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0167-5273/$ - see front matter
doi:10.1016/j.ijcard.2009.02.041

Characterisation of isoform-specific tryptic peptides of rat cardiac myosin heavy chains using automated liquid chromatography-matrix assisted laser desorption ionisation (LC-MALDI) mass spectrometry

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Received 25 May 2008; accepted 16 November 2008
Available online 19 December 2008

Abstract

Proteomics investigations using 2-dimensional electrophoresis (2-DE) cannot resolve the entire cardiac proteome because some proteins, including myosin heavy chains (MyHC), are insoluble in the buffers required for isoelectric focusing. Here, we report an automated mass spectrometry (MS) method complementary to 2-DE and capable of yielding important additional information. Rat myocardium was homogenised in standard lysis solution and centrifuged to produce a supernatant fraction, suitable for 2-DE. The pelleted fraction, which is normally discarded, was used for the current analysis. Proteins were digested with trypsin and the peptides fractionated by HPLC. Automated spotting of eluent fractions onto 384-well target plates and matrix-assisted laser desorption tandem time of flight (MALDI-ToF/ToF) MS were directed by dedicated software. Peptide ions were fragmented by collision-induced dissociation and the MS/MS spectra searched against the NCBI database using Mascot. This approach confidently identified 13 tryptic peptides specific to cardiac α-MyHC and 4 specific to β-MyHC, which can be used to differentiate these highly homologous protein isoforms in future quantitative MS analyses.

Keywords: Automated analysis; Cardiac muscle; Liquid chromatography-matrix assisted laser desorption ionisation tandem time of flight mass spectrometry; Myosin heavy chain isoforms

1. Introduction

Two-dimensional electrophoresis (2-DE) has been optimised for cardiac muscle [1] and investigations [2–6] combining 2-DE and mass spectrometry have advanced cardiac biology. However, a weakness of 2-DE is that a

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proportion of cardiac proteins are insoluble in the solvent parameters [7] required for isoelectric focusing [8,9]. This insoluble fraction comprises membrane proteins and myofibrillar proteins, in particular myosin heavy chains (MyHC). Two MyHC isoforms, α-MyHC and β-MyHC, are expressed in the heart and their ratio alters during development and in pathological states [10] and influences cardiomyocyte power output [11]. MyHC aggregate with actin postmortem and cannot be adequately disassociated by 2-DE. Typically, an indiscriminate MyHC fragment with a mass (~120 kDa) approximately half that of the complete polypeptide is observed. Difficulties associated with protein insolubility can be circumvented by using proteases, such as trypsin, to produce peptide solutions that can be separated using HPLC. Large-scale 'shotgun' profiling of whole tissue using this approach requires sophisticated two-dimensional liquid chromatography [12], and data analysis can become a significant obstacle. Compared to whole muscle, the cardiac insoluble protein fraction is likely to be reasonably simple, and we hypothesised that automated profiling of this fraction can be used to resolve the 2 highly homologous cardiac MyHC isoforms.

2. Materials and methods

A heart from a male Wistar rat (290 g body weight) was rapidly isolated in accordance with the British Home Office Animals (Scientific Procedures) Act 1986. The ventricles were pulverised in liquid nitrogen and a portion (100 mg) homogenised on ice in 10 volumes of 8 mol urea, 4% (w/v) CHAPS, 40 mmol Tris base and Complete™ protease inhibitor (Roche Diagnostics, Lewes, UK) at 4 °C, consistent with the requirements for 2-DE [13,14]. After centrifugation at 12,000 ×*g*, 4 °C for 45 min, the supernatant was decanted and the pellet washed 3 times and heat denatured (5 min at 96 °C) in 500 µl of 25 mmol ammonium bicarbonate. Pelleted proteins were diluted in 250 µl of 8 mol urea and 50 µl of 45 mmol dithiothreitol and heated for 15 min at 50 °C. After cooling to room temperature 50 µl of 100 mmol iodoacetamide was added and the solution incubated in the dark at room temperature for 15 min. After the addition of 600 µl of de-ionised water, trypsin was added at a 1:50 enzyme to substrate ratio in 50 mM ammonium bicarbonate.

A 500 fmol aliquot of the tryptic peptide solution was diluted to 20 µl in mobile phase A and loaded directly onto a reversed phase LC column (300 µm i.d.×15 cm, 3 µm, Pepmap C_{18}; Dionex, Sunnyvale, CA) and separated using the following gradient: (*A*=5% acetonitrile+0.05% TFA, *B*=80/20 acetonitrile+0.05% TFA)

T = 3 min, 0% *B*
T = 5 min, 15% *B* (~12% organic)
T = 20 min, 27% *B* (~21% organic)
T = 25 min, 45% *B* (~36% organic)
T = 28 min, 100% *B* (~80% organic).

Table 1
Abridged sequence alignments showing cardiac MyHC isoform specific peptides identified by LC-MALDI analysis.

