Purification and Some Properties of Porcine Erythrocyte Adenylate Kinase

Norinaga MIWA

Public Health Station, Numazu-shi, Shizuoka 410

Kensuke CHIKAMORI

*Department of Anatomy, Faculty of Medicine, Tokushima University,
Tokushima-shi, Tokushima 770*

Bunei SYUTO and Shuichiro KUBO

*Department of Biochemistry, Faculty of Veterinary Medicine,
Hokkaido University, Sapporo-shi, Hokkaido 060*

(Received for publication October 24, 1977)

Abstract. Porcine erythrocyte adenylate kinase showed three components, AK_a, AK_b and AK_c, designated on the basis of the distance of migration from their origin to the anode in starch gel electrophoresis. By isoelectrofocusing, 96% of the total erythrocyte adenylate kinase focused at pH 9.25, and this predominant enzyme form corresponded to AK_a in starch gel electrophoresis. The predominant enzyme form, AK_a, was purified at approximately 31,000 fold from the porcine erythrocyte in an overall yield of 45% by affinity chromatography on a column of Blue Dextran-Sepharose 4B, substrate elution from a column of phosphocellulose, and by isoelectrofocusing. The purified AK_a had a specific activity of 2,100 units per mg of enzyme and migrated in gel electrophoresis as a single band having a molecular weight of 21,500. The amino acid composition of AK_a was the same as that of the skeletal muscle enzyme. Peptide mapping showed a correspondence of all spots of the tryptic peptides from the erythrocyte and skeletal muscle enzyme preparations. These results strongly suggest that the predominant adenylate kinase in porcine erythrocyte is identical to porcine skeletal muscle adenylate kinase.

Adenylate kinase (ATP:AMP phosphotransferase, EC, 2.7.4.3), an ubiquitous enzyme, exists in multiple molecular forms in various animal tissues [13]. The organ specificity of the adenylate kinase isozymes was observed in several animals on the bases of antibody and sulfhydryl reactivity and of the differences of enzyme distribution pattern in isoelectrofocusing [15]. The erythrocyte enzyme in humans has been used as a genetic marker enzyme to determine human population [2], and attempts

made to use the enzyme as a diagnostic tool for muscular diseases [9, 19] and myocardial infarction [11] have met with good success. Kaffarnick and Klaus [7], however, tested the adenylate kinase in the serum of patients with acute hepatitis, liver metastases and myocardial infarction and did not find it to be sensitive enough as a diagnostic tool. The relatively high activity of adenylate kinase in erythrocytes seems to have complicated the evaluation.

Our ultimate aim is to see if adenylate

ブタ赤血球アデニレートキナーゼの分離と性質について：三輪憲永（静岡県沼津保健所），近森憲助（徳島大学医学部解剖学教室），首藤文栄・久保周一郎（北海道大学獣医学部家畜生化学講座）

kinase in porcine blood is useful for determining whether pigs have PSE (pale soft exudative) muscle or not. The determination of the adenylate kinase level in erythrocytes and the characterization of the erythrocyte enzyme are required first. In this paper porcine erythrocyte adenylate kinase was analyzed for determining multiple forms and genetic variance by using starch gel electrophoresis and isoelectrofocusing. The predominant enzyme form in the erythrocyte was purified and a comparison of this enzyme to that from porcine skeletal muscle [5, 17, 18] is made.

Materials and Methods

Porcine erythrocyte: Porcine blood samples were obtained from a Sapporo slaughter house, and the washed erythrocytes were stored at -20°C before use.

Enzyme assay and protein determination: Adenylate kinase activity was determined as the rate of ADP formation from ATP and AMP in a coupled enzyme system at 25°C . The reaction mixtures contained 20 mM triethanolamine buffer at pH 7.5, 75 mM KCl, 8 mM MgSO_4 , 3 mM EDTA, 1.2 mM ATP, 0.375 mM phosphoenolpyruvate, 0.133 mM NADH, 2.5 units of lactic dehydrogenase, 2.5 units of pyruvate kinase, and a rate-limiting amount of adenylate kinase in a volume of 0.5 ml. The samples were diluted with 0.1% bovine serum albumin in 20 mM Tris-HCl buffer at pH 7.5 and immediately assayed. Hemoglobin was determined as the cyanomethemoglobin complex [1]. The protein was routinely estimated by the method of Lowry et al. [12] with bovine serum albumin as a standard. For the purified erythrocyte and skeletal muscle enzymes, a value of $A_{279\text{ nm}}^{1\%} = 5.38$ was used.

