Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography B, 806 (2004) 95–100
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Heterogeneity of mitochondrial creatine kinase

Fusae Kanemitsu [a,*], Takeshi Kageoka [a,*], Shohei Kira [b]

[a] *Clinical Laboratories, Kurashiki Central Hospital, 1-1-1 Miwa, Kurashiki 710-8602, Japan*
[b] *Department of Public Health, Okayama University Graduate School of Medicine and Dentistry, 2-5-1 Shikata-cho, Okayama 700-8558, Japan*

Received 11 September 2003; received in revised form 10 March 2004; accepted 17 March 2004

Available online 07 May 2004

Abstract

The heterogeneity of cardiac sarcomeric mitochondrial creatine kinase (creatine *N*-phosphotransferase, EC 2.7.3.2, sMi-CK), namely, brain ubiquitous Mi-CK (uMi-CK) and an atypical Mi-CK detected in the serum of a patient with ovarian cancer, was studied by isoelectric focusing. These Mi-CKs were found to be slightly different from each other with respect to their p*I*s under the examined conditions. The atypical Mi-CK was found to be an atypically oxidized form of uMi-CK. Results suggest that these heterogeneities of Mi-CK are caused by the genotypes, structures, biological functions and metabolism/dissimilation of Mi-CKs in the mitochondria and intravascular circulation.

Keyword: Creatine kinase

1. Introduction

Creatine kinase (creatine *N*-phosphotransferase, CK, EC 2.7.3.2) is found in the cytosol and mitochondria of cells, and catalyzes the reversible exchange of high-energy phosphate between ATP and phosphocreatine (PCr) [1–3]. Cytosolic CKs are dimers consisting of muscle-type (M) and/or brain-type (B) subunits. CK-MM and CK-BB are expressed at high levels in the skeletal muscle, and in the brain and smooth muscle, respectively, and hybrid CK-MB is found in the cardiac muscle.

On the other hand, two genotypes of mitochondrial CK (Mi-CK) are known, that is, the ubiquitous Mi-CK (uMi-CK) and the sarcomeric Mi-CK (sMi-CK) [4–6]. uMi-CK and sMi-CK are encoded by two different genes on human chromosomes 15 and 5 [7–9], respectively, and are expressed in a tissue-specific manner; uMi-CK is expressed in the brain and smooth muscle, while sMi-CK is expressed in the cardiac and skeletal muscles [7–9].

The monomeric Mi-CK molecule displays a two-domain organization and Mi-CK exists as both octamers and dimers [10,11]. The dimeric Mi-CK is a banana-shaped structure, and tryptophan 206 in one of the monomer/monomer contact regions of Mi-CKs contributes to the stability of dimers [12–15]. An octamer is composed of four active and stable dimers. Octamer formation has been postulated to occur via the interaction of charged amino acids in the N-terminal peptide of Mi-CKs [12–15]. Structural differences between the four crystallographically independent monomers in an octamer are rare [12].

Mi-CKs are found exclusively in mitochondrial intermembrane spaces, attached to the outer surface of the inner mitochondrial membrane, but only octamers can interact with the inner and outer mitochondrial membranes [16–18]. In the mitochondrial compartment, the CK/PCr system plays important roles in various functions, such as energy buffering functions, regulatory functions, and energy transport functions [19].

We have characterized cardiac sMi-CK, brain uMi-CK, and an atypical Mi-CK detected in the serum of a patient with ovarian cancer, by isoelectric focusing (IEF) [20,21]. On the basis of our previous studies, in our present paper, the heterogeneity of octameric and dimeric Mi-CKs was examined in relation to recent findings regarding the structures, biological functions [12–15] and metabolism/dissimilation of Mi-CKs.

* Corresponding author.
E-mail address: fk1306@kchnet.or.jp (F. Kanemitsu).

1570-0232/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2004.03.065

2. Experimental

2.1. Materials

The materials used in this study were as follows: Autopack CK NAC, marker proteins for IEF and molecular weight (Roche Diagnosis, Tokyo, Japan), Sephadex G-200 superfine, DEAE Sephacel, agarose and carrier ampholytes for IEF (Amersham Pharmacia Biotech, Tokyo, Japan), carboxypeptidase B (Sigma Chemical, St. Louis, MO, USA) and Freund's adjuvant (Difco Laboratories, Detroit, MI, USA).

