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アプリケーションノート

Quantitative Determination of Noncovalent Protein-Ligand Interactions Automatic Chip-Based Nanoelectrospray

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Abstract

The purpose of this experiment is to demonstrate automated nanoESI/MS analysis to determine micromolar and submicromolar dissociation constants as well as to measure the solution binding constants for the Ribonuclease A (RNase) complexes with cytidilic acid ligands.

Benefits

- Allows extended acquisition time for better data quality
- Increases sample throughput
- Improves spray stability and reproducibility
- Automates nanoelectrospray with one time spray optimization
- No carryover

Introduction

Automated Chip-Based Nanoelectrospray

Advion BioSciences, Inc. (Ithaca, NY) has developed a method and demonstrated the capabilities of the NanoMate System for quantitative determination of noncovalent interactions between proteins and ligands. The NanoMate 100 is a chip-based automated nanoelectrospray ionization system for mass spectrometry, and is readily integrated with the Waters Micromass Q-Tof micro.

Investigations of noncovalent protein-ligand interactions by nanoelectrospray ionization mass spectrometry (nanoESI/MS) are of great interest because of their relevance to molecular recognition and to combinatory ligand library searching. This application note from Advion Biosciences introduces an experiment where automated nano ESI/MS analysis has been used to determine micromolar and submicromolar dissociation constants as well as to measure the solution biding constants for the Ribonuclease A (RNase) complexes with cytidilic acid ligands.

Waters Micromass Q-Tof micro Mass Spectrometer and the Advion NanoMate 100.

Experimental

Determination of Noncovalent Protein-Ligand Interactions

RNase complexed with cytidine 2’ -monophosphate and cytidine triphosphate (see Figure 1), a well characterized model system, was used to demonstrate the method.

Titration Experiments

RNase protein was maintained at 10 µM and 4 µM, respectively, in 10 mM ammonium acetate pH 6.8 for titration of 2’ -CMP (1 to 20 µM) and CTP (1 to 8 µM), respectively. The solutions were then incubated at room temperature for 15 minutes prior to MS analysis.

$$Kd = \frac{[R] * [L]}{[RL]} = \frac{[R] * ([Li] - [RL])}{[RL]}$$

$$[RL]/[R] = 1/Kd * ([Li] - [RL])$$

Competitive Binding Experiments

Equimolar solutions of 2’ -CMP and CTP (4 µM) were mixed with 4 µM of RNase in 10 mM ammonium acetate pH 6.8. The solutions were then incubated at room temperature for 15 minutes prior to nanoelectrospray MS analysis.

$$Kd_{RL1} = [R] * ([R] + [RL_2]) / [RL_1]$$

$$Kd_{RL2} = [R] * ([R] + [RL_1]) / [RL_2]$$

NanoESI/MS Analytical Conditions

Sample size:	3 µL
Flow rate:	100 nL/minute

Spray voltage:	1.5 kV
Pressure:	0.3 psi
Acquisition time:	2 minutes
Instrumentation:	NanoMate100 with ESI Chip Micromass Q-Tof micro
Sample cone voltage:	30 V
Source temp.:	45 °C

Results and Discussion

Each ligand was detected using the NanoMate100 System (Figures 2 and 3) and as a result titration and competitive binding experiments were performed (Figure 4). The results presented are in agreement with previously published results of circular dichroism (CD).[1]

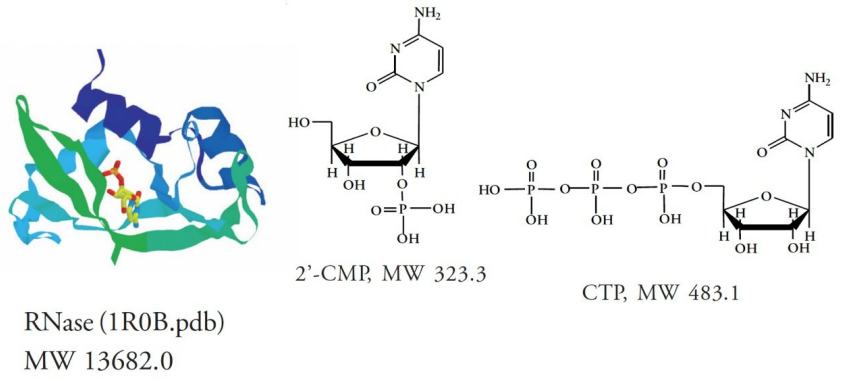

Figure 1. Protein and ligand structures.

