A Method for the Fractionation of Oligopeptides Based on their Molecular Sizes

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Amino-acid sequence studies of proteins are of interest for the elucidation of mechanism of their biological activities. In any such study, a large number of peptides produced by partial hydrolysis of protein must first be separated from one another. Although several methods of their fractionation, such as ion exchange chromatography, ionophoresis based on their electrochemical properties, countercurrent distribution utilizing the difference of their partition coefficients between two immissible solvents and adsorption chromatography, are available, no method based on their molecular size is known.

Partridge[1] has suggested the molecular sieve effect of ion exchange resins as a tool for the fractionation of a series of various substances which have only different molecular sizes, and Thompson[2] showed that C-terminal leucine liberated from lysozyme by the action of carboxypeptidase could be separated from the remaining large protein fragment with the aid of strongly acidic ion exchange resin. Further, Deuel et al.[3] found that polygalacturonic, polymannuronic and polymetaphosphoric acid could be separated by such a method from their low molecular components. Richardson[4] has used ion exchange resin for the purification of crude dyestuffs which contained low molecular coupling substances. Kressman[5] has studied fundamental problems of the separation of various dyestuffs based on their ionic sizes.

The present authors attempted to establish a general method to fractionate various oligopeptides based on their hydrated molecular sizes by the molecular sieve of ion exchange resin.

It is a well-known fact that various amino acids are adsorbed on the hydrogen form of sulfonic acid type ion exchangers from neutral or slightly acidic solutions. In general, polystyrene type ion exchange resin has a three dimensional network structure corresponding to the extent of cross-linkage which is attributed to its divinylbenzene content. In order to be exchanged and adsorbed on the resin, the solute has to possess suitable polar group(s), and it has to be able to penetrate into the resin molecule and to react with it. Consequently large molecules, such as proteins, can not diffuse into highly cross-linked ion exchange resin and no adsorption will result, because the network is too small even though suitable dissociable groups are present in the solute molecules. On the contrary, small molecules will be adsorbed easily. On such a principle, when a solution of a peptide mixture is put on the top of the highly cross-linked ion exchange column which is assembled in series with stepwise decreasingly cross-linked resin columns, peptides will be adsorbed separately on the resins which possess the proper pore size respectively.

In the present study Dowex 50-X 16, X 12, X 8, X 4, X 2 and X 1 were used in order to study the behavior of some amino acids, synthetic peptides and the partial hydrolysates of modified silk fibroin[6] and casein towards ion exchange resins of various cross-linkages; it appears that this method was useful for the preliminary separation of peptides based on the difference of their molecular sizes.

Experimental

Materials.—Dowex 50-X 16 (20-50 mesh, lot 5754), X 12 (20-50 mesh, lot 8106), X 6 (20-50 mesh, lot 181), X 4 (50-100 mesh, lot 3458-40), X 2 (50-100 mesh, lot 3458-42) and X 1 (50-100 mesh, lot no. unknown) were used in the hydrogen form after several cycles from sodium to hydrogen forms have been performed.

The oxidative degradation product of silk fibroin, which was prepared by the reaction with hydrogen peroxide as described in earlier papers[6], contains only glycine, alanine and serine in

1) S. M. Partridge, *Nature*, **169**, 496 (1952).
2) A. R. Thompson, ibid., **169**, 495 (1952).
3) H. Deuel, J. Solms and L. Anayas-Weisz, *Helv. Chim. Acta*, **33**, 2171 (1950).
4) R. W. Richardson, *Nature*, **164**, 916 (1949); *J. Chem. Soc.*, **1951**, 910.
5) T. R. E. Kressman, *J. Phys. Chem.*, **56**, 118 (1952).
6) K. Narita, *J. Chem. Soc. Japan, Pure Chem. Sec.* (*Nippon Kagaku Zassi*), **75**, 487 (1954); S. Akabori, K. Satake and K. Narita, *Proc. Japan Acad.*, **25**, 206 (1949).

molar ratio of about 5 : 4 : 1 as constituent amino acids.