2.2. Specimens

Normal human cardiac muscle and brain tissues were obtained within 6 h of death and stored at −80 °C until use. Serum samples were obtained from a 62-year-old woman with advanced carcinoma of the ovary (carcinoma of germ cell origin, probably hepatoid yolk sac tumor).

2.3. Partial purification of sMi-CK and uMi-CK

The tissues were cut into small pieces and homogenized in four volumes of normal human serum. The homogenates were centrifuged at 10,000 × *g* for 15 min at 4 °C. The obtained extracts were applied on a Sephadex G-200 superfine column (2.5 × 30 cm) equilibrated with 50 mM phosphate buffer, pH 7.4. CK was eluted with phosphate buffer, and sMi-CK and uMi-CK were collected [20].

2.4. Assay methods

The heterogeneity of Mi-CK was determined using agarose gel IEF. The agarose plate used (124 mm × 80 mm × 0.5 mm) consisted of 1% agarose and 2% ampholytes (pH range 5–9). Five microliters of serum sample was applied to the agarose plate, and 0.2 M citric acid and 0.5 M ethanolamine were used as anode and cathode solutions, respectively [21]. Focusing was carried out at 4 °C and 300–1200 V for 120 min (power: 0.8–4.8 W), and Mi-CK isoforms were detected by tetrazolium staining [21]. Counter-immuno-IEF was carried out by applying 5 μl of anti-sMi-CK antiserum at the anodic position 1.0 cm away from the sample application point [21].

To examine the heterogeneity of Mi-CK, Mi-CKs were subjected to chromatography on a Sephadex G-200 superfine column and were treated separately with 2 M urea, 0.0–0.2% hydrogen peroxide, or 0–250 mM 2-mercaptoethanol (2-ME) at 27 °C for 15 min, or with 900 U/l carboxypeptidase B at 37 °C for 30 min [21].

2.5. Preparation of anti-sMi-CK antibody

Approximately 5 mg of Mi-CK was emulsified with an equal volume of complete Freund's adjuvant. One milliliter of the emulsion was injected intramuscularly into the back of a rabbit and booster injections were administered with the adjuvant 7, 14 and 28 days after the initial injection. Seven days after the last booster injection, the rabbit was bled from the ear vein, and serum containing an anti-human Mi-CK antibody was collected. Nonspecific antibodies in the antiserum were absorbed by incubation with normal human serum at 37 °C for 2 h, followed by incubation at 4 °C for 24 h and centrifugation. CK and complements in the antiserum were inactivated by heating at 56 °C for 30 min. The anti-Mi-CK antiserum was confirmed to react with only Mi-CK and not with CK-MM or CK-BB by the Octerlony test [20].

2.6. Hybridization of sMi-CK and uMi-CK

sMi-CK and uMi-CK solutions prepared at the final concentrations of 5000–10,000 U/l in 0.3 ml were mixed in 1 ml of 100 mM Tris–HCl buffer, pH 8.0. Then, 50 mg of guanidine hydrochloride and 0.01 ml of 2-mercaptoethanol (2-ME) were added to the mixture. The mixture was incubated at 28 °C for 1 h and dialyzed against 100 mM Tris–HCl buffer, pH 8.0 [20].

3. Results

Fig. 1 shows the IEF patterns of Mi-CKs. Cardiac sMi-CK was focused as several bands at p*I*s 6.55–8.0 by agarose gel IEF (Fig. 1a). After urea treatment, the bands at p*I*s 7.1–8.0 disappeared and four bands remained at p*I*s 6.55, 6.75, 6.85 and 6.95 (Fig. 1b). Thus, it was found that the bands at p*I*s 7.1–8.0 were octamers and the bands at p*I*s 6.55, 6.75, 6.85 and 6.95 were dimers.

Brain uMi-CK was focused as several bands at p*I*s 6.6–7.9 by IEF (Fig. 1b). After urea treatment, the bands at p*I*s 7.1–7.9 disappeared and five bands at p*I*s 6.6, 6.7, 6.8, 6.9 and 7.0 remained (Fig. 1b). Thus, the bands at p*I*s 7.1–7.9 were found to be octamers and the bands at p*I*s 6.6, 6.7, 6.8, 6.9 and 7.0 were dimers. The p*I*s of octameric and dimeric uMi-CKs were found on a slightly more anodic side than those of sMi-CK.