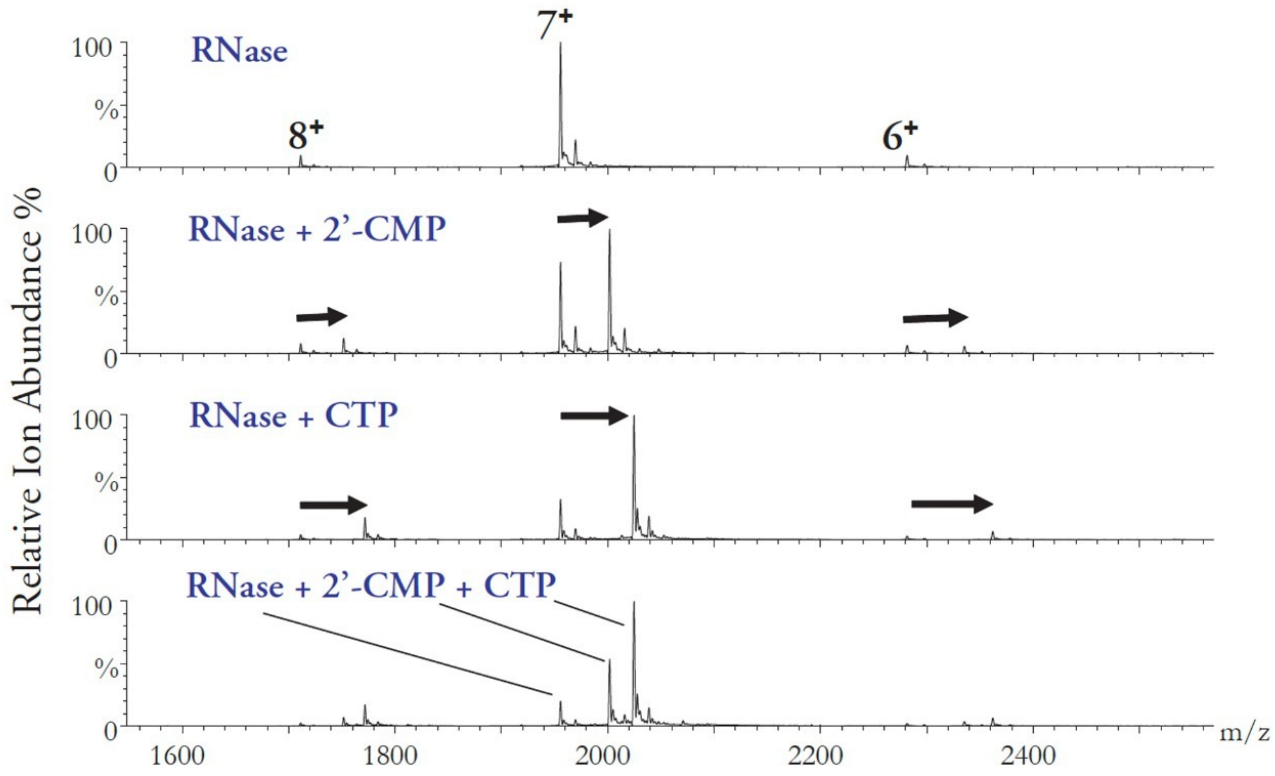

Figure 2. NanoESI mass spectra.