Enzymatic digests of casein were kindly supplied by Daigo Nutritive Chemicals, and in this case a mixture of bovine pancreas enzymes was used as a hydrolytic agent. Whale insulin was also supplied by Shimizu Pharmaceutical Co. and various authentic amino acids were supplied by Ajinomoto Co. Ltd.

A part of synthetic peptides (DL-leucylglycylglycine and DL-alanylglycylglycine) was supplied by Professor H. Tani of Osaka University and other peptides were synthesized by one of the authors (K. Narita). To confirm the purity of peptides, these were checked with paper chromatography by the use of several solvents and it was found that these were homogeneous.

Procedure.—*Multicolumn of Dowex 50.* Six columns (inner diameter 0.9 cm.) containing, from top to bottom, Dowex 50-X 16, X 12, X 8, X 4, X 2 and X 1 respectively were assembled in series. To fractionate peptides or to investigate on which column a single peptide was mainly adsorbed, 10 ml. of sample solution was put on the top column (X 16 column), and, after the solution sank under the resin surface, the developer (water or diluted hydrochloric acid) was added with a flow rate of 0.2 ml. per min. at the lowest column (X 1 column) until the effluent amounted to 250 ml.

Water, 0.1 and 0.3% hydrochloric acid were used as solvent for peptide solutions. Prior to the addition of the peptide solution, all columns were equilibrated with the solvent by passing it through them overnight. The effluent was concentrated at 40°C in vacuo to 15 ml., transferred to a 25 ml. volumetric flask and filled with water to the marked level. After the disassembly of the multicolumn system, adsorbed peptides were eluted from each column with 2 N sodium hydroxide using the same flow rate as for the development. It was necessary to use 1.5 fold excess of the sodium ion over the total exchange capacity of the resin, in order to displace the peptide completely under the given conditions; the column was then washed with water. The eluate was collected in a 25 ml. or 50 ml. volumetric flask. Aliquots were used for total and α-amino nitrogen determinations. The analyses were performed by the micro Kjeldahl and the manometric van Slyke method respectively and the average peptide length (number of amino acids in the peptide) in the eluate was represented as follows.

$$\text{average peptide length} = \frac{\text{total nitrogen}}{\alpha\text{-amino nitrogen}}$$

When preparative fractionation of peptides in mixture is attempted, it is preferable to use 10% aqueous ammonia solution instead of sodium hydroxide solution as the eluting solvent, to avoid the trouble of desalting. The total exchange capacities of each resin column were listed in the tables. The amount of sample to be fractionated is preferably equivalent to 1/5 to 1/20 of the total exchange capacity of each resin column.

Batch method.—To get the knowledge about the maximum adsorption amount of some amino acids and peptides on the resins, a batch method was used. Into 30 ml. of the solution containing about 150 mg. of amino acid or peptide, the resin was added in an amount corresponding to the theoretical for the exchange of the entire solute, stirred occasionally, kept to stand over-night and an aliquot of the supernatant was pipetted. The amount of unadsorbed solute was estimated by ninhydrin method using color yield of each solute to leucine and the adsorbed quantity was calculated. In Table VIII the ratios of adsorbed amounts of amino acids or peptides to the sodium ion (exchange capacity) are shown.

