-alumina, silica-magnesia, and silica-titania gels have been investigated in relation to the technique described above.[10,11] We have here attempted the separation of the mixed amino acids by means of adsorption and elution with the mixed-oxide gels.

Silica-containing mixed-oxide gel was precipitated from a mixture of sodium metasilicate solution and aluminum, magnesium, or titanium chloride solution by adding sodium hydroxide up to the prescribed pH at room temperature, as in a previous paper.[10] The sodium metasilicate solution was initially acidified to pH 1 with hydrochloric acid. After aging for 10 h, the resultant precipitate was filtered by suction, washed thoroughly with deionized water, and then dried in an oven at 110 °C overnight. The dried gel was sieved to 20-42 mesh size (average diameter ca. 0.6 mm) after grinding. Amino acids of reagent-grade quality (Wako Pure Chemical Ind., Ltd.) were used without further purification. Adsorption tests on the mixed solutions composed of equimolar L-glutamic acid (acidic amino acid, L-GLU), glycine (neutral amino acid, GLY), and L-lysine (basic amino acid, L-LYS) (the total amino acid concentration 1.2×10^{-3} mol dm^{-3}) were conducted by shaking 400 mg of gel with 100 cm^3 of solution at 25 °C for 24 h, when the adsorption of amino acid had already reached equilibrium. The initial pH of amino acid solution was adjusted by the addition of hydrochloric acid or sodium hydroxide solution. Adsorption tests were also performed on the mixed amino acids (L-glutamic acid and L-aspartic acid, or L-histidine and L-lysine) solutions. Elution tests on adsorbed gels were conducted by means of a batch or a column operation with alkaline solution. After adsorption or elution, the solution was filtered

through a 1.0 μm membrane filter. The concentration of amino acid was determined by means of HPLC, and the adsorption and elution percentages were calculated from the concentration of the solution after the test.

It has already been clarified that the adsorption behavior has changed variously depending on the pH of solution as well as the combination of gel and amino acid.[11] We have, therefore, attempted to separate mutually from an equimolar L-glutamic acid, glycine, and L-lysine mixed solutions with silica-containing binary gels, making use of the different adsorption behaviors of the amino acids with the change of the pH of solution. Figure 1 shows a typical result obtained using silica-magnesia of molar ratio 1:1 (SM1:1), in which the peak height corresponds to the fraction of the residual amino acid in the solution. Both L-glutamic acid and glycine were completely adsorbed on the gel up to pH 2.4, thus making it possible for L-lysine to be separated from these two amino acids. The adsorption of L-glutamic acid on the gel occurred by formation of an electrostatic bond between the negative carboxyl ion of the acidic amino acid (pKa_1 of L-glutamic acid 2.19) and the positively charged surface of the gel (pzc of the gel 8.9).[11,12] The adsorption of electrically neutral glycine resulted from physical adsorption (hydrogen and/or van der Waals bonding). However, the decrease in the adsorption of glycine over pH8.0 seems to be mainly caused by the dissolution of silica component of this binary gel in basic solution.[13] Silica-magnesia gel has been applicable to a packing material of column for basic organic compounds in normal phase liquid chromatography.[14] The present work also suggests the possibility of the mutual separation among these three amino acids using the gel in terms of the gradient alteration of the pH of eluent from 2 to 8.

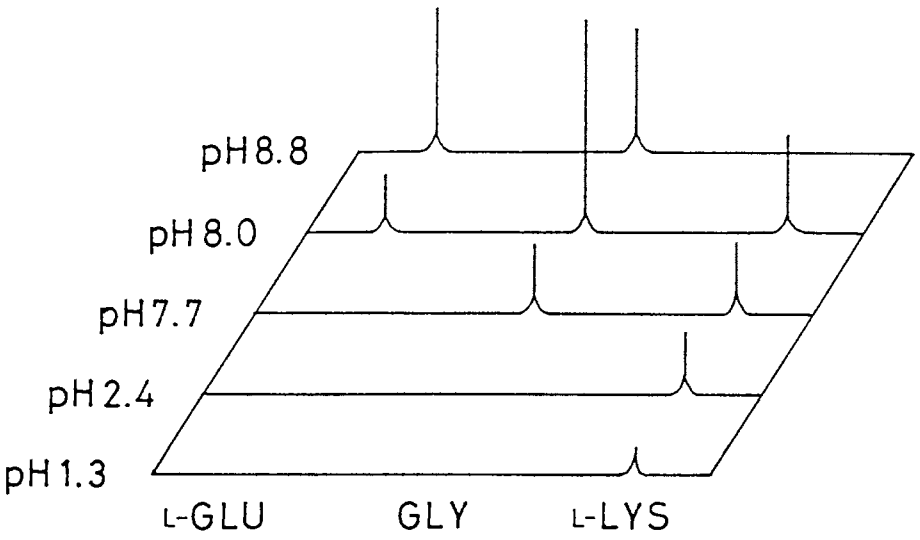

Fig. 1. Adsorption selectivity of amino acids on SM1:1.

