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疎水性ペプチドに適したMALDIマトリックスalkylated trihydroxyacetophenone
Alkylated Trihydroxyacetophenone as a MALDI matrix for Hydrophobic Peptides

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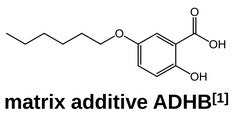
1. Introduction

- 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. But, 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.[2]

2. Experimental Section

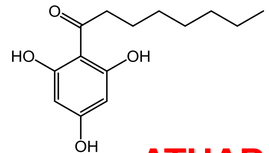


Fig 1. A novel matrix ATHAP

2-1. Alkylated trihydroxyacetophenone (ATHAP).

- ATHAP incorporating a C8 (or C6, C10, C12) 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.

2-2. 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.

2-3. Analyte solution.

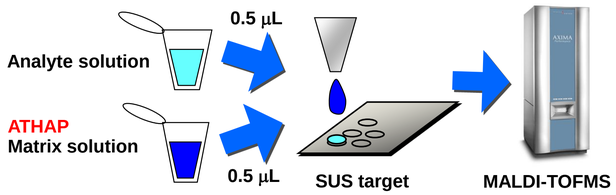
- The peptides were dissolved in 50%ACN/0.1% aqueous TFA (v/v) at appropriate concentrations.

2-4. 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**).

2-5. MALDI-MS.

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



Scheme 1. Sample preparation

3. Results and Discussion

3-1. ATHAP for Hydrophobic peptides

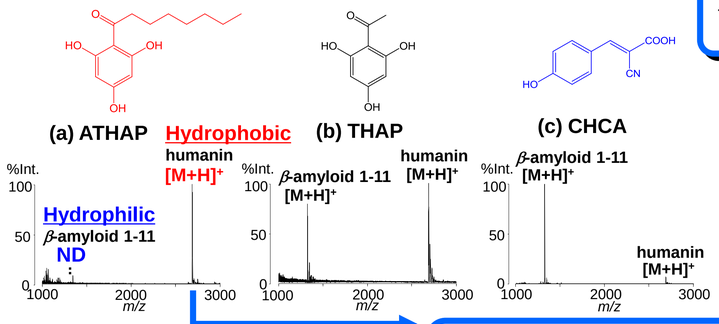


Fig 2. Mass spectra for 100 fmol equimolar mixture of hydrophobic peptide (humanin) and hydrophilic peptide (β-amyloid 1-11). ND indicates that the ions were not detected.

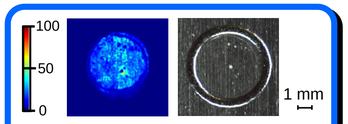


Fig 3. MS imaging of $[M+H]^+$ for 100 fmol humanin.

Homogeneous dried spot

- ◆ **Hydrophobic peptide was preferentially ionized** using ATHAP.
- ◆ The detection of hydrophilic peptide ions was suppressed using ATHAP.
- ◆ **Hydrophobic peptide ions were detected throughout the entire matrix/analyte dried spot** using ATHAP.
  - → Raster scanning was used in all the experiments.

Table 1. Sensitivity improvement by ATHAP for peptides

	analytes				sensitivity improvement rate by ATHAP to CHCA (fold)
	no.	name	SSRCalc Hydrophobicity[3]	*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**

Sensitivity improvement rate by ATHAP to CHCA (fold) = Detection limit using CHCA / Detection limit using ATHAP

Higher sensitivity with ATHAP

Lower sensitivity with ATHAP

3-2. Digestion analyses

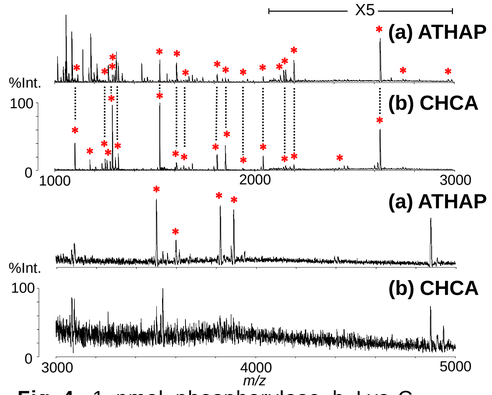


Fig 4. 1 pmol phosphorylase b Lys-C digestion fragments using ATHAP or CHCA.

- ◆ The **intensity was increased for hydrophobic peptides** with higher SSRCalc Hydrophobicity and **decreased for hydrophilic peptides** with lower SSRCalc Hydrophobicity **using ATHAP**.
- ◆ **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 or 49% with ATHAP
  - 55% with both the results of ATHAP and CHCA

Table 2. The Ion detection for 1 pmol 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
Hydrophobic	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	+	++
Hydrophilic	25	**9.3**	1102.2	-	++

Hydrophobic

Higher sensitivity with ATHAP

Lower sensitivity with ATHAP

Hydrophilic

[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.

Table 3. Comparison of the Ion detection for 1 pmol phosphorylase b Lys-C digestion fragments using C6-, C8-, C10- or C12-ATHAP

phosphorylase b Lys-C digests		detection (+/-)				
no.	SSRCalc Hydrophobicity	CHCA	C6-ATHAP	C8-ATHAP	C10-ATHAP	C12-ATHAP
1	55.9	-	-	++	++	-
2	53.9	-	-	++	++	-
3	53.1	-	-	++	+	-
4	51.0	-	-	++	++	-
5	50.7	+	+	++	++	-
6	45.8	+	+	++	++	-
7	45.1	+	-	+	+	-
8	42.8	-	-	+	+	-
9	38.9	-	-	++	++	-

- ◆ **Hydrophobic peptide was detected with higher sensitivity using C8- (or C10-)ATHAP** compared with C6-, C12-ATHAP or CHCA.

4. 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**.

References

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[2] Fukuyama.Y.; Nakajima,C.; Furuichi,K.; Taniguchi,K.; Kawabata,S.; Izumi,S.; Tanaka, K. *Anal. Chem.* submitted.

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