

Selective Sampling of Phosphopeptides Based on Isoelectric Point (pI) for Ultra-Sensitive MALDI MS Analysis

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Overview

Purpose: Selective sampling of phosphopeptides based on their isoelectric points (pI) for MALDI-TOF-MS analysis.

Methods:

- Isolation of target peptides is performed based on the pI.
- Selected peptides are introduced into silica capillary by electrokinetic injection.
- The performance is evaluated by MALDI MS.

Results: Selective sampling of phosphopeptides was achieved with the synthetic phosphopeptides, tryptic digest of β -casein and α -casein.

Introduction

Protein phosphorylation plays a very important role in cell signal transduction, metabolism and apoptosis. However, their MS signals are always suppressed due to their low abundance as well as the highly acidic properties.

Recent efforts on separation/preconcentration of phosphopeptides:

- Chromatography: IMAC (Immobilized Metal Affinity Chromatography) or LC.
- β -elimination followed by chemical modification.

Drawbacks: need high volume of sample; complex procedure, with significant sample loss.

Principle of selective sampling method for MALDI MS (Fig 1)

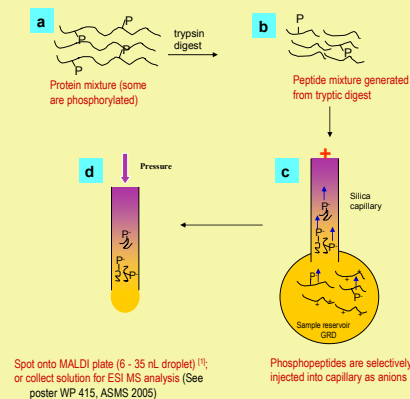


Fig 1. Principle of selective sampling of phosphopeptides.

Methods

Instrumentation: Agilent ¹³CE; Bruker REFLEX IV MALDI-TOF mass spectrometer. All mass spectra were recorded as sums of 20 individual shots.

Chemicals: 1,2-Dimyristoyl-sn-glycero-3-phosphocholine (DMPC, Avanti) was used to suppress the EOF; Citric acid (Aldrich, 15 mM, pH adjusted with ammonium) buffer; synthetic peptides: DSSLK (P_1), DpSSLK (P_2), DpSpSSLK (P_3), β -casein & α -casein (Sigma).

Matrix: 2,5-Dihydroxybenzoic acid (DHB, Aldrich). Three-layer method for matrix deposition ²¹.

Results and Discussion

1. Selective sampling of synthetic phosphopeptides

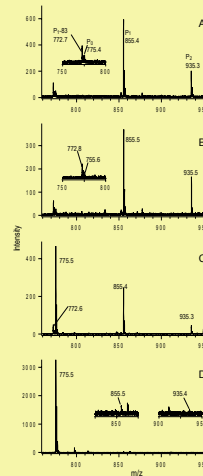


Fig 2. Positive ion MALDI mass spectra of selectively sampled fractions from mixtures of P_1 , P_2 , and P_3 at pH 3.0 (A), 4.0 (B), 5.2 (C) and 6.5 (D) respectively. K denotes possible assignments to keratin peptides based on m/z. U denotes unidentified signals. Sample quantity, 14 fmol in 35 nL spot, based on initial protein concentration. — indicates the 83 Da difference due to post-source loss of phosphate according to the manufacturer.

- In the original peptide mixture (D), phosphopeptide signals were suppressed by the non-phosphorylated counterparts.
- Minor P_3 was observed due to carryover during the selective sampling.
- Selective sampling of phosphopeptides was achieved at P_3 up to 10^6 times higher in concentration.
- Sample recovery was performed by comparing the signal of 500 amol P_3 obtained from traditional injection and the selective injection separately, a 98% recovery was recorded based on 15 replicates.

2. Selective sampling of β -casein tryptic digest

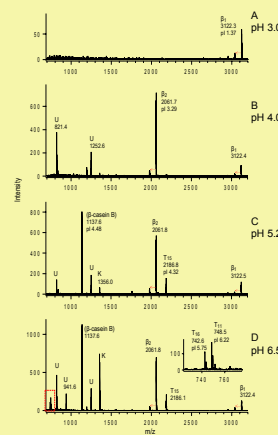


Fig 3. Positive ion MALDI mass spectra of selectively sampled fractions from a tryptic digest of β -casein adjusted to various pH: 3.0 (A), 4.0 (B), 5.2 (C) and 6.5 (D) respectively. K denotes possible assignments to keratin peptides based on m/z. U denotes unidentified signals. Sample quantity, 14 fmol in 35 nL spot, based on initial protein concentration. — indicates the 83 Da difference due to post-source loss of phosphate according to the manufacturer.

- Peptides were gradually injected into capillary based on their pI, cationic peptides were rejected out of the capillary.
- Injection/rejection of peptides occurred at 0.3 pH difference.
- Detection limit was 1.4 fmol for $\beta 1$ and 140 amol for $\beta 2$ in the positive ion mode, about 10-100 times higher than those reported data ^{8,9}.

