

LaserToF LT3 Plus

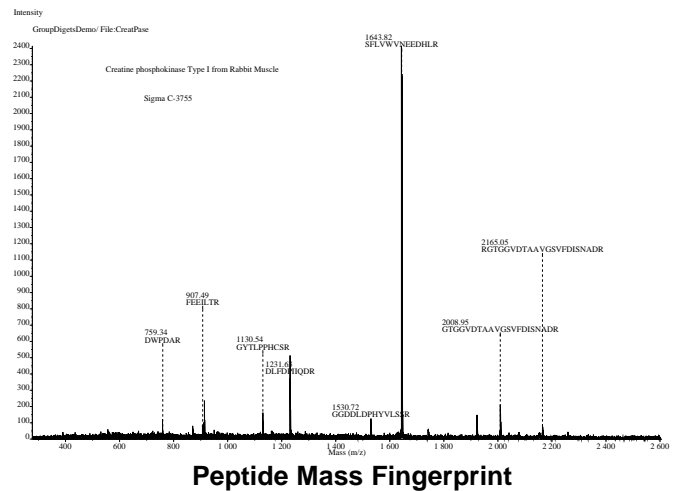
Unambiguous Protein Identification Using a Combination of Peptide Mass Fingerprinting and Partial Peptide Sequencing

Introduction

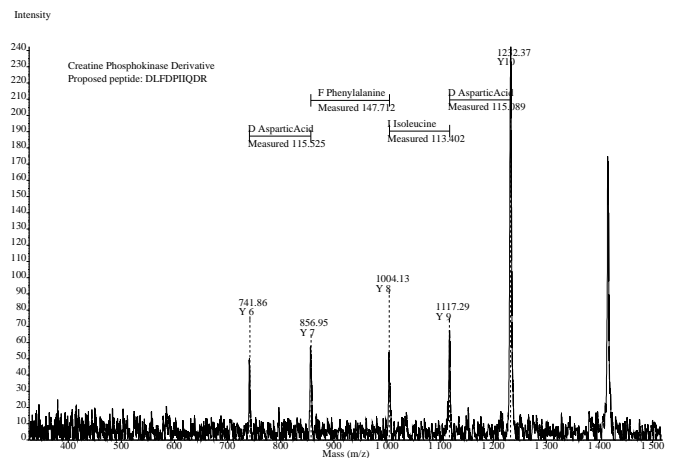
The technique of peptide mass fingerprinting can be used to positively identify proteins for which entries exist in the protein database. However, on occasion the identification may be ambiguous. The ambiguity stems from the presence of isobaric peptide sequences in the fingerprint database. Isobaric peptides have the same nominal mass to charge ratio but differ in the sequence of amino acids. Such isobaric peptides can be sequenced in the mass spectrometer. When these sequence data are combined with the mass fingerprint the ambiguity is resolved.

Materials and Methods

A peptide mass fingerprint was obtained for the protein creatine phosphokinase type I, found in rabbit muscle. This process is described in the application note LTAN1. The peptides were derivatized using sulphonamide chemistry as this promotes a single fragmentation pathway thus revealing the amino acid sequence. See Sheet LTAN3. The peptide at mass 1231.6 Th was chosen because it appears in the mass fingerprint for the likely candidates. This peptide was sequenced from the PSD spectrum which was obtained from the LaserToF LT3 Plus in a seamless scan using the harmonic field reflectron.



Peptide Mass Fingerprint



PSD Peptide Sequence

Peptide Mass Fingerprint			Partial Peptide Sequence (1231.63 Th)			Peptide Mass Fingerprint and Partial Peptide Sequence		
Rank	Protein	Score	Rank	Protein	Score	Rank	Protein	Score
1	..KCRM_RABIT	...245.0	1	..KCRB_HUMAN	...26.0	1	..KCRM_RABIT	...245.0
2	..KCRM_CANFA	...187.0	2	..KCRB_RAT	...26.0	2	..KCRM_CANFA	...187.0
3	..RF1_STRGC	...182.0	3	..KCRM_BOVIN	...26.0	3	..KCRM_MOUSE	...94.0
4	..PSD2_BOVIN	...158.0	4	..KCRM_CANFA	...26.0	4	..KCRM_RAT	...94.0
5	..Y158_RICPR	...127.0	5	..KCRM_MOUSE	...26.0	5	..KCRM_BOVIN	...68.0
6	..KCRM_MOUSE	...94.0	6	..KCRM_RABIT	...26.0	6	..KCRB_HUMAN	...5.0
7	..KCRM_RAT	...94.0	7	..KCRM_RAT	...26.0	7	..KCRB_RAT	...5.0
8	..RL21_XENLA	...83.0						
9	..C2_OXYNO	...69.0						
10	..KCRM_BOVIN	...68.0						

Table 1 Protein Identification using combinations of Peptide Mass Fingerprinting and Peptide Sequencing

Discussion

Identification was performed using the peptide fingerprinting module of the LaserToF software application suite. Table 1 compares the results obtained using:

- 1 Peptide Mass Fingerprint
- 2 Sequence Data from the peptide 1231.6 Th
- 3 Peptide Mass Fingerprint in conjunction with the Sequence data

The peptide mass fingerprint was sufficient to identify the protein as the most probable match from the database. However, proteins which are unrelated to creatine phosphokinase (KCRM) are present in the top ten candidates. The score correlates the set of measured masses to the set of theoretical masses calculated from the entry in the database.

The peptide database tool was used to select an isobaric peptide mass from the candidate proteins. The following possible candidates were found to contain the isobaric mass 1231.6.

Candidate	Sequence
KCRM_RABIT	DLFDPIIQDR
KCRM_CANFA	DLFDPIIQDR
Y158_RICPR	DETIYIPPRK
RL21_XENLA	HGAVPLSTYMR

Table 2 Candidate Fragment sequence for 1231.6± 0.2

A search of the protein sequence database using the peptide mass and the partial amino acid sequence data returns only seven possible matching proteins out of 300,000! Furthermore, all seven are closely related.

When the peptide mass fingerprint and the sequence data are combined in a single search, the protein is identified correctly and there is greater differentiation of other unrelated proteins.

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It should be noted that the searches were not restricted by taxa or protein mass.

Conclusion

This approach to protein identification allows the protein to be identified with more confidence than is the case when the peptide mass fingerprint is used alone.

Local Representative

