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Alkylated Trihydroxyacetophenone as a Novel MALDI Matrix for Hydrophobic Peptides

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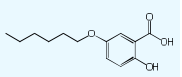
1. Overview.

- Alkylated trihydroxyacetophenone (ATHAP) as a novel matrix for hydrophobic peptides was reported.
- ATHAP is a 2,4,6-trihydroxyacetophenone derivative incorporating a hydrophobic alkyl chain on the acyl group which was expected to have affinity for hydrophobic peptides.
- As a result, ATHAP increased the sensitivity of hydrophobic peptides by 10-fold and decreased the sensitivity of hydrophilic peptides compared with α-cyano-4-hydroxycinnamic acid (CHCA).
- The peptides were detected throughout the entire matrix/analyte dried spot using ATHAP.

2. Introduction.

- Hydrophobic peptides are difficult to be detected in MALDI-MS because the detection hindrance appertains to hydrophilic peptides of conventional matrices.
- Recently, we reported alkylated dihydroxybenzoic acid (ADHB) as a matrix additive for hydrophobic peptides. However, it still remains the following issues:
  1. Hydrophobic peptide ions were detected in the rim of matrix/analyte dried spot, which has difficulty in finding the "sweet spot".
  2. ADHB was an additive, thus unavailable without conventional matrices.
  3. Hydrophilic peptide ions were also detected, which may limit the detection of hydrophobic peptide ions.



A matrix additive ADHB[1]

- To solve the issues, we launched a study to develop a novel matrix for hydrophobic peptides.

3. Methods.

3-1. Alkylated trihydroxyacetophenone (ATHAP).

- ATHAP incorporating a C8 acyl chain (**Figure 1**) was synthesized.
- ATHAP solution was prepared in 75% acetonitrile (ACN)/0.1% aqueous trifluoroacetic acid (TFA) (v/v) at 5 mg/mL.

3-2. Matrix solution.

- α-Cyano-4-hydroxycinnamic acid (CHCA) was purchased from LaserBio Labs.
- Trihydroxyacetophenone (THAP) was purchased from Sigma-Aldrich.
- Each matrix was dissolved in 50% ACN/0.1% aqueous TFA (v/v) at 10 mg/mL.

3-3. Analyte solution.

- The peptides were dissolved in 50%ACN/0.1% aqueous TFA (v/v) at appropriate concentrations.

3-4. Sample preparation.

- The analyte solution (0.5 μL) and the matrix solution (0.5 μL) were mixed on a stainless-steel plate to be analyzed by MALDI-TOFMS (**Scheme 1**).

3-5. MALDI-MS.

- MALDI-TOFMS measurement was performed using *AXIMA Performance* (Shimadzu/Kratos, UK) mass spectrometer in linear, positive ion mode.

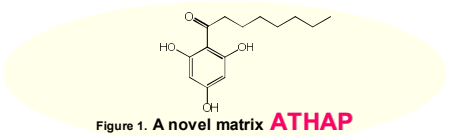
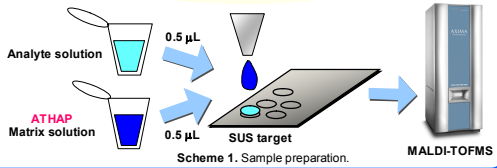


Figure 1. A novel matrix ATHAP



Scheme 1. Sample preparation.

4. Results.

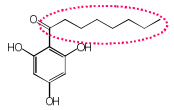
4-1. Sensitivity Increase for Hydrophobic Peptides by ATHAP.

Table 1. Detection limit of hydrophobic peptide.

	humanin (fmol/well)
	positive mode
C12-ATHAP	10
C10-ATHAP	**1**
C8-ATHAP	**1**
C6-ATHAP	10
THAP	10
CHCA	10

- C8-ATHAP was used in this study.

- The sensitivity for hydrophobic peptide was increased by 10-fold using C8- or C10-ATHAP compared with THAP or CHCA.



4-2. Preferential Ionization of Hydrophobic Peptides by ATHAP.

- Hydrophobic peptide was preferentially ionized using ATHAP.
- The detection of hydrophilic peptide ions was suppressed using ATHAP.

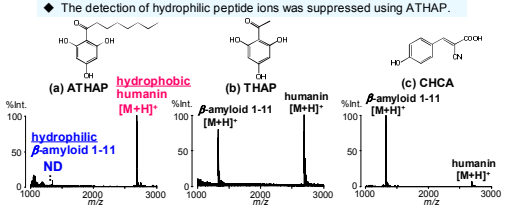


Figure 2. Positive mass spectra for 100 fmol equimolar mixture of hydrophobic peptide (humanin) and hydrophilic peptide (β-amyloid 1-11). ND indicates that the ions were not detected.

4-3. Enhanced Detection of [M+H]+ throughout the Entire Spot by ATHAP.

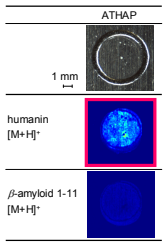


Figure 3. MS imaging for 100 fmol equimolar mixture of hydrophobic peptide (humanin) and hydrophilic peptide (β-amyloid 1-11).

- Hydrophobic peptide ions were detected throughout the entire matrix/analyte dried spot using ATHAP.
  ⟶ Raster scanning was used in all the experiments.
- The detection of [M+H]+ was enhanced using ATHAP.