On the other hand, Fig. 1c shows the IEF patterns of the atypical CK. Broad CK bands were detected at p*I*s 7.0–7.8, and the cathodic side of the broad band was detected at p*I* 0.1 toward the anodic side compared with the typical uMi-CK. After urea treatment, a band at p*I* 6.75 and several minor bands on the anodic side of p*I* 6.75 were detected, in addition to the typical bands of uMi-CK at p*I*s 6.9 and 7.0; however, the bands at p*I*s 6.7 and 6.8 were not detected (Fig. 1d).

Counter-immuno-IEF was carried out using sMi-CK and anti-sMi-CK antibodies. A new band was detected at p*I* 5.8 while the bands at p*I*s 7.8–8.0 disappeared (Fig. 2a). Thus, octameric sMi-CK reacted with anti-sMi-CK antibodies and migrated to p*I* 5.8. To examine the C-terminus of sMi-CK, sMi-CK was treated with carboxypeptidase B. After the

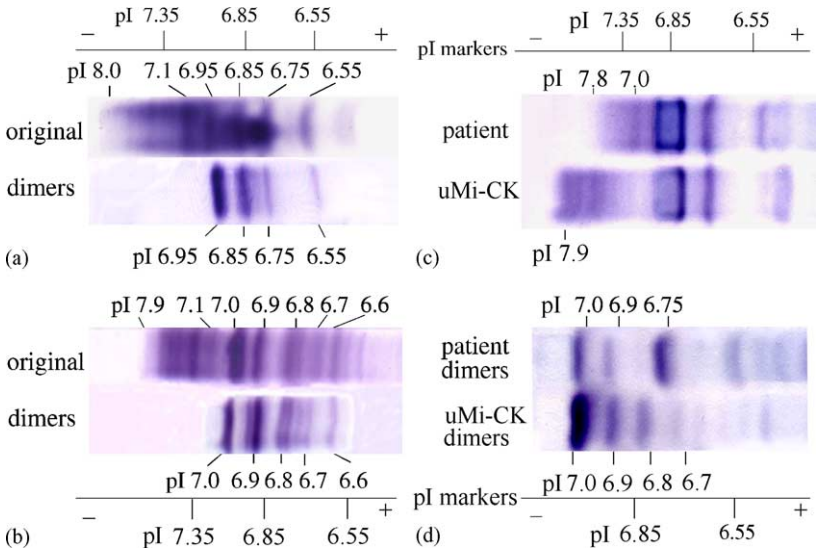

Fig. 1. IEF patterns of octameric and dimeric Mi-CKs. (a) Cardiac sMi-CK was focused at p*I*s 6.55–8.0. Dimers were focused at p*I*s 6.55, 6.75, 6.85 and 6.95 after urea treatment. (b) Brain uMi-CK was focused at p*I*s 6.6–7.9. Dimers were focused at p*I*s 6.6, 6.7, 6.8, 6.9 and 7.0 after urea treatment. (c) The atypical octamer was focused at p*I*s 7.0–7.8. The cathodic edge of the atypical CK was at p*I* 0.1 toward the anodic side compared with the typical uMi-CK. (d) An atypical dimer was focused at p*I* 6.75 whearas typical dimers of uMi-CK were focused at p*I*s 6.7–7.0.

treatment, new bands appeared at p*I*s 6.55 and 6.75 while the original sMi-CK bands disappeared (Fig. 2a). Therefore, the new bands were considered to be the delysined forms of sMi-CK.

After counter-immuno-IEF of uMi-CK, a new band appeared at p*I* 5.8 (Fig. 2b); therefore, uMi-CK reacted with the sMi-CK antibody. After uMi-CK treatment with carboxypeptidase B, the bands of uMi-CK appeared at p*I*s 6.3, 6.4, 6.8 and 6.9 (Fig. 2b); therefore, the new bands were considered to be the delysined forms of uMi-CK.

sMi-CK with bands at p*I*s 6.75, 6.95 and 7.1–8.0 was mixed with uMi-CK with bands at p*I*s 6.8, 6,9 7.0 and 7.1–7.9 and hybridization was carried out. Hybrid Mi-CKs appeared at p*I*s 6.78, 6.98 and 7.1–7.95 (Fig. 2c). The p*I*s of the hybrid Mi-CKs were in between those of sMi-CK and uMi-CK.