β-MyHC	1	MAD**R**EMAAFGAGAPFL**RK**SE**K**E**R**LEAQT**R**PFDL**KK**DVFVPDD**K**EEFV**K**A**K**IVS**R**EGG**K**VT	60
		M D +MA FGA A +L**RK**SE**K**E**R**LEAQT**R**PFD++ + FVPDD**K**EE+V**K**A**K**IVS**R**EGG**K**VT	
α-MyHC	1	MTDAQMADFGA-A**R**YL**RK**SE**K**E**R**LEAQT**R**PFDI**R**TECFVPDD**K**EEYV**K**A**K**IVS**R**EGG**K**VT	59
β-MyHC	181	GAG**K**TVNT**KR**VIQYFAVIAAIGD**R**S**KK**DQ-TPG**K**GTLEDQIIQANPALEAFGNA**K**TV**R**ND	239
α-MyHC	180	GAG**K**TVNT**KR**VIQYFA IAAIGD**R**S**KK**D **K**GTLEDQIIQANPALEAFGNA**K**TV**R**ND	239
		GAG**K**TVNT**KR**VIQYFASIAAIGD**R**S**KK**DNPNAN**K**GTLEDQIIQANPALEAFGNA**K**TV**R**ND	
β-MyHC	659	MTNL**R**STHPHFV**R**CIIPNET**K**SPGVMDNPLVMHQL**R**CNGVLEGI**R**IC**RK**GFPN**R**ILYGDF	718
		MTNL**R**+THPHFV**R**CIIPNE **K**+PGVMDNPLVMHQL**R**CNGVLEGI**R**IC**RK**GFPN**R**ILYGDF	
α-MyHC	660	MTNL**R**TTHPHFV**R**CIIPNE**RK**APGVMDNPLVMHQL**R**CNGVLEGI**R**IC**RK**GFPN**R**ILYGDF	719
β-MyHC	719	**R**Q**R**Y**R**ILNPAAIPEGQFIDS**RK**GAE**K**LLGSLDIDHNQY**K**FGHT**K**VFF**K**AGLLGLLEEM**R**D	778
		RQ**R**Y**R**ILNPAAIPEGQFIDS **K**GAE**K**LLGSLDIDHNQY**K**FGHT**K**VFF**K**AGLLGLLEEM**R**D	
α-MyHC	720	**R**Q**R**Y**R**ILNPAAIPEGQFIDSG**K**GAE**K**LLGSLDIDHNQY**K**FGHT**K**VFF**K**AGLLGLLEEM**R**D	779
β-MyHC	1259	H**R**S**K**AEETQ**R**SVNDLT**R**Q**R**A**K**LQTENGELS**R**QLDE**K**EALISQLT**R**G**K**LTYTQQLEDL**KR**Q	1318
		+**R K** EE Q**R**S+ND T Q**R**A**K**LQTENGEL+**R**QL+E**K**EALI QLT**R**G**K**L+YTQQ+EDL**KR**Q	
α-MyHC	1260	Y**R**V**K**LEEAQ**R**SLNDFTTQ**R**A**K**LQTENGELA**R**QLEE**K**EALIWQLT**R**G**K**LSYTQQMEDL**KR**Q	1319
β-MyHC	1439	AAALD**KK**Q**R**NFD**K**ILVEWXXXXXXXXXXXXXXXXXA**R**SLSTELF**K**LK**K**NAYEESLEHLETF	1498
		AAALD**KK**Q**R**NFD**K**IL EW**K**Q**K**YEESQSELESSQ**K**EA**R**SLSTELF**K**LK**K**NAYEESLEHLETF	
α-MyHC	1440	AAALD**KK**Q**R**NFD**K**ILAEW**K**Q**K**YEESQSELESSQ**K**EA**R**SLSTELF**K**LK**K**NAYEESLEHLETF	1499
β-MyHC	1619	EGDLNEMEIQLSHAN**R**MAAEAQ**K**QV**K**SLQSLL**K**DTQIQLDDAV**R**ANDDL**K**ENIAIVE**RR**N	1678
		EGDLNEMEIQLS AN**R**+A+EAQ**K** +**K**+ Q+ L**K**DTQ+QLDDAV**R**ANDDL**K**ENIAIVE**RR**N	
α-MyHC	1620	EGDLNEMEIQLSQAN**R**IASEAQ**K**HL**K**NAQAHL**K**DTQLQLDDAV**R**ANDDL**K**ENIAIVE**RR**N	1679
β-MyHC	1679	NLLQAELEEL**R**AVVEQTE**R**S**RK**LAEQELIETSE**R**VQLLHSQNTSLINQ**KKK**MDADLSQLQ	1738
		LLQAELEEL**R**AVVEQTE**R**S**RK**LAEQELIETSE**R**VQLLHSQNTSLINQ**KKK**MDADLSQLQ	
α-MyHC	1680	TLLQAELEEL**R**AVVEQTE**R**S**RK**LAEQELIETSE**R**VQLLHSQNTSLINQ**KKK**MDADLSQLQ	1739

Trypsin cleavage sites at c-terminal side of lysine (K) and arginine (R) are shown in bold font. Underlined residues represent the tryptic peptides identified by LC-MALDI TOF/TOF analysis (listed in Table 1) and found to be specific for either the α- or β-isoform of cardiac myosin heavy chain (MyHC). Sequences of the alpha (gi|8393804) and beta (gi|8393807) isoforms of cardiac MyHC were aligned using the NCBI blastp 2 sequence tool. Peptides specific for either α-MyHC or β-MyHC were identified by manually comparing the aligned sequences with the peptides identified in Table 1.