Electrophoresis: Starch gel electrophoresis was carried out in a starch gel at pH 7.4 and 4°C using the buffer system and staining procedure of Fildes and Harris [2]. Isoelectrofocusing was carried out in an LKB column of 110 ml volume at 0°C using 1% carrier ampholytes and a sucrose gradient. All solutions and gradients were prepared according to the LKB instruction manual. Disc electrophoresis was carried out by the method of Gabriel [3] using 7.5% polyacrylamide gels containing 4.4 M urea and 0.1 mM 2-mercaptoethanol at pH 4.3 with 50 mM β -alanine-acetic acid buffer as a running buffer. Electrophoresis in sodium dodecylsulfate (SDS) was

carried out by the method of Weber et al. [26] using 10% polyacrylamide gels at 8 mA per gel. All gels were stained with 0.03% Coomassie Brilliant Blue R-250 in 25% isopropyl alcohol-10% acetic acid solution overnight, and destained in 10% acetic acid solution.

Absorbent for column chromatography: Phosphocellulose (Brown Paper Co., Berlin, N.H., U.S.A., Lot No. 2184) was equilibrated in 0.1 M acetate buffer at pH 5.0. Blue Dextran-Sepharose 4B was prepared from Blue Dextran 2,000 (Pharmacia Fine Chemicals) and Sepharose 4B (Pharmacia Fine Chemicals) by the method of Ryan and Vestling [16], then equilibrated in 20 mM Tris-HCl buffer at pH 8.5.

Purification of porcine skeletal muscle adenylate kinase: The method of Noda et al. [14] for carp muscle adenylate kinase was used to purify the porcine muscle enzyme. The enzyme was homogeneous in disc and SDS gel electrophoresis.

Amino acid analyses: Samples of 0.4 mg of enzyme were hydrolyzed for 24, 48 and 72 hr at 105°C with constant-boiling glass-distilled HCl in sealed evacuated tubes. Analyses were carried out on a Hitachi amino acid analyzer, model KLA-3, by the method of Spackmen et al. [20]. Half-cystine was determined as the cysteic acid after oxidation with performic acid [6]. Tryptophan was determined by the spectrophotometric method of Goodwin and Morton [4].

Tryptic digestion and peptide maps: The enzyme was dissolved in 0.5% NH_4HCO_3 buffer at pH 8.0 to a concentration of 10 mg/ml, and digestion was performed for 6 hr at 37°C using trypsin (Washington TRTPCK 34K920), which was equal to 4% of the sample weight. Peptide mapping was carried out by the method of Svasti and Milstein [21] and Heil et al. [5]. Electrophoresis was carried out on the peptide mixture using Whatman 3 MM at pH 6.5 (pyridine-acetic acid-water, 25:1:225 by volume, cooled with Cool-X, Toyo Kagaku Sangyo, Tokyo). After cutting out the neutral band, descending chromatography was carried out by using a solvent system of butan-1-ol-acetic acid-water-pyridine (15:3:10:12, by volume) at right angle to the first direction. The neutral band was submitted to descending chromatography and rerun in electrophoresis at pH 1.9 (formic acid-acetic acid-water, 1:4:45, by volume, cooled with Cool-X).

Results

Electrophoretic patterns of adenylate kinase isozymes in the porcine erythrocyte: Adenylate kinase existed in three compo-

Fig. 1. Comparative electrophoretic patterns in starch gel

Remarks.

A: hemolysate of porcine erythrocyte; B: extract of porcine skeletal muscle; C: isolated enzyme form AK_a in porcine erythrocyte; D: hemolysate of human erythrocyte (AK_1 phenotype). After diluting samples were applied to the gel on filter paper strips to contain 10 units (hemolysate and muscle extract) and 5 units (isolated enzyme form) of activity per ml in 0.1% bovine serum albumin. Electrophoresis was carried out at pH 7.0 and 4°C for 4 hr at a constant 25 mA. Enzyme stain was developed for 1 hr at 37°C. Arrow indicates origin.

nents in the porcine erythrocyte in starch gel electrophoresis, as shown in Fig. 1; the components were designated AK_a , AK_b and AK_c , respectively, depending on their migration from origin to anode. All components migrated from origin to anode; no different isozyme pattern was observed in this experimental scale of 50 specimens. The erythrocyte pattern was similar to that of the porcine skeletal muscle.