Results and Discussion

As mentioned already, chemically modified silk fibroin contained only the neutral amino acids of small molecule, glycine, alanine and serine[6]. Consequently, in order to test the possibility of the success of fractionation of peptides based on their hydrated molecular sizes with the aid of a molecular sieve, it seemed suitable to use the partial hydrolysate of this protein. The peptide mixture obtained by hydrolysis of the sample (500 mg.) with 25 ml. of concentrated hydrochloric acid at 40°C for 2 hours was concentrated in vacuo at 40°C to 15 ml. The concentrate was diluted with four volumes of water and treated with free form of Amberlite IR-45 column to remove the remaining hydrochloric acid. The effluent was concentrated in vacuo to 30 ml. An aliquot of this concentrate was diluted with water to 20 ml. and another aliquot was adjusted to 0.1% hydrochloric acid concentration by adding diluted acid to 20 ml. Both samples were fractionated separately with the Dowex 50 multi-column system as described in the experimental section. As listed in Table I, these results satisfied the authors' expectation.

When the tendencies of adsorption of petidpes from water are compared in Table I with those from 0.1% hydrochloric acid solution, one can find that larger peptides are much more adsorbed from water than from acid solution on the same resin column. This fact suggests that the pore size in the resin molecule decreases in hydrochloric acid. It is a generally well-known fact that ion exchange resins shrink to some extent in the presence of electrolytes and that the degree of shrinkage depends upon the concentration of electrolyte in the surrounding medium. The shrinkage of low cross-linked resin is so particularly

TABLE I

THE FRACTIONATION OF PEPTIDES IN THE PARTIAL ACID HYDROLYSATE OF MODIFIED SILK FIBROIN BY MOLECULAR SIEVE

Developer*		H_2O			0.1 % HCl**		
Cross-linkage	Total exchange capacity of column	Total N	α-NH_2 N	Average peptide length	Total N	α-NH_2 N	Average peptide length
	meq.	mg	mg		mg	mg	
X 16	9.4	16.2	6.92	2.4	5.77	2.28	2.6
12	8.6	1.85	0.58	3.2	4.18	1.65	2.4
8	9.5	1.50	0.28	5.4	5.00	1.73	3.4
4	6.1	1.50	0.17	8.8	6.23	1.11	5.6
2	3.8	0.69	0.03	23	1.67	0.26	6.4
1	1.8	0.40	0.01	40	0.71	0.08	8.4
Effluent	—	5.17	0.10	52	3.20	0.15	21.4
Total		27.3	8.09	—	26.8	7.11	—
Charged mixture		29.7	8.25	3.6	29.7	8.25	3.6
Recovery		92.7%	89.1%	3.4***	89.9%	86.1%	3.8***

* Flow rate, 0.2 ml./min.

** pH 1.7

*** The value was calculated from the analytical results.

TABLE II

THE EFFECT OF THE DEVELOPER TO THE ADSORBABILITY OF DL-ALANYLGLYCYLGLYCINE ON DOWEX 50

Developer*		H_2O		0.1 % HCl**		0.3 % HCl**	
Concentration of inflow		0.403 mM		0.314 mM		0.396 mM	
Cross-linkage	Total exchange Capacity of column	Amount adsorbed	Per cent. adsorbed	Amount adsorbed	Per cent. adsorbed	Amount adsorbed	Per cent. adsorbed
	meq.	mM	%	mM	%	mM	%
X 16	7.2	0.400	99.2	0.124	39.6	0.015	3.8
12	6.2	0	0	0.176	56.2	0.090	22.8
8	6.0	0	0	0	0	0.219	55.5
4	4.5	0	0	0	0	0.071	18.0
2	2.9	0	0	0	0	0	0
1	1.5	0	0	0	0	0	0
Total		0.400	99.2	0.300	95.8	0.395	99.8

* Flow rate, 0.2 ml./min.