The adsorptions of two acidic amino acids, L-glutamic and L-aspartic acids, depended on the pH of solution in almost the similar way to each other by a batch operation using silica-alumina of molar ratio 1:3 (SA1:3); the separation of these two acidic amino acids was impossible. However, it was found that the difference of the adsorption percentages of the acidic amino acids on silica-alumina of molar ratio 1:1 (SA1:1) calcined at 400 °C

was relatively large compared with that on the SA1:3, and that sodium hydrogen carbonate solution was better than sodium hydroxide solution or aqueous ammonia as an alkaline eluent. Based on these results, we have further attempted the separation of the two acidic amino acids by means of a column operation using the SA1:1 calcined as an adsorbent (Fig. 2). As a result, L-aspartic acid was eluted solely below 120 cm^3 of the eluent amount, while L-glutamic acid between 180 cm^3 and 220 cm^3 of the eluent amount. The recovery percentage of pure L-aspartic acid was 40% and that of pure L-glutamic acid 25%, respectively.

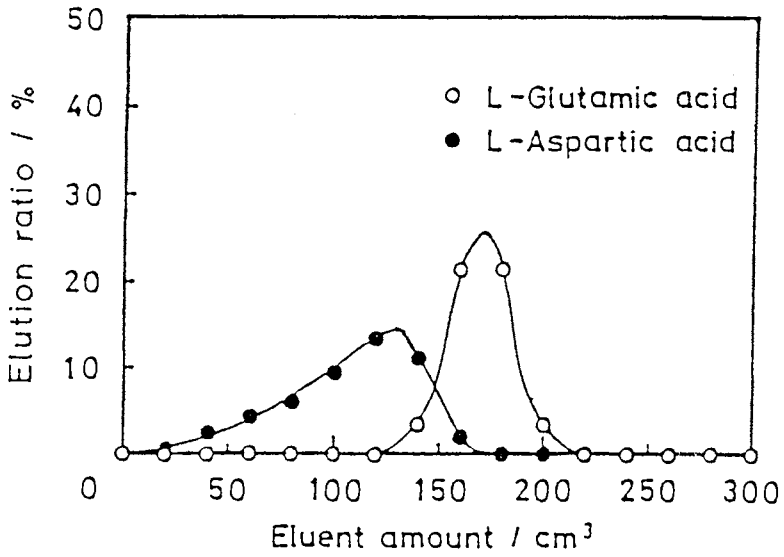

Fig. 2. Elution curves of acidic amino acids on SA1:1 calcined at 400 °C using $NaHCO_3$ solution.

On the other hand, the adsorptions of two basic amino acids, L-histidine and L-lysine on silica-titania of molar ratio 3:1 (ST3:1) were diversely affected by the pH of solution, as shown in Fig. 3. The difference of the adsorption percentages between the two basic amino acids was larger at the pH's 6.5 and 8.3, and the separation of these two basic amino acids may be possible at the pH 8.3. Furthermore, we have attempted the separation of these basic amino acids by means of an elution process. The elution was then conducted on the gel which adsorbed the two basic amino acids sufficiently in the solution at pH 8.3. Figure 4 shows that the elution percentage of L-histidine was the highest and that of L-lysine negligible at pH 2; the separation of these two basic amino acids was almost complete and the efficiency was relatively high (the recovery percentage ca. 75%).

The initial pH of amino acid solutions as well as the combination of the amino acids and the binary gels had an influence on the adsorption. The elution of the amino acid adsorbed on the gel was also affected by the pH of eluent. Consequently, we have succeeded in the separation of several kinds of mixed amino acids with the gels by means of batch or column operations.

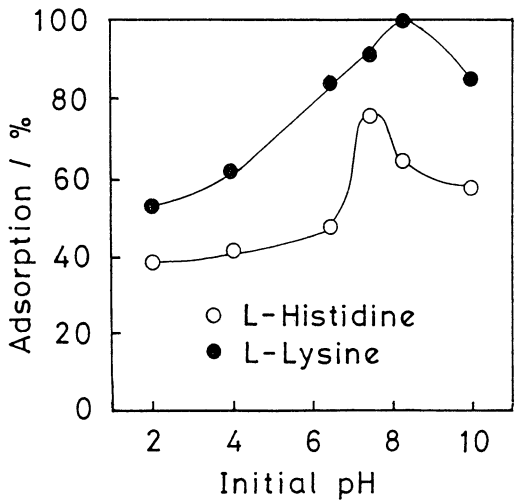

Fig. 3. Effect of initial pH on adsorption of basic amino acids on ST3:1.

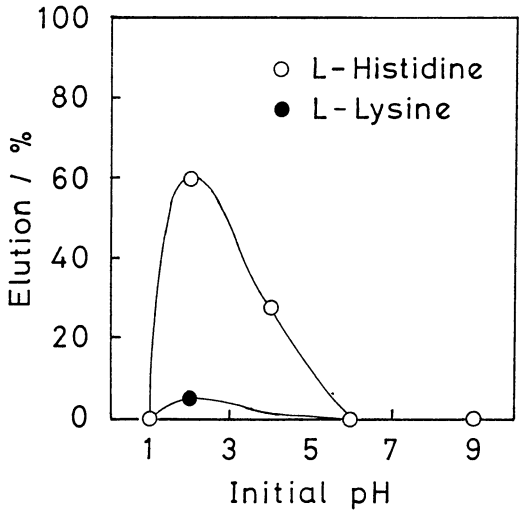

Fig. 4. Effect of initial pH on elution of basic amino acids on ST3:1.

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