Figure 2 shows the isoelectrofocusing pattern of adenylate kinase in hemolysate, and 96% of the total activity was seen as a sharp peak at pH 9.25. This fraction corresponded to AK_a in starch gel electrophoresis (Fig. 1). The residual activity focused as a broad peak from pH 6.5 to 7.5. The distribution of the activity in the erythrocyte was also similar to that of the porcine skeletal muscle.

Fig. 2. Isoelectrofocusing pattern of adenylate kinase in hemolysate

Remarks.

The enzyme solution of 130 units in 1% ampholine of pH 5-9.5 was applied to a column of 110 ml capacity. Isoelectrofocusing was carried out at 500 V for the first 5 hr, at 1,000 V for the next 6 hr, and at 1,500 V for 50 hr at 0°C. (●) pH; (○) adenylate kinase activity (U/ml).

Purification procedure of predominant enzyme form, AK_a : All procedures were carried out in a cold room or in an ice bath. The washed erythrocytes, which were kept frozen, were thawed in a cold room overnight. Cold water, equal to 6 times the volume of 900 ml of erythrocytes, was added and stirred. Then, 300 g of washed Celite 535 was mixed with the hemolysate, and a red solution was obtained by filtration through a 25 cm inner diameter Buchner funnel to give fraction I.

The pH of fraction I was adjusted to 8.5 with 2 M Tris, and the enzyme was absorbed on a column (38 by 5.5 cm) of Blue Dextran-Sepharose 4B previously equilibrated with 20 mM Tris-HCl buffer at pH 8.5 at a flow rate of 900 ml/hr. After washing with 3 column volumes of the same buffer, the activity was eluted with 0.4 M NaCl-20 mM Tris-HCl buffer at pH 8.5 to give fraction II. The activity was stable on freezing because of the high concentra-

tion of NaCl. Fraction II, which was obtained from three runs of 900 ml of erythrocytes, was stored at -80°C .

Phosphocellulose chromatography of fraction II was carried out by the method of Noda et al. [14]. Stored fraction II was thawed and diluted by the addition of 3 more volumes of cold water to lower the concentration of NaCl. The pH of diluted fraction II was reduced to 5.0 with N acetic acid, and the enzyme was absorbed at a flow rate of 300 ml/hr on a column (7.0 by 3.7 cm) of phosphocellulose equilibrated previously with 0.1 M acetate buffer at pH 5.0. After absorption of the activity, the column was washed by a) 1 column volume of 0.1 M sodium acetate buffer pH 5.0; b) 2 column volumes of 0.1 M sodium acetate buffer pH 5.7; c) 5 column volumes of 15 mM NaCl–50 mM morpholinopropane sulfonic acid-NaOH (MOPS) buffer pH 7.0; and d) 4 column volumes of 6 mM NaCl–20 mM MOPS buffer pH 7.0. Then, the enzyme was eluted by 0.5 mM ATP–0.5 mM AMP in 20 mM MOPS buffer at pH 7.0 to give fraction III.

To remove AK_b in fraction III, the fraction was concentrated to 50 ml by diaflo membrane (Amicon UM-10) and then iso-electrofocused in a column of 110 ml capacity in 1% ampholine of pH 8–9.5 and 2 mM dithiothreitol. The focusing was

carried out at 500 V for the first 5 hr and at 1,000 V for the next 6 hr, and continued at 1,500 V to obtain a constant current at 0°C . The activities focused at pH 9.25 as a sharp peak and also at 7.10. The fraction focused at pH 9.25 contained 96% of the total activity of fraction III and migrated at the position of AK_a in starch gel electrophoresis. The fraction focused at pH 7.10 had 4% of the total activity of fraction III and corresponded to AK_b in starch gel electrophoresis. The fraction showing isoelectric point 9.25 was applied at a flow rate of 10 ml/hr to a column (9 by 1.5 cm) of Blue Dextran-Sepharose 4B equilibrated previously with 20 mM Tris-HCl buffer pH 8.5. After washing with 5 column volumes of the same buffer, the activity was eluted with 0.5 mM ATP–0.5 mM AMP in 20 mM Tris-HCl buffer at pH 8.5 and collected to give fraction IV.

The result of this purification procedure is summarized in Table 1. The predominant enzyme form AK_a was purified by these procedures to approximately 31,000 fold from porcine erythrocyte in an overall yield of 45% to a final specific activity of 2,100 units per mg of enzyme.