** pH 1.7

*** pH 1.3

remarkable that it sometimes shrinks to about half of its initial volume. A natural consequence of the decreasing resin volume is that the pore size of network structure of the resin is decreased. Therefore, when hydrochloric acid concentration is increased, it is expected that the pore size of the resin becomes much smaller. In this respect, the behavior of DL-alanylglycylglycine in water, 0.1 and 0.3% hydrochloric acid solutions towards resin was studied. As shown in Table II, it is possible to change the pore size of the resin artificially, but to increase the acid concentration above 0.3% is not practicable because the dissociation of sulfonic acid groups of the resin will be suppressed. However, when organic acids, such as formic or acetic, are used, this defect will be avoided. The extent of the change of the pore size was remarkable in proportion to the decrease of divinylbenzene content as shown in Table I. As an alternate explanation of the above mentioned facts, one can not exclude the change of the shape and the size of the peptide in the presence of the electrolyte. However it appears probable that the change in the pore size of the resin is more important than that of the shape and the size of peptide in this case. On the other hand one must consider that the suppression of the dissociation of sulfonic acid groups in the resin molecule may cause the decrease in adsorption quantity of peptide in the acidic solution.

TABLE III
EXCHANGE CAPACITIES* OF DOWEX 50-X 16, X 12 AND X 8** IN WATER AND DILUTED HYDROCHLORIC ACID

Dowex 50	H_2O		0.1% HCl***		0.3%HCl****	
	meq.	%	meq.	%	meq.	%
X 16	3.95	(100.0)	3.82	(96.7)	3.82	(96.7)
12	3.15	(100.0)	3.07	(97.5)	3.03	(96.2)
8	3.65	(100.0)	3.51	(96.2)	3.45	(94.5)

* These were measured by batch method and the values were expressed for Na ion.
** The resins used were dried at 80°C in the hydrogen form for 10 hours.
*** pH 1.7
**** pH 1.3

But this possibility was excluded because of the finding of no marked differences between exchange capacities of the resins for the sodium ion in water and diluted hydrochloric acid as shown in Table III.

Table IV and V show various kinds of behavior of some amino acids in 0.3% hydrochloric acid towards the resin columns. Although the molecular size of L-leucine should be greater than that of L-alanine, the former was much more adsorbed on X 16 resin than the latter contrary to the authors' expectation as illustrated in Table IV. Similar phenomena were also found by the batch method

TABLE IV
ADSORPTION OF SOME AMINO ACIDS ON DOWEX 50 MULTICOLUMN SYSTEM FROM 0.3% HYDROCHLORIC ACID* SOLUTION

Amino acid		Glycine		L-Alanine		L-Leucine		L-Aspartic acid		L-Lysine	
Concentration of inflow		0.563 mM		0.503 mM		0.416 mM		0.396 mM		0.482 mM	
Cross-linkage	Total exchange capacity meq.	A** mM	B*** %	A** mM	B*** %	A** mM	B*** %	A** mM	B*** %	A** mM	B*** %
X 16	7.2	0.468	6.5	0.124	1.7	0.335	4.7	0.015	0.21	0.387	5.4
12	6.2	0.093	1.5	0.388	6.3	0.065	1.0	0.018	0.29	0.020	0.34
8	6.0	0	0	0	0	0	0	0.288	4.8	0	0
4	4.5	0	0	0	0	0	0	0.041	0.91	0	0
2	2.9	0	0	0	0	0	0	0	0	0	0
1	1.5	0	0	0	0	0	0	0	0	0	0
Total		0.561		0.512		0.400		0.362		0.407	
Recovery		99.6%		101.7%		96.2%		91.4%		84.6%	

* Flow rate, 0.2 ml./min.; pH 1.3.
** Amount adsorbed.
*** Ratio of adsorption of amino acid to the Na ion.