After the counter-immuno-IEF of the atypical CK, it reacted with anti-Mi-CK antibodies and migrated toward the anodic side (Fig. 3a), thus, the atypical CK was found to be Mi-CK. After the treatment of the atypical Mi-CK with carboxypeptidase B, the oligomeric atypical Mi-CK migrated toward the anodic side and was detected at p*I*s 6.75 and 6.9 (Fig. 3b). In contrast, the octameric typical uMi-CK migrated toward the cathodic side of the patient's Mi-CK

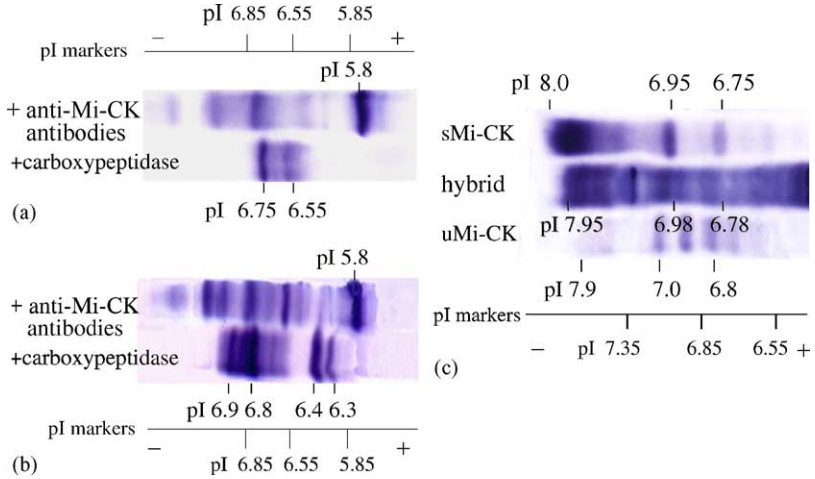

Fig. 2. IEF patterns of Mi-CKs after reaction with the anti-sMi-CK antibodies, after treatment with carboxypeptidase B, and formation of hybrid Mi-CKs. (a) Cardiac sMi-CK reacted with the anti-sMi-CK antibodies and the immune complexes were focused at p*I* 5.8. The delysined forms were focused at p*I*s 6.55 and 6.75 after treatment with carboxypeptidase B. (b) Brain uMi-CK reacted with the anti-sMi-CK antibodies and the immune complexes were focused at p*I* 5.8. The delysined forms were focused at p*I*s 6.3, 6.4, 6.8 and 6.9 after treatment with carboxypeptidase B. (c) sMi-CK and uMi-CK formed hybrid Mi-CKs and the p*I*s of the hybrids were in between those of sMi-CK and uMi-CK.

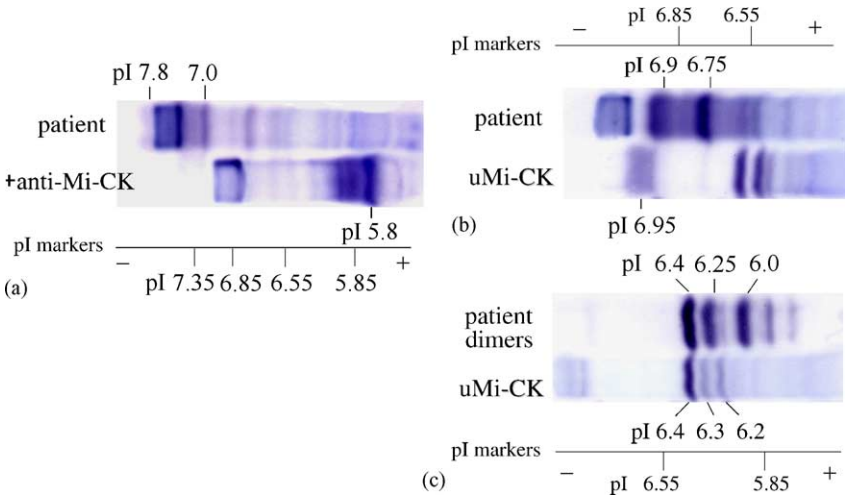

Fig. 3. IEF patterns of the atypical Mi-CK after reaction with the anti-sMi-CK antibodies, and treatment with carboxypeptidase B. (a) Atypical Mi-CK reacted with anti-sMi-CK antibodies. (b) and (c): The p*I*s of the delysined oligomeric (b) and dimeric (c) atypical Mi-CKs differed from that of the typical uMi-CK.