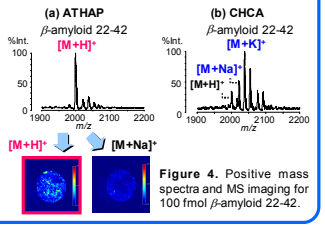


Figure 4. Positive mass spectra and MS imaging for 100 fmol β-amyloid 22-42.

4-4. Sensitivity Improvement by ATHAP and SSRCalc Hydrophobicity.

- The sensitivity improvement rate by ATHAP to CHCA was increased for hydrophobic peptides with higher SSRCalc Hydrophobicity and decreased for hydrophilic peptides with lower SSRCalc Hydrophobicity.

Table 2. Sensitivity improvement by ATHAP for peptides with SSRCalc Hydrophobicity from 5.2 to 54.8.

	analytes				sensitivity improvement rate by ATHAP to CHCA (fold) = Detection limit using CHCA / Detection limit using ATHAP
	no.	name	SSRCalc Hydrophobicity	m/z (Ave.)	
Hydrophobic	1	NF-κB inhibitor	**54.8**	2782.6	**10**
	2	OVA-BIP hybrid peptide	**50.2**	2291.6	**10**
	3	humanin	**50.0**	2688.3	**10**
	4	β-amyloid 22-42	**42.4**	2000.4	**10**
	5	catestatin	**38.1**	2327.7	**1**
	6	ACTH 18-39	**37.9**	2466.7	**1**
	7	nocistatin	**29.8**	1928.1	**1**
	8	neuropeptide S	**22.3**	2188.5	**0.1**
	9	β-amyloid 1-16	**18.2**	1956.1	**0.1**
	10	β-amyloid 1-11	**13.5**	1326.3	**0.001**
Hydrophilic	11	β-conglycinin 165-178	**5.2**	1848.8	**0.0001**

Higher sensitivity using ATHAP than CHCA

Lower sensitivity using ATHAP than CHCA

4-5. Phosphorylase b Lys-C Digestion Fragments Analysis using ATHAP.

- The intensity was increased for hydrophobic peptides with higher SSRCalc Hydrophobicity and decreased for hydrophilic peptides with lower SSRCalc Hydrophobicity using ATHAP.

Table 3. The Ion detection for phosphorylase b Lys-C digestion fragments and the SSRCalc Hydrophobicity.[a]

phosphorylase b Lys-C digestion fragments			detection (+/-)	
No.	SSRCalc Hydrophobicity	m/z (Ave.)	ATHAP	CHCA
1	**55.9**	3602.2	++	-
2	**53.9**	3823.5	++	-
3	**53.1**	3890.3	++	-
4	**51.0**	3823.5	++	-
5	**50.7**	2198.6	++	+
6	**45.8**	2155.6	++	+
7	**45.1**	2742.0	+	+
8	**42.8**	2969.5	+	-
9	**38.9**	3504.9	++	-
10	**35.1**	1855.1	++	++
11	**33.9**	1657.0	+	+
12	**33.6**	2130.5	++	-
13	**33.4**	2629.0	++	++
14	**31.4**	1610.9	++	++
15	**31.4**	1814.1	++	++
16	**31.2**	1526.8	++	++
17	**30.9**	2043.3	++	++
18	**30.4**	1942.3	++	++
19	**29.9**	2449.7	-	+
20	**28.4**	1304.7	++	++
21	**25.8**	1263.4	-	++
22	**24.7**	1178.3	-	++
23	**24.5**	1290.5	+	++
24	**18.9**	1254.5	+	++
25	**9.3**	1102.2	-	++

[a] "++" indicates that the ions were detected with S/N ≥5, "+" indicates that the ions were detected with S/N = 2~5, and "-" indicates that the ions were not detected.

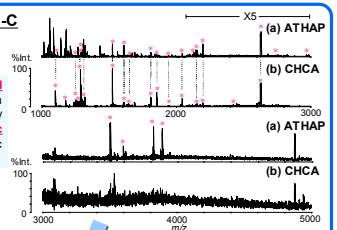


Figure 5. Positive mass spectra of 1 pmol phosphorylase b Lys-C digestion fragments using ATHAP or CHCA.

- Hydrophobic peptide ions, which could not be detected by CHCA, were detected by ATHAP.
- The sequence coverage was increased by using the both results of ATHAP and CHCA.
  - 30% with CHCA
  - 45% (or 49%) with ATHAP
  - 51% (or 55%) with both the results of ATHAP and CHCA

Higher intensity using ATHAP than CHCA.

Lower intensity using ATHAP than CHCA.

References.

[1] Fukuyama, Y.; Tanimura, R.; Maeda, K.; Watanabe, M.; Kawabata, S.;Iwamoto, S.; Izumi, S.; Tanaka, K. *Anal. Chem.* **2012**, *84*, 4237-4243.

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5. Conclusions.

- A novel matrix ATHAP increased sensitivity of hydrophobic peptides by 10-fold.
- All the issues of ADHB were solved by ATHAP as follows:
  1. The peptide ions were detected throughout the entire matrix/analyte dried spot.
  2. ATHAP works as a matrix itself.
  3. The detection of hydrophilic peptide ions was suppressed.