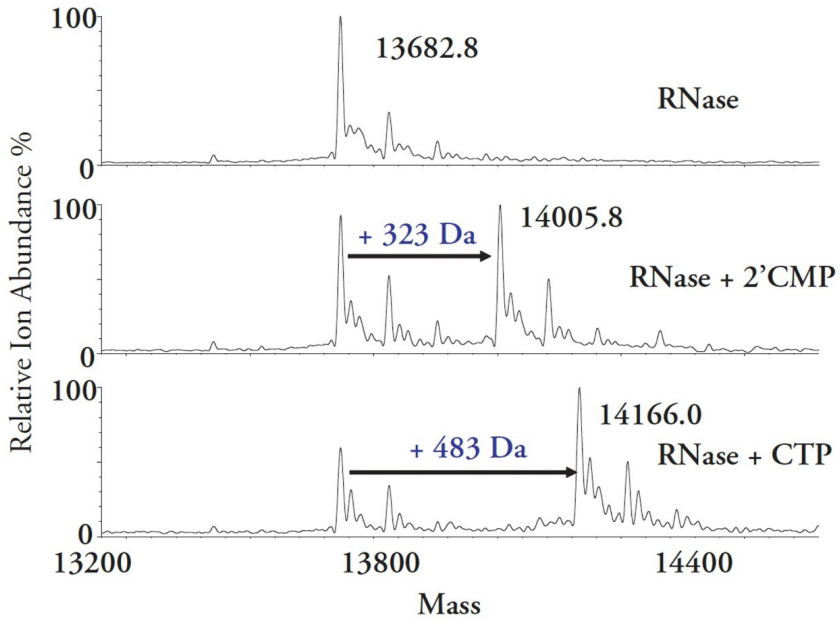

Figure 3. Deconvoluted mass spectra of the RNase-Ligand complexes.

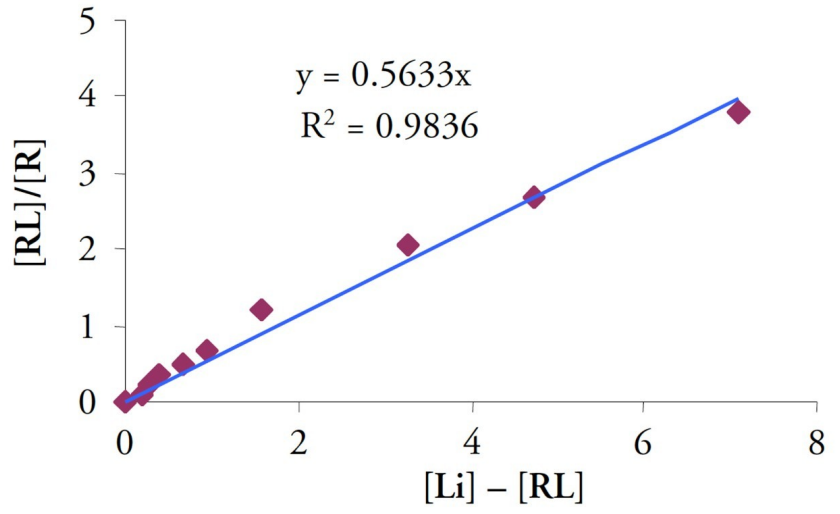

Figure 4. Titration assay for RNase (10mM) with 2'-CMP.

Ligand	Kd (µM)		
	Titration experiment		Competitive binding experiment
	Ave. of individual points	Plot	
Cytidine 2'- monophosphate (2'-CMP)	1.71 ± 0.33	2.00 ± 0.43	2.3 ± 0.4
Cytidine triphosphate (CTP)	0.80 ± 0.2	0.74 ± 0.3	0.75 ± 0.4

Table 1. Summary of binding assay for RNase and cytidine nucleotide ligands using automated NanoESI/MS.

Conclusion

The NanoMate100 System can be used to determine micromolar and submicromolar dissociation constants. In addition, an automated nanoESI/MS method can be used to measure solution binding constants for the RNase complexes with cytidilic acid ligands.

References

1. Jones, C.L.; Fish, F.; Muccio, D.D. *Anal BioChem* 2002, 302, 184–190.
2. Application note based on Zhang, S.; Van Pelt, C.K.; Wilson, D.B. *Anal Chem* 2003, 75, 3010–3018.

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