Homogeneity and molecular weight of AK_a ; The purified AK_a showed a single protein band in SDS or disc electrophoresis, as shown in Fig. 3. A mixture of the puri-

Table 1. Purification of predominant adenylate kinase in porcine erythrocyte

Fraction	Total volume (ml)	Total protein (mg)	Total activity (units)	Yield (%)	Specific activity (units/mg)	Purification
I. Hemolysate, 1 : 6	17,500	579,000	42,000	100	0.07	1
II. Blue Dextran-Sepharose 4B	2,250	990	38,000	90	38	543
III. Phosphocellulose	134	30	29,000	69	966	13,800
IV. Blue Dextran-Sepharose 4B	6	9	19,000	45	2,111	30,159

Remarks.

The erythrocyte used for the starting material was 2,700 ml. Protein was estimated by hemoglobin measurement [1] in Fraction I and by the method of Lowry et al. [12] through Fraction II to IV.

Fig. 3. Polyacrylamide gel electrophoresis of AK_a

Remarks.

A: SDS gel electrophoresis. Fifteen μ g of enzyme was loaded on a gel. B: Disc electrophoresis. Twenty μ g of enzyme was loaded on a gel.

fied AK_a and adenylate kinase prepared from the skeletal muscle also migrated as a single band in SDS electrophoresis. To determine the molecular weight, a plot of electrophoretic mobilities of marker proteins against the logarithm of their weights gave a molecular weight of 21,500 for the purified AK_a , as shown in Fig. 4.

Amino acid composition and peptide map of AK_a : For comparison the amino acid composition of AK is presented in Table 2 together with that of the skeletal muscle enzyme, and it was found to be the same as that of the muscle enzyme. There were two cysteic acids per mole of AK_a oxidized by performic acid. When AK_a was treated with 5,5'-dithiobis-(2-nitro-benzoic acid), 1.7 moles sulfhydryl group per mole of enzyme reacted, and the activity was lost completely.

Peptide mapping of AK_a was carried out parallel to that of the skeletal muscle enzyme. For comparison the peptide map of the acidic and basic peptides of AK_a in electrophoresis at pH 6.5 are shown in Fig.

 Fig. 4. Determination of the molecular weight of AK_a by SDS electrophoresis

Remarks.

(●) Marker proteins; (○) AK_a . The condition of treatments of AK_a and marker proteins was the same as that in Fig. 3.

5 together with that of the skeletal muscle enzyme. Acidic peptide 12 and basic peptide 13 of AK_a migrated further in descending chromatography than did those of the skeletal muscle enzyme, but the amino acid compositions of peptide 13 (Ser₁, Pro₁, Gly₄, Val₂, Ile₂, Phe₁, Lys₁) and peptide 12 (Asp₁, Thr₁, Glu₂, Pro₁, Gly₁, Val₂, Met₁, Leu₄, Arg₁) of AK_a were identical to those of corresponding peptides of the skeletal muscle enzyme. The neutral peptides obtained from electrophoresis at pH 6.5 were further fractionated by chromatography and by electrophoresis at pH 1.9, as shown in Fig. 6. The number and location or amino acid composition of all major peptide spots from AK_a of the map were essentially identical to those from the muscle enzyme.

Discussion

Porcine erythrocyte adenylate kinase existed in three components, all of which migrated from their origin to the anode, and no different isozyme pattern could be observed in this experimental scale, unlike the

Table 2. Amino acid composition of predominant adenylate kinase in porcine erythrocyte

Amino acid	Residues	Integral number of residues	Skeletal muscle adenylate kinase [5]
Aspartic acid	13.1*	13*	13
Threonine ^a	13.7	14	14
Serine ^a	11.2	11	11
Glutamic acid	25.3	25	25
Proline	6.3	6	6
Glycine	18.7	19	19
Alanine	7.9	8	8
Valine ^b	16.9	17	17
Methionine	5.7	6	6
Isoleucine ^b	8.8	9	9
Leucine	18.0	18	18
Tyrosine	6.6 (7.1) ^c	7	7
Phenylalanine	4.9	5	5
Histidine	1.8	2	2
Lysine	20.6	21	21
Arginine	11.1	11	11
Tryptophan ^c	0.05	0	0
Cysteine ^d	1.8	2	2
Total		194	194

Remarks.