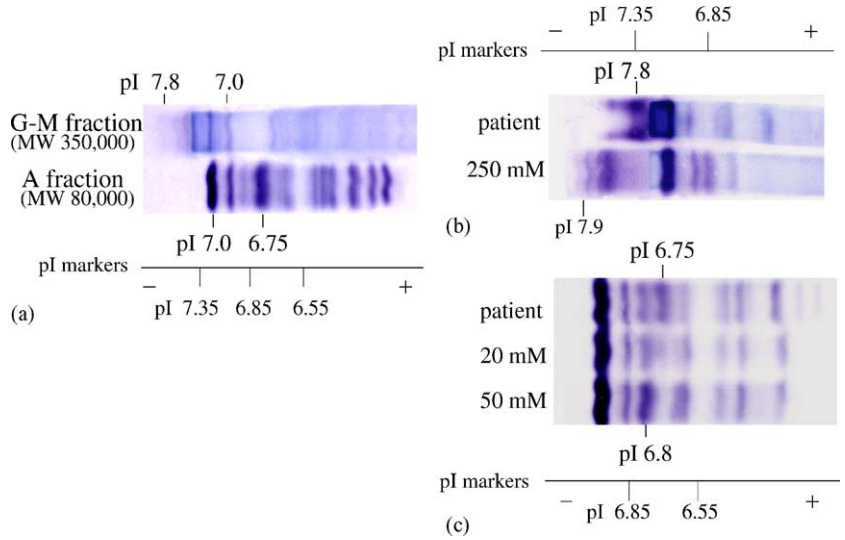

Fig. 4. IEF patterns of the atypical Mi-CK in the eluate from the Sephadex G-200 superfine column, and after treatment with 2-ME. (a) Atypical CK was detected in the fractions of G-M (MW 350,000) and A (MW 80,000). (b) and (c): The p*I*s of the oligomeric (b) and dimeric (c) atypical Mi-CKs migrating toward the cathodic side after treatment with 2-ME at 27 °C for 15 min.

(Fig. 3b). Dimeric atypical Mi-CKs were detected at p*I*s 6.0 and 6.25 after treatment with carboxypeptidase B (Fig. 3c); however, dimeric typical uMi-CKs were detected at p*I*s 6.2, 6.3 and 6.4 (Fig. 3c). Therefore, the p*I*s of the delysined atypical Mi-CK differed from those of the typical uMi-CK.

To determine the molecular size of the atypical Mi-CK, the patient's Mi-CK was subjected to chromatography using a Sephadex G-200 superfine column. The atypical CK at p*I* 7.0–7.8 was detected in the G-M fraction (MW 350,000), and the atypical Mi-CK at p*I* 6.75 and the typical Mi-CK at p*I* 7.0 were detected in the A fraction (MW 80,000; Fig. 4a). Therefore, it was found that the molecular sizes of the atypical Mi-CK were similar to that of the octameric or dimeric typical uMi-CK, and the atypical Mi-CK at p*I* 6.75 was not a tetramer but consisted of dimeric uMi-CK at p*I*s 6.7 and 6.8.

The atypical Mi-CK was separately treated with 2-ME and hydrogen peroxide. After treatment with 2-ME, the octameric atypical Mi-CK migrated toward the cathodic side (Fig. 4b), and the dimeric atypical Mi-CK at p*I* 6.75 migrated to p*I* 6.8 (Fig. 4c). In contrast, after treatment with hydrogen peroxide, the IEF pattern of the atypical Mi-CK did not change.