3. Selective sampling of α -casein tryptic digest

peptide ID	sequence	mass M ⁺	calc. pI	peptide observed in ^a	
				original digest	selectively injected sample
T α -s1	QMEAESt ¹⁹ S ²⁰ SPSEEV ²¹ PN ²² VEQK ²³	2720.91	0.72	—	+/-
T α -s2	NANEVEY ²⁴ S ²⁵ SP ²⁶ SP ²⁷ SAEVA ²⁸ TEEK ²⁹ V	3008.02	0.90	—	+/-
T α -s2	NTEH ³⁰ Y ³¹ SP ³² SP ³³ SEISGICE ³⁴ TYK ³⁵	2618.91	1.37	—	+/-
T α -s1	DG ³⁶ SE ³⁷ SDQAMEDIK ³⁸ *	1927.68	2.16	+/-	+/-
T α -s2	EQL ³⁹ ST ⁴⁰ SE ⁴¹ NSK ⁴² *	1411.50	2.28	—	—
T α -s2	TYD ⁴³ ME ⁴⁴ ST ⁴⁵ EF ⁴⁶ TK ⁴⁷ *	1485.60	3.52	+/-	+/-
T α -s1	VP ⁴⁸ LE ⁴⁹ VP ⁵⁰ SAE ⁵¹ ER ⁵² *	1660.79	3.62	+/-	+/-
T α -s2	EQL ⁵³ ST ⁵⁴ SE ⁵⁵ NSK ⁵⁶ *	1539.60	3.62	+/-	+/-
T α -s1	TYD ⁵⁷ ME ⁵⁸ ST ⁵⁹ EF ⁶⁰ TK ⁶¹ *	1598.71	4.08	+/-	+/-
T α -s1	ED ⁶² PS ⁶³ ER	831.38	4.09	++	++
T α -s2	L ⁶⁴ TEEEK ⁶⁵	748.37	4.20	+	+/-
T α -s1	YK ⁶⁶ QLE ⁶⁷ VP ⁶⁸ NS ⁶⁹ SAE ⁷⁰ ER ⁷¹ *	1951.95	4.20	+/-	+/-
T α -s1	EP ⁷² MI ⁷³ GN ⁷⁴ QEL ⁷⁵ AY ⁷⁶ PEL ⁷⁷ FR	2316.13	4.20	+/-	+/-
T α -s1	HQ ⁷⁸ GL ⁷⁹ QLE ⁸⁰ V ⁸¹ EN ⁸² LL ⁸³ RY	1789.94	5.62	+/-	+/-
T α -s1	FN ⁸⁴ YAP ⁸⁵ PE ⁸⁶ Y ⁸⁷ Q ⁸⁸ IK	1384.72	6.22	+/-	+/-
T α -s1	Y ⁸⁹ LY ⁹⁰ LE ⁹¹ QL ⁹² LR	1267.70	6.22	+/-	+/-
T α -s2	AL ⁹³ ME ⁹⁴ Y ⁹⁵ Q ⁹⁶ IK	1367.69	6.22	+/-	+/-
T α -s1	EG ⁹⁷ RA ⁹⁸ G ⁹⁹ Q ¹⁰⁰ IK	910.47	7.25	+	+
T α -s2	F ¹⁰¹ AL ¹⁰² PO ¹⁰³ Y ¹⁰⁴ LK	979.55	8.83	+	+
T α -s2	NAV ¹⁰⁵ PT ¹⁰⁶ PL ¹⁰⁷ NR	1195.67	10.00	++	++
T α -s2	AIR ¹⁰⁸ PW ¹⁰⁹ G ¹¹⁰ IK	1085.61	10.01	+	+

Table 1. Selective sampling of α -casein digest @ pH 5.2. * Sequence verified by ESI MS/MS data, a theoretical monoisotopic masses. \pm Relative intensity of the peptide signals recorded with MALDI MS: (+) weak or positive ion spectrum; (-) strong in positive ion spectrum; (++) weak or negative ion spectrum; (+/-) strong in negative ion spectrum; (blank) not observed.

- Two variants were present in the original sample: α -casein s1 and s2.
- In the original peptide mixture, most peptides observed in MALDI MS have high pI values, only 8 phosphorylation sites were detected.
- In the selective sampled solution, most peptides with lower pI values were detected (pI < 5.2), more importantly, we first record all the known phosphorylation sites from s1 & s2 (19) even at femtomole-level.
- Sequence coverage for both variants together increased from 45% to 59%.

Overall conclusions

- Selective sampling of phosphopeptides was achieved in the presence of high concentration of non-phosphorylated counterparts based on the pI.
- Lower detection limit of β -casein phosphopeptides was recorded after selective sampling combining nanoliter volume sample spotting technique.
- Sequence coverage of α -casein was enhanced by combining the selective sampled data.
- Properties includes simple instrumentation, minimum sample consumption/lost, which is ideal for microscale sample analysis.

References

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Acknowledgments

NSERC, UWO, CFI-OT for funding. Thanks also to the following individuals from Department of Biochemistry, UWO: Yinyin Liao for synthesizing phosphopeptides, and Suyu Liu for useful comments and suggestions.