The data was obtained from 24, 48 and 72 hr hydrolysates. a: Extrapolated value to 0 time. b: Values are for the 72 hr hydrolysis. c: Determined by the method of Goodwin and Morton [4]. d: Determined as cysteic acid [6].

*: Per 21,500 g enzyme.

isozyme patterns of different adenylate kinase phenotypes observed in the human erythrocyte [2]. In order to observe genetic variants, however, the experimental scale and selection of porcine species must be increased.

The predominant enzyme form AK was purified in an overall yield of 45% to a final specific activity of 2,100 units per mg of enzyme. Tsuboi and Chervenka [24] purified the predominant form of adenylate kinase in the human erythrocyte in an overall yield of 14% to a final specific activity of 3,200 units per mg of enzyme. Comparing the two methods of purification, Blue Dextran-Sepharose 4B and phosphocellulose chromatography in this purification procedure was very effective, especially the former, which was very useful in removing large amounts of hemoglobin and in

purifying enzymes showing a dinucleotide fold structure [22] in the erythrocyte. The activity level of AK_a was less than that of the human erythrocyte enzyme [24] but equal to that of human [23, 25], rabbit [10] and carp [14] muscle enzymes. At this point, we can not attribute these differences in the activity level to the differences in species or to the methods employed.

The molecular properties and amino acid composition of AK_a are in agreement with those of the skeletal muscle enzyme. The peptide spots of the fingerprint of the enzyme accounted for all of the peptide spots identified on the fingerprint of the skeletal muscle enzyme. There are 21 lysines and 11 arginines per mole of enzyme, however, the tryptic digest of enzymes showed 38 peptide spots in the fingerprint. This excess number of peptide spots reflects the

existence of a continuous sequence of lysine or arginine in the molecule, as shown by Heil et al. [5].

On the basis of anti-rabbit muscle adenylate kinase and sulfhydryl reactivity studies, Khoo and Russel [8] and Russel et al. [15] proposed that erythrocyte adenylate kinase in humans is similar to the enzyme of the skeletal muscle. Tsuboi and Chervenka [24] purified the predominant form of adenylate kinase in the human erythrocyte and established that the purified enzyme is similar in physicochemical and kinetic properties to rabbit muscle adenylate kinase. Because of the correspondences of molecular weights, isoelectric points, amino acid compositions and fingerprints, it may be concluded that the erythrocyte and skeletal muscle adenylate kinases in the same species are identical.