TABLE V
ADSORPTION OF SOME PEPTIDES ON DOWEX 50 MULTICOLUMN SYSTEM FROM 0.3% HYDROCHLORIC ACID* SOLUTION

Peptide		Gly-L-Leu		DL-Leu-Gly		Gly-L-Leu-Gly		Gly-L-Leu-L-Leu		DL-Leu-Gly-Gly		DL-Ala-Gly-Gly	
Concentration of inflow		0.365 mM		0.422 mM		0.361 mM		0.230 mM		0.292 mM		0.396 mM	
Cross-linkage	Total exchange capacity meq.	A** mM	B*** %	A** mM	B*** %	A** mM	B*** %	A** mM	B*** %	A** mM	B*** %	A** mM	B*** %
X 16	7.2	0.086	1.2	0.060	0.83	0.049	0.68	0.041	0.57	0.013	0.18	0.015	0.21
12	6.2	0.241	3.9	0.210	3.4	0.227	3.7	0.148	2.4	0.080	1.3	0.090	1.4
8	6.0	0.036	0.6	0.122	2.0	0.027	0.45	0.035	0.58	0.189	3.2	0.219	3.6
4	4.5	0	0	0	0	0	0	0	0	0.011	0.24	0.071	1.6
2	2.9	0	0	0	0	0	0	0	0	0	0	0	0
1	1.5	0	0	0	0	0	0	0	0	0	0	0	0
Total		0.363		0.392		0.303		0.224		0.293		0.395	
Recovery		99.6%		93.1%		83.9%		97.5%		100.3%		99.8%	

* Flow rate, 0.2 ml./min.; pH 1.3.
** Amount adsorbed.
*** Ratio of adsorption of peptide to Na ion.

(Table VIII). To explain this fact one must consider that the hydrated molecular size of L-alanine is much larger than that of L-leucine in acid solution. In the case of L-aspartic acid, Table IV suggests that the effect of side chain carboxyl group can not be ignored under these conditions.

When the adsorption quantities of glycyl-L-leucine and DL-leucylglycine are compared with one another, it is clear that the effect of the size of the N-terminal amino acid is greater than that of the C-terminal one, as expected. The results of the tripeptides listed in Table V also satisfy the authors' expectation; namely the larger the molecular size of the peptide, the more the decrease in the adsorption quantity of peptide on the resins.

TABLE VI

THE BEHAVIOR OF WHALE INSULIN IN 0.1% HYDROCHLORIC ACID* TOWARDS DOWEX 50 MULTICOLUMN SYSTEM

Concentration of inflow		5.48 mg N	
Cross-linkage	Total exchange capacity meq.	Amount adsorbed mg N	Per cent adsorbed %
X 16	7.2	>0.011	>0.2
12	6.2	>0.025	>0.5
8	6.0	>0.020	>0.4
4	4.5	0.431	7.9
2	2.9	0.683	12.6
1	1.5	1.11	20.3
Effluent	—	2.74	50.0
Total		5.01	91.4

* pH 1.7, flow rate, 0.2 ml./min.

As shown in Table VI, whale insulin was hardly ever adsorbed from 0.1% hydrochloric acid solution on high cross-linked resins. In such an acid solution, it will be considered that insulin is dissociated to monomer molecules (molecular weight 6,000). If the insulin molecule were spherical, the hydrated particle diameter could be calculated as roughly 27Å[7] from the data of Fredericq et al.[8] Thus it seems that Dowex 50-X 16, X 12 and X 8 do not have the pore of diameter of above 27Å in 0.1% hydrochloric acid solution.

In a further experiment, the authors attempted to separate the synthetic mixture of glycine, DL-leucylglycine and DL-alanylglycine in 0.3% hydrochloric acid solution from one another by the molecular sieve. After the elution of adsorbed materials with 2 N sodium hydroxide, the desalting and then one dimensional chromatography in phenol-water system, it was found that glycine was adsorbed on X 16 resin and a part on X 12 resin, DL-leucylglycine was adsorbed mostly on X 12 resin and partly on X 8 resin, and most of the tripeptide was adsorbed on X 8 and a small amount of X 4 resin.

