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Measurement of Carbohydrate Contents in Multiple Forms of Hog Pancreatic Kallikrein and Their Behavior on Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis[1]

Carbohydrate content of kallikrein B was about twice as much as that of kallikrein A in neutral hexsose and glucosamine. Total sugar contents of kallikrein B-III and B-IV (main micro-heterogeneous forms derived from kallikrein B) were also about twice as much as that of kallikrein A-II (one of the main micro-heterogeneous forms derived from kallikrein A). Contents of sialic acid in heterogeneous form, A, (more acidic than B) contain rather less amount than that of B, while contents of sialic acid in the molecules of the microheterogeneous forms derived from both kallikreins A and B were different from each other and this difference was seemed to reflect their isoelectric points.

After the reduction of hog pancreatic kallikreins A and B with 0.04% 2-mercapto-ethanol in 0.01 M Tris-HCl buffer pH 7.4 containing 1% SDS, two protein bands were detected in each sample on polyacrylamide gel electrophoresis. However, although staining of carbohydrate moiety of the reduced preparation of kallikrein B by PAS reagent also showed two bands identical with the protein bands, only one of the protein bands of the reduced preparation of kallikrein A was stained for carbohydrate. From these results, it was revealed that both kallikreins A and B are consisted of two peptide chains, and one of the peptide chains of kallikrein A has no carbohydrate moiety, whereas the carbohydrate moieties are bound to both peptide chains in case of kallikrein B.

Keywords——hog pancreatic kallikrein; multiple forms of kallikrein; micro-heterogeneous forms; carbohydrate contents of kallikreins; sialic acid; isoelectric focusing; sodium dodecyl sulfate-polyacrylamide gel electrophoresis

Recently, it has been revealed that the glandular kallikreins, such as human urinary, hog pancreatic, rat submandibular kallikreins and so on, have multiple forms which are usually separable on Ampholine isoelectric focusing.[2] Multiple forms of hog pancreatic kallikrein were early observed by Moriya and Shimazawa (called a_1 and a_2 on paper electrophoresis Fig. 3. in ref.[3]) which were separable by electrophoresis with starch gel.[3] Afterwards, these two forms of kallikrein were certainly clarified and separated by other groups[4,5] named as kallikreins A and B. Moreover, kallikreins A and B were further separated into several

1) Enzymes: kallikrein (EC 3.4.21.8); neuraminidase (EC 3.2.1.18). Abbreviations: sodium dodecyl sulfate, SDS; Kallikrein Unit, KU; periodic acid-Schiff reagent, PAS.
2) K. Nustad, K.M. Gautvik, and J.V. Pierce, "Chemistry and Biology of the Kallikrein-Kinin System in Health and Disease," eds. by J.J. Pisano and K.F. Austen, U.S. Government Printing Office, Washington D.C., 1977, pp. 77—92.
3) H. Moriya and E. Shimazawa, *Yakugaku Zasshi*, **79**, 374 (1959).
4) C. Kutzbach and G. Schmidt-Kastner, *Hoppe-Seyler's Z. Physiol. Chem.*, **353**, 1099 (1972).
5) M. Zuber and E. Sache, *Biochemistry*, **13**, 3098 (1974).

micro-heterogeneous forms having different isoelectric points.[5,6] On the other hand, it was found that hog pancreatic kallikrein contains carbohydrate components.[5,7,8] In our previous paper,[9] the authors investigated the carbohydrate contents of kallikreins A and B and only some of the combined micro-heterogeneous forms derived from kallikrein B, and discussed the relationship between the multiple forms of kallikrein and their carbohydrate contents.

Recently the authors have purified further more amounts of these highly pure preparations, so that the present paper describes the investigation in detail of the carbohydrate contents and the compositions of the main micro-heterogeneous forms derived from kallikrein A (A-II and A-III) and B (B-III, B-IV and B-V) which were obtained by the isoelectric focusing method. Furthermore, the analysis of carbohydrate bound peptide chains of kallikreins A and B was performed by SDS-polyacrylamide gel electrophoresis. Hog pancreatic kallikreins A and B and their micro-heterogeneous forms (A-II, A-III, B-III, B-IV and B-V) were prepared mainly according to the method previously described[9] and used in our present experiments. The purified preparations of kallikreins A and B were homogeneous in disc electrophoresis with 10% (w/w) polyacrylamide gel, pH 8.9, and revealed their specific activities of 1350 and 1400 KU/mg, respectively.

Table I shows the carbohydrate compositions of kallikreins A and B and their micro-heterogeneous forms indicated (A-II, A-III, B-III, B-IV and B-V). The total sugar content of kallikrein B was about twice as much as that of kallikrein A. This observation was closely resemble with that of Fiedler *et al.*[8] In the micro-heterogeneous forms of kallikrein B (B-III and B-IV) roughly twice or a little less of the total sugar as also contained as compared with that of the micro-heterogeneous form of kallikrein A (A-II). Moreover, within each components of kallikreins A and B, the more acidic micro-heterogeneous components, the higher sialic acid contents were observed.