References

- [1] Cartwright, G. E. (1963). In Diagnostic Laboratory Hematology, Grune and Stratton, New York, 42-51.
- [2] Fildes, R. A., and Harris, H. (1966). Genetically determined variation of adenylate kinase in man. *Nature*, **209**, 261-263.
- [3] Gabriel, O. (1971). Analytical disc gel electrophoresis. In *Methods in Enzymology*, **22**, Jakoby, W. B., editor, Academic Press, New York, 565-578.
- [4] Goodwin, T. W., and Morton, R. A. (1946). The spectrophotometric determination of tyrosine and tryptophan in proteins. *Biochem. J.*, **40**, 628-632.
- [5] Heil, A., Müller, G., Noda, L., Pinder, T., Schirmer, H., Schirmer, I., and von Zabern, I. (1974). The amino acid sequence of porcine adenylate kinase from skeletal muscle. *Eur. J. Biochem.*, **43**, 131-144.
- [6] Hirs, C. H. W. (1956). The oxidation of ribonuclease with performic acid. *J. biol. Chem.*, **219**, 611-621.
- [7] Kaffarnick, H., and Klaus, D. (1962). Adenylatkinase, Adenosin-triphosphate-phosphatase und Kreatinphosphokinase im Serum von Gesunden und Kranken. *Artzl. Forsch.*, **16**, 361-365.
- [8] Khoo, J. C., and Russell, P. J. (1972). Isoenzyme of adenylate kinase in human tissue. *Biochim. Biophys. Acta*, **268**, 98-101.
- [9] Kitchin, S. E., and Watts, D. C. (1974). Comparison of adenylate kinase from liver and muscle of normal mice and those with an hereditary muscular dystrophy. *Biochim. Biophys. Acta*, **364**, 272-283.
- [10] Kress, L. F., Bono, Jr. V. H., and Noda, L. (1966). The sulfhydryl groups of rabbit muscle adenosine triphosphate-adenosine monophosphate phosphotransferase: Activity of enzyme treated with mercurials. *J. biol. Chem.*, **241**, 2293-2300.
- [11] Lehmann, F. G., Schneider, K. W., and Menge, H. (1966). The enzymic diagnosis of myocardial infarction. II. The measurement of organ specific enzymes: Creatinine phosphokinase and myokinase. *Enzymol. Biol. Clin.*, **6**, 36-51.
- [12] Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951). Protein measurement with the folin phenol reagent. *J. biol. Chem.*, **193**, 265-275.
- [13] Noda, L. (1973). Adenylate kinase. In *The Enzymes*, 3rd ed., Vol. 8, Boyer, P. D., editor, Academic Press, New York, 279-305.
- [14] Noda, L., Schulz, G. H., and von Zabern, I. (1975). Crystalline adenylate kinase from carp muscle. *Eur. J. Biochem.*, **51**, 229-235.
- [15] Russell, P. J., Horenstein, J. M., Goins, L., Jones, D., and Laver, M. (1974). Adenylate kinase in human tissues. I. Organ specificity of adenylate kinase isoenzymes. *J. biol. Chem.*, **249**, 1874-1879.
- [16] Ryan, L. D., and Vestling, C. S. (1974). Rapid purification of lactic dehydrogenase from rat liver and hepatoma: A new approach. *Arch. Biochem. Biophys.*, **160**, 279-284.
- [17] Schulz, G. E., Biedermann, K., Kabsch, W., and Schirmer, R. H. (1973). Low resolution structure of adenylate kinase. *J. mol. Biol.*, **80**, 857-864.
- [18] Schulz, G. E., Elzinga, M., Marx, F., and Schirmer, R. H. (1974). Three-dimensional structure of adenylate kinase. *Nature*, **250**, 120-123.
- [19] Schirmer, R. H., and Thuma, E. (1972). Sensitivity of adenylate kinase isozymes from normal and dystrophic human muscle to sulfhydryl reagents. *Biochim. Biophys. Acta*, **268**, 92-97.
- [20] Spackman, D. H., Stein, W. H., and Moore, S. (1958). Automatic recording apparatus for use in the chromatography of amino acids. *Anal. Chem.*, **30**, 1190-1206.

- [21] Svasti, J., and Milstein, C. (1972). The complete amino acid sequence of a mouse κ light chain. *Biochem. J.*, **128**, 427-444.
- [22] Thompson, S. T., Cass, K. H., and Stellwagen, E. (1975). Blue dextran-sepharose: An affinity column for the dinucleotide fold in proteins. *Proc. Nat. Acad. Sci. USA*, **72**, 669-672.
- [23] Thuma, E., Schirmer, R. H., Schirmer, I. (1972). Preparation and characterization of crystalline human ATP:AMP phosphotransferase. *Biochim. Biophys. Acta*, **268**, 81-91.
- [24] Tsuboi, K. K., and Chervenka, C. H. (1975). Adenylate kinase of human erythrocyte: Isolation and properties of the predominant inherited form. *J. biol. Chem.*, **250**, 132-140.
- [25] von Zabern, I., Wittmann-Liebold, B., Untucht-Grau, R., Schirmer, R. H., and Pai, E. F. (1976). Primary and tertiary structure of the principal human adenylate kinase. *Eur. J. Biochem.*, **68**, 281-290.
- [26] Weber, K., Pringle, J. R., and Osborn, M. (1972). Measurement of molecular weights by electrophoresis on SDS-acrylamide gel. In *Methods in Enzymology*, Vol. 26, Hirs, C. H. W., and Timasheff, S. N., editors, Academic Press, New York, 3-27.

Explanation of Figures

Fig. 5. Peptide maps of acidic and basic peptides. A: AK_a. B: Skeletal muscle enzyme. The tryptic digest was subjected to electrophoresis at pH 6.5, and the paper was divided into three sections: acidic peptides, neutral peptides and basic peptides. The acidic peptides and basic peptides were run in chromatography. The

color of peptide spots marked by the solid line was dense; it was lighter near the dotted line.

Fig. 6. Peptide maps of neutral peptides. A: AK_a. B: Skeletal muscle enzyme. Neutral peptides obtained from electrophoresis at pH 6.5 were run in chromatography and rerun in electrophoresis at pH 1.9.

→ D.C.

→ pH 1.9

—

A

B

⑥

→ D.C.

→ pH 1.9

—