In Table VII, two examples of the result which was obtained by the treatment of enzymatic digests of casein with molecular sieve column are listed. Digest I represents an almost completely digested mixture with an average peptide length of 1.78, while Digest II represents a partial digest mixture of average peptide length of 4.66. These data seem to show that the peptides are poorly separated. However, when the behavior of L-aspartic acid shown in Table IV is considered, one can suppose that the behavior of acidic amino acids and their peptides is not necessarily controlled only by their molecular sizes. It is a well-known fact that casein contains a large amount of acidic amino acids and especially strongly acidic phosphoserine residues. In fact, large quantities of phosphoric acid, serine, proline, aspartic and glutamic acid, leucine and alanine were detected in the complete hydrolysate of the effluent fraction which passed through the whole column system. This fact seems to indicate that the phosphopeptides in casein digests contain these amino acids, and that they are resistant to enzymatic attack as the average peptide length in the effluent fraction is quite great.

Moreover, the maximum adsorption quantities of some amino acids and peptides on resins were measured by the batch method (Table VIII). Comparing these figures with those of Table IV and V, one can see that the adsorption quantities of the solutes in column method are only 7 to 15% of those in the batch method. These facts indicate that the adsorption equilibrium is not reached at the flow rate of 0.2 ml. per min. in the column method and that for the solute to react completely with the resin a much slower rate is required. Table VIII shows that there is only a slight difference between maximum adsorption quantities of DL-leucylglycine and DL-alanylglycylglycine in 0.3% hydrochloric acid solution, but in the column method both peptides

7) The calculation was done by Dr. Y. Kawade of the Virus Research Institute, Kyoto University and the authors express their thanks to him.

8) E. Fredericq and H. Neurath, *J. Am. Chem. Soc.*, **72**, 2684 (1950).

TABLE VII
THE FRACTIONATION OF PEPTIDES IN ENZYMATIC DIGEST OF CASEIN*

Sample		Digest I			Digest II		
Cross-linkage	Total exchange capacity	Total N	α-N_2H N	Average peptide length	Total N	α-N_2H N	Average peptide length
		mg	mg		mg	mg	
		22.98	12.90	1.78	80.24	17.24	4.66
	meq.	mg	mg		mg	mg	
X 16	7.2	7.19	5.20	1.38**	12.34	4.73	2.51**
12	6.2	4.94	4.26	1.16	5.71	1.78	3.21
8	6.0	2.89	2.76	1.05	10.41	2.89	3.60
4	4.5	1.10	0.95	1.16	25.98	6.34	4.10
2	2.9	0.31	0.31	1.00	4.44	0.97	4.57
1	1.5	0.0	0.0	—	1.50	0.32	4.69
Effluent	—	4.20	0.30	14.0	6.95	0.22	31.58
Total		20.63	13.78		67.33	17.25	
Recovery		89.8%	106.8%	1.49***	83.9%	100.0%	4.90***

* Developed with 0.3% hydrochloric acid, pH 1.3, at flow rate of 0.2 ml./min.
** In this fraction, basic amino acids are also contained.
*** The value was calculated from the analytical results.

TABLE VIII
MAXIMUM ADSORPTION QUANTITIES OF SOME AMINO ACIDS AND PEPTIDES ON DOWEX 50
(Figures show adsorbed solutes in percentages of exchange capacities in water.)

	Cross-linkage	H_2O	0.1% HCl*	0.3% HCl**
		%	%	%
Glycine	X 16	85.2	85.5	55.3
	12	86.9	87.7	58.0
	8	87.2	86.6	54.5
L-Alanine	X 16	—	—	32.8
	12	—	—	40.0
	8	—	—	36.1
L-Leucine	X 16	—	—	38.8
	12	—	—	48.5
	8	—	—	41.8
L-Aspartic acid	X 16	39.8	—	13.4
	12	—	—	21.4
	8	—	—	24.4
L-Lysine	X 16	—	—	37.6
	12	—	—	39.9
	8	—	—	37.1
DL-Leu-Gly	X 16	58.5	34.1	14.0
	12	78.8	46.8	22.8
	8	88.5	53.7	29.8
DL-Ala-Gly-Gly	X 16	62.4	20.2	13.8
	12	78.2	28.3	20.2
	8	85.5	35.6	24.8