4. Discussion

Two types of Mi-CK exist, which are designated as sMi-CK and uMi-CK due to the tissue specificity of their expression. In the case of chicken Mi-CKs, sMi-CK and uMi-CK are also referred to as Mib-CK and Mia-CK,

respectively (b stands for basic and a stands for acidic) on the basis of their isoelectric points [22–24]. In the present study, the p*I*s of the dimeric cardiac sMi-CK were 6.55–6.95 and those of the dimeric brain uMi-CK were 6.6–7.0; these values are identical to those previously reported [25,26]. The p*I*s of sMi-CK and uMi-CK seemed to differ due to their differences in amino acid composition; sMi-CK and uMi-CK share 82–85% amino acid identity, whereas cytoplasmic CKs and Mi-CKs share 60–65% identity [26]. It was reported that the p*I*s of the dimeric uMi-CK in the serum of a patient with rectal cancer are 6.7–7.0, and that the band at p*I* 7.0 is the reduced form of uMi-CK and the bands at p*I*s 6.7–6.9 are the oxidized forms [27]. Therefore, the most basic bands at p*I* 6.95 of the dimeric sMi-CK and at p*I* 7.0 of the dimeric uMi-CK in the present study probably represent their reduced forms. The bands of sMi-CK at p*I*s 6.55–6.85 and those of uMi-CK at p*I*s 6.7–6.9 probably represent their oxidized forms.

The octamers had wide p*I* ranges, p*I*s 7.1–8.0 for sMi-CK and 7.1–7.9 for uMi-CK; thus, the octamers were found to be heterogenous with respect to their charges. The octameric Mi-CK has a cube-like structure with a central channel [28], and considerable changes in the shape and size of molecules of the octamers occur upon substrate binding [29]; The radius of gyration of Mi-CKs is reduced from 55.6 Å (free Mi-CK) to 48.9 Å (Mi-CK-Mg-ATP) [29]. However, creatine alone does not induce significant changes in the radius of gyration, neither does free ATP or ADP. Therefore, it was suggested that structural changes produce the heterogenous charges of octameric molecules, and octameric Mi-CKs have wide p*I* ranges.

Octameric Mi-CKs bind to membranes mainly by electrostatic interactions due to charged residues located in the C-terminus and have another function as structural proteins [12]. In chicken Mib-CK, the C-terminus contains six positive charges, consists of five lysines and one arginine, and electrostatically interacts with negatively charged cardiolipin and other phospholipids in mitochondrial membranes [30]. In the present study, the lysine residues in the C-terminus were removed by carboxypeptidase, and the p*I*s of delysined Mi-CKs changed to the more acidic side than those of the original Mi-CKs. The amino acid of the C-terminus of two cytosolic CKs (CK-M and CK-B) is also lysine, and is dissimilated by delysination with carboxypeptidase existing in serum at 267 ± 45 IU/l (mean ± standard deviation) in the intravascular circulation [31,32]. Therefore, the results suggest that Mi-CKs are dissimilated by carboxypeptidase in the intravascular circulation similar to cytosolic CKs.

On the other hand, an octamer can be converted to dimers by dilution at an alkaline pH [22] or urea treatment [20–22] in vitro, however, dimers are very stable and dissociate into monomers only under chaotropic conditions [20]. In the present study, the octameric sMi-CK and uMi-CK were converted to dimers by treatment with 2 M urea, and the dimeric Mi-CKs were dissociated into monomers by treatment with guanidine hydrochloride. However, the dissociation from a dimer to monomers was reversible and the dissociated monomers were hybridized into dimeric sMi-uMi-CK, and then formed octamers in vitro. As shown in Fig. 2c, the p*I*s of the hybrid octamer and dimer were between those of sMi-CK and uMi-CK.

Anti-sMi-CK rabbit polyclonal antibodies reacted not only with sMi-CK but also with uMi-CK. The antibody reacted well with the octamer but negligibly with the dimer probably because the octamer was predominant in the antigen preparation for rabbit immunization. It was reported that antibodies against purified pig and rabbit cardiac octameric Mi-CK exhibits a marked specificity for dimers, while antibodies against rabbit cardiac octameric Mi-CK react with dimers and octamers [33]. This suggests that octameric and dimeric Mi-CKs may or may not be distinguished by polyclonal antibodies in experiments.