Table I. Carbohydrate Compositions of Hog Pancreatic Kallikreins (Residues/mol of Kallikrein)

	Fuc	Man	Gal	GlcNAc	NANA	pI	Total sugar
Kallikrein A	0.80	2.20	0.76	2.69	0.24	—	4.56%
B	2.44	3.92	1.78	4.63	0.47	—	8.40%
A-II	1.47	1.36	1.38	2.77	0.57	4.13	5.26%
A-III	2.35	3.41	0.19		0.16	4.24	
B-III	2.93	1.71	1.90	5.30	1.77	4.13	9.19%
B-IV	3.14	1.52	1.35	3.96	0.95	4.19	7.10%
B-V	3.21	4.14	0.48		0.33	4.29	

The values were calculated on the basis that the molecular weight of kallikreins A and B are 26800 and 28600, respectively.[8a,b]

Neutral sugars were estimated on gas chromatography (Shimadzu CG 6A; column: 3% OV-225) according to the procedures of Spiro.[a] Glucosamine was estimated with the amino acid analyzer (Hitachi automatic liquid chromatography 034; column: Hitachi custum ion exchanger resin # 2611). Sialic acid was determined by the periodate-resorcinol method described by Jourdian *et al.*[b]

a) R.G. Spiro, *Methods in Enzymol.*, **28**, 3 (1972).

b) G.W. Jourdian, L. Dean, and S. Roseman, *J. Biol. Chem.*, **246**, 430 (1971).

Fig. 1 shows the electrophoregrams of kallikreins A and B, treated with and without 1% SDS and 0.04% 2-mercaptoethanol in 0.01 M Tris-HCl buffer, pH 7.4 for 30 minutes at

6) F. Fiedler, C. Hirschauer, and E. Werle, *Hoppe-Seyler's Z. Physiol. Chem.*, **351**, 225 (1970).
7) H. Moriya, *Yakugaku Zasshi*, **79**, 1390 (1959).
8) *a*) F. Fiedler, C. Hirschauer, and E. Werle, *Hoppe-Seyler's Z. Physiol. Chem.*, **356**, 1879 (1975); *b*) F. Fiedler, *Methods in Enzymol.*, **45**, 289 (1976).
9) H. Moriya, Y. Fukuoka, Y. Hojima, and C. Moriwaki, *Chem. Pharm. Bull.*, **26**, 3178 (1978).

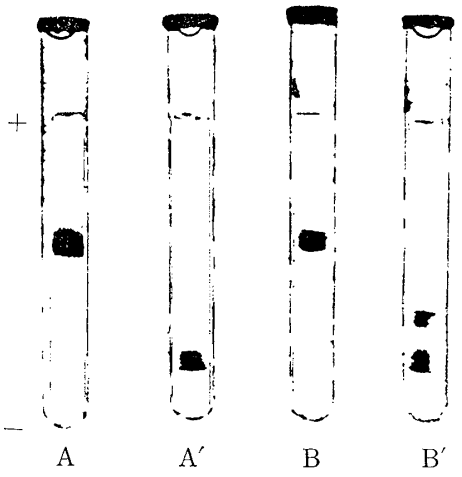

Fig. 1. Electrophoregrams of Kallikreins A and B before and after the Treatment of 2-Mercaptoethanol on SDS-Polyacrylamide Gel

Sixty μg of samples were applied in each gel. After the electrophoresis,[a] gels were stained with PAS reagent as described by Zacharius *et al.*[b]
A, B: kallikreins A and B, respectively,
A′, B′: reduced kallikreins A and B, respectively.
a) K. Weber and M. Osborn, *J. Biol. Chem.*, **244**, 4406 (1969).
b) R.M. Zacharius, T.E. Zell, J.H. Morrison, and J.J. Woodlock, *Anal. Biochem.*, **30**, 148 (1969).

37°. Gels were stained for sugars with PAS reagent. Both kallikreins A and B showed one band. In contrast to this, reduced samples of kallikreins A and B showed one (A′) and two (B′) bands, respectively. On the other hand, both kallikreins exhibited two bands when gels were stained for proteins with Coomassie Brilliant Blue R-250. The band detected with the reduced kallikrein A (A′) and faster migration band of the reduced kallikrein B (one of B′) were quite identical with the bands stained for sugars with PAS reagent. These results suggested that both kallikreins A and B are consisted with two polypeptide chains linked with disulfide bond, and one of the chains of kallikrein A has no carbohydrate moiety. While kallikrein B was consisted of two polypeptide chains, both which contain carbohydrate chains.

Fiedler *et al.*[8a] reported that the highly purified kallikreins A and B were prepared after treatment with neuraminidase and the amino acid compositions of kallikrein A were almost identical with kallikrein B. Both in our previous[9] and present investigations it was showed that the contents of neutral hexose and glucosamine of kallikrein B were about twice as much as those of kallikrein A, and especially sialic acid content in more acidic kallikrein A which showing faster migration on disc electrophoresis, was rather less amount than that of kallikrein B. In addition, it was revealed by our experiments that the sialic acid content of micro-heterogeneous components of kallikreins (A-II, A-III, B-III, B-IV and B-V) was closely related to their isoelectric points, so that micro-heterogeneity of kallikreins showing different acidity should be due to the contents of sialic acid. Judging from these results, it is concluded that the acidities of heterogeneous levels of A and B are not owing to the amount of sialic acid, while those of micro-heterogeneous forms are owing to that amount in their molecules.

Studies of the structure of the carbohydrate moiety of each micro-heterogeneous forms of kallikreins A and B are now under investigation.

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