* pH 1.7
** pH 1.3

are successfully separated from each other (also see Table V). This fact indicates the adsorption velocity of DL-alanylglycylglycine is lower than that of DL-leucylglycine. Contrary to the authors' expectation, this concept, unfortunately, was incompatible with experimental results

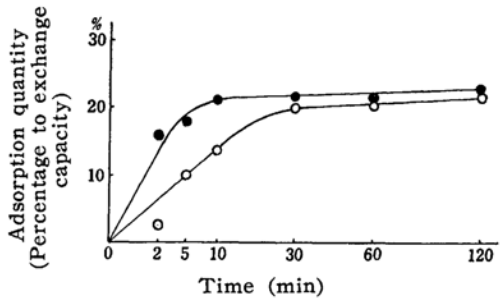

Fig. 1. The rates of adsorption of DL-alanylglycylglycine and DL-leucylglycine on Dowex 50-X 12 from 0.3 per cent. hydrochloric acid solutions.
●—● DL-Alanylglycylglycine
○—○ DL-Leucylglycine

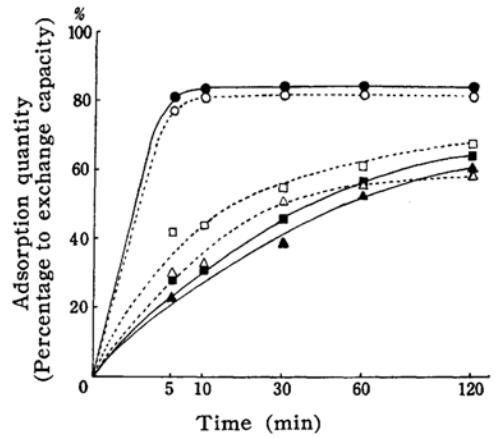

Fig. 2. The rates of adsorption of glycine, L-phenylalanine and DL-alanylglycylglycine on Dowex 50-X 16 from water. The black and the white marks show results obtained at room temperature and at 50°C respectively.
—●···○ Glycine
—■···□ L-Phenylalanine
—▲···△ DL-Alanylglycylglycine

shown in Fig. 1.

The adsorption velocities of glycine, L-phenylalanine and DL-alanylglycylglycine in aqueous solutions were also studied at room temperature (25°C) and 50°C (Fig. 2). No appreciable difference was found between velocities of low molecular glycine at either of the temperatures, while a slight difference was observed between those of L-phenylalanine and DL-alanylglycylglycine. Besides the ion exchange reaction, L-phenylalanine is adsorbed on the resin by van der Waals' force which is reduced at a high temperature, and thus aromatic amino acids may diffuse into a resin molecule easily at a higher temperature and their adsorption velocities will be increased. Generally speaking, the diffusion of solutes into the resin particles is favored by an elevated temperature. Consequently, so far as the solutes are not decomposed at high temperatures in contact with the resin, the fractionation of peptides in the column system will be much more favorable at a high temperature than at room temperature.

In the fractionation of peptides by molecular sieve, it is most important to use resins the size of whose particle is large as possible in order to reduce the surface area of the resin particle[1]; otherwise large peptides will be also adsorbed on the surface of high cross-linked resin as well as small molecular peptides. Another necessary condition is a problem of flow rate. When the flow rate is too great, the solute can not diffuse into properly cross-linked resin particles and then fractionation will be unsuccessful. By this method, not only sulfonic acid-type resins but also those of othe types may be used for peptides and another substances.

Summary

A method to fractionate peptides based on their hydrated molecular sizes by the molecular sieve effect of sulfonic-acid type ion exchange resins, Dowex 50, is described. The behavior of some amino acids, peptides and partial protein hydrolysates towards molecular sieve is also studied. Various factors which control the fractionation of solutes by molecular sieve are discussed.

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