Mi-CK is sometimes detected in sera of patients with advanced ovarian cancer, and the type of Mi-CK expressed in the ovarian tissue is uMi-CK [19]. However, the p*I*s of the atypical Mi-CK in the present study were not identical to those of the typical uMi-CK [21]. As described above, Mi-CK with p*I*s similar to those of the dimeric atypical Mi-CK was obtained by hybridizing sMi-CK and uMi-CK in vitro; however, the atypical Mi-CK was not identical to the hybrid Mi-CK. Also the atypical Mi-CK was not a tetramer formed with two typical dimers, because the molecular weights of the atypical Mi-CKs were determined to be 350,000 and 80,000. It was reported that a tetramer is formed by the association of two dimers, which react rapidly to form an octamer, but this tetramer is unstable [34,35]. The atypical part of Mi-CK was also not found in the C-terminal region because the octameric and dimeric atypical Mi-CKs reacted with carboxypeptidase B, and the p*I*s of the atypical Mi-CKs after treatment with carboxypeptidase B differed from those of the typical uMi-CK. After the treatment of the atypical Mi-CK with 2-ME, the octameric atypical Mi-CK migrated toward the cathodic side from its original position, and the dimeric atypical uMi-CK at p*I* 6.75 migrated to p*I* 6.8, which is the p*I* of the typical uMi-CK (Fig. 4b and c). These results suggest that the atypical Mi-CK is an atypically oxidized form of the typical uMi-CK [21].

The octameric atypical uMi-CK had an atypical p*I*; therefore, it is possible that the atypical uMi-CK is oxidized in the mitochondria. Mi-CKs are located in mitochondrial intermembrane spaces and functionally couple with oxidative phosphorylation via the inner mitochondrial membrane ATP/ADP translocator [36–38]. The reaction of Mi-CKs utilizes H^+ resulting in the decrease in cell pH [36–38]. Oxidative phosphorylation conjugates with the electron transport system, and superoxide is produced from oxygen in this system [36–38]. The superoxide anion reacts with nitrogen monoxide (NO), which is one of the inhibitors of CKs, most likely by promoting nitrosylation of the critical sulfhydryl group of the enzyme [39], producing peroxynitrite (PN). PN is a powerful oxidant of thiols [7,40], and Mi-CKs are a prime target of inactivation by PN in the mitochondria [41].

The active-site cystine 278 of Mi-CKs is involved in the process, and the inactivation of Mi-CKs by PN is reversed by 2-ME [41]. Moreover, it was reported that free radicals induce the inactivation of octameric and dimeric Mi-CKs [41]. These previous and our present findings taken together suggest that the atypical Mi-CK is influenced by oxidative phosphorylation and the electron transport system, and the oxidized site of the atypical uMi-CK may be the active site. It was supposed that oxidation was one pathway for the metabolism of Mi-CKs in the mitochondria.

In conclusion, our results suggest that the heterogeneity of Mi-CK is caused by the genotypes, structures, biological functions and metabolism/dissimilation of Mi-CKs; that is, Mi-CKs are the structural proteins that mediate the adhesion between inner and outer mitochondrial membranes and the oxidative phosphorylation conjugated with the electron transport system.

References

[1] T. Wallimann, M. Wyss, D. Brdiczka, K. Nicolay, H.M. Eppenberger, Biochem. J. 281 (1992) 21.
[2] T. Wallimann, M. Dolder, U. Schlattner, M. Eder, T. Hornemann, E. O'Gorman, A. Ruck, D. Brdiczka, Biofactors 18 (1998) 229.
[3] P. Kakdis, R. Furter, T. Wallimann, Biochemistry 33 (1994) 952.
[4] W. Qin, Z. Khuchua, J. Cheng, J. Boero, R.M. Payne, A.M. Strauss, Mol. Cell. Biochem. 184 (1998) 153.
[5] K. Anflous, V. Veksler, P. Mateo, F. Samson, V. Saks, R. Ventura-Clapier, Biochem. J. 322 (1997) 73.
[6] M. Eder, K. Fritz-Wolf, W. Kabsch, T. Wallimann, U. Schlattner, Proteins 39 (2000) 216.
[7] R.L. Stallings, E. Olson, A.W. Strauss, L.H. Thompson, L.L. Bachinski, M.J. Siciliano, Am. J. Hum. Genet. 43 (1988) 144.
[8] R.C. Haas, C. Korenfeld, Z. Zhang, B. Perryman, D. Roman, A.W. Strauss, J. Biol. Chem. 24 (1989) 2890.
[9] R.C. Haas, A.W. Strauss, J. Biol. Chem. 265 (1990) 6921.
[10] W. Kabsch, K. Fritz-Wolf, Curr. Opin. Struct. Biol. 7 (1997) 811.
[11] K. Fritz-Wolf, T. Schnyder, W. Wallimann, W. Kabsch, Nature 381 (1996) 341.
[12] U. Schlattner, M. Forstner, M. Edder, O. Stachowiak, K. Fritzwolf, T. Wallimann, Mol. Cell. Biochem. 184 (1998) 125.
[13] Z.A. Khuchua, W. Qin, J. Beoro, J. Cheng, R.M. Payne, V.A. Saks, A.W. Strauss, J. Biol. Chem. 273 (1998) 22990.
[14] M. Gross, E.M. Furter-Graves, T. Wallimann, H.M. Eppenberger, R. Furter, Protein Sci. 3 (1994) 1058.
[15] M. Gross, T. Wallimann, Biochemistry 34 (1995) 6660.
[16] O. Stachowiak, M. Dolder, T. Wallimann, Biochemistry 35 (1996) 15522.
[17] U. Schlattner, T. Wallimann, J. Biol. Chem. 275 (2000) 17314.
[18] W.R. Ellington, K. Roux, O. Agustine, A.O. Pineda Jr., FEBS Lett. 425 (1998) 75.
[19] T. Wallimann, W. Hemmer, Mol. Cell. Biochem. 133–134 (1994) 193.
[20] F. Kanemitsu, J. Mizushima, T. Kageoka, T. Okigaki, K. Taketa, S. Kira, Electrophoresis 21 (2000) 266.
[21] F. Kanemitsu, T. Kageoka, S. Kira, J. Chromatogr. B 783 (2003) 191.
[22] J. Schlegel, M. Wyss, U. Schürch, T. Schnyder, A. Quest, G. Wegmann, M.H. Eppenberger, T. Wallimann, J. Biol. Chem. 363 (1988) 16963.
[23] J.P. Hossle, J. Schegel, G. Wegmann, M. Wyss, P. Böhlen, H.M. Eppenberger, T. Wallimann, J.C. Perriard, Biochem. Biophys. Res. Commun. 151 (1988) 408.
[24] M. Wyss, J. Schlegel, P. James, H.M. Eppenberger, T. Wallimann, J. Biol. Chem. 265 (1990) 15900.
[25] R.A. Wevers, C.P.M. Reutelingsperger, B. Dam, J.B.J. Soons, Clin. Chim. Acta 119 (1981) 209.
[26] R.M. Payne, R.C. Haas, A.W. Strauss, Biochim. Biophys. Acta 1089 (1991) 352.
[27] F. Kanemitsu, S. Kira, J. Chromatogr. B 721 (1999) 171.
[28] T. Schnyder, H. Gross, H. Winkler, H.M. Eppenberger, T. Wallimann, J. Cell. Biol. 112 (1991) 95.
[29] M. Forstner, M. Kriechbaum, P. Laggner, T. Wallimann, Biophys. J. 75 (1998) 1016.
[30] P. Kaldis, R. Furter, T. Wallimann, Biochemistry 33 (1994) 952.
[31] M.B. Perrymann, J.K. Knell, R. Roberts, Clin. Chem. 30 (1984) 662.
[32] D.R. Abendschein, H. Serota, T.H. Plummer Jr., K. Amiraian, A.W. Strauss, B.E. Sobel, A.S. Jaffe, J. Lab. Clin. Med. 110 (1987) 798.
[33] E. Quemeneur, D. Eichenberger, C. Vial, FEBS Lett. 262 (1990) 275.
[34] M. Gross, T. Wallimann, Biochemistry 32 (1993) 13933.
[35] O. Stachowiak, U. Schlattner, M. Dolder, T. Wallimann, Mol. Cell. Biochem. 184 (1998) 141.
[36] M. Echegaray, M. Rivera, Sports Med. 31 (2001) 919.
[37] W.S. Kunz, Biochem. Biophys. Acta 1504 (2001) 12.
[38] J. Vina, A. Gimeno, J. Sastre, C. Desco, M. Asensi, F.V. Pallardo, A. Cuesta, J.A. Ferrero, L.S. Terada, J.E. Repine, IUBMB Life 49 (2000) 539.
[39] H. Wolosker, R. Panizzutt, S. Englender, FEBS Lett. 392 (1996) 274.
[40] M.C. Kennedy, W.E. Antholine, H. Beinert, J. Biol. Chem. 272 (1997) 20340.
[41] O. Stachowiak, M. Dolder, T. Wallimann, C. Richter, J. Biol. Chem. 273 (1998) 16694.