

Direct Detection of Non-covalent Complexes of Oligo(tyrosine sulfate)s with Synthetic Heparin-binding Peptides by MALDI-TOF-MS

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Introduction

Members of the heparin/heparan sulfate-like glycosaminoglycan (HLGAG) family play important roles in regulation of many biological processes. However, their controlled application has been limited because of their heterogeneity and difficulty to synthesis. To solve these problems, many synthetic mimics including sulfated peptides have been prepared. Since the discovery of anti-human immunodeficiency virus (HIV) activity in oligo(tyrosine sulfate)s (Fig. 1) in our laboratory [1, 2], we have been interested in their potential as HLGAG mimics. We have previously reported their interactions with synthetic heparin-binding peptides using surface noncovalent affinity mass spectrometry [3, 4]. In this study, we present direct detection of noncovalent complexes of oligo(tyrosine sulfate)s with a heparin-binding peptide by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS).

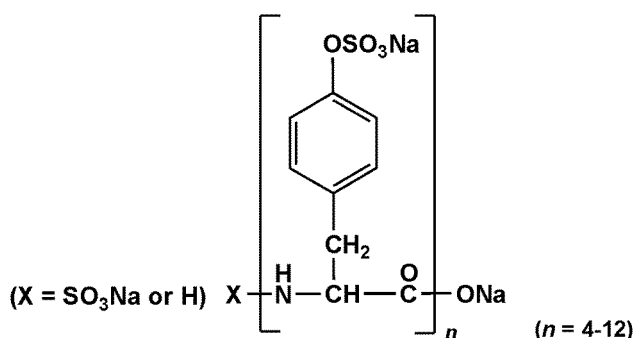


Fig. 1. Structure of oligo(tyrosine sulfate)s.

Results and Discussion

First, we tried to establish a method to directly detect complexes of an oligo(tyrosine sulfate) with a heparin-binding peptide. Recently, Woods *et al.* reported the formation of a noncovalent complex between sulfated tyrosine-containing peptides and peptides containing two or more adjacent arginines using MALDI-TOF-MS [5]. In that study, saturated solution of 2,4,6-trihydroxyacetophenone (THAP) was used as a matrix. Therefore, we selected a high-concentration solution (20 mg/mL) of THAP as the matrix and analyzed a 1:1 mixture of *N*-sulfated nonamer NaO₃S-[Tyr(SO₃Na)]₉-ONa and hAT III (123–139), a heparin-binding domain of human antithrombin III (hAT III) [6]. We detected successfully a 1:1 complex and fragment ions corresponding to loss of SO₃ in both positive

and negative modes (data not shown). We next analyzed mixtures of an *N*-sulfated nonamer and various amounts of hAT III (123–139). When the amount of hAT III (123–139) was less than or equimolar to that of the nonamer, a 1:1 complex was observed. Increasing the amount of hAT III (123–139) further caused the appearance of a 1:2 complex [a nonamer + 2 hAT III (123–139)] (data not shown).

To compare the relative binding affinities of oligo(tyrosine sulfate)s to hAT III (123–139), we analyzed mixtures of three oligo(tyrosine sulfate)s of different chain length [*N*-nonsulfated tetramer, octamer, and dodecamer, H-[Tyr(SO₃Na)]_n-ONa (n = 4, 8, 12)] and an increasing amount of hAT III (123–139). When the amount of hAT III (123–139) was small, only a complex with the longest *N*-nonsulfated dodecamer was observed (Fig. 2b). This result showed that the *N*-nonsulfated dodecamer had the highest binding affinity for hAT III (123–139) of the three oligomers. As the amount of hAT III (123–139) increased, a complex with *N*-nonsulfated dodecamer disappeared and a complex with the *N*-nonsulfated octamer appeared (Fig. 2c). It seemed that the complex with the *N*-nonsulfated octamer could ionize much more easily than that with the *N*-nonsulfated dodecamer. A complex with the *N*-nonsulfated tetramer was observed after adding more hAT III (123–139) (Fig. 2d and 2e). From these results, the relative binding affinities of oligo(tyrosine sulfate)s to hAT III (123–139) could be easily determined. Thus, we succeeded in observing chain-length dependence in the binding affinity of oligo(tyrosine sulfate)s to hAT III (123–139).

In summary, noncovalent complexes between oligo(tyrosine sulfate)s and hAT III (123–139) were detected directly by MALDI-TOF-MS, and this allowed us to compare the relative binding affinities of oligo(tyrosine sulfate)s to hAT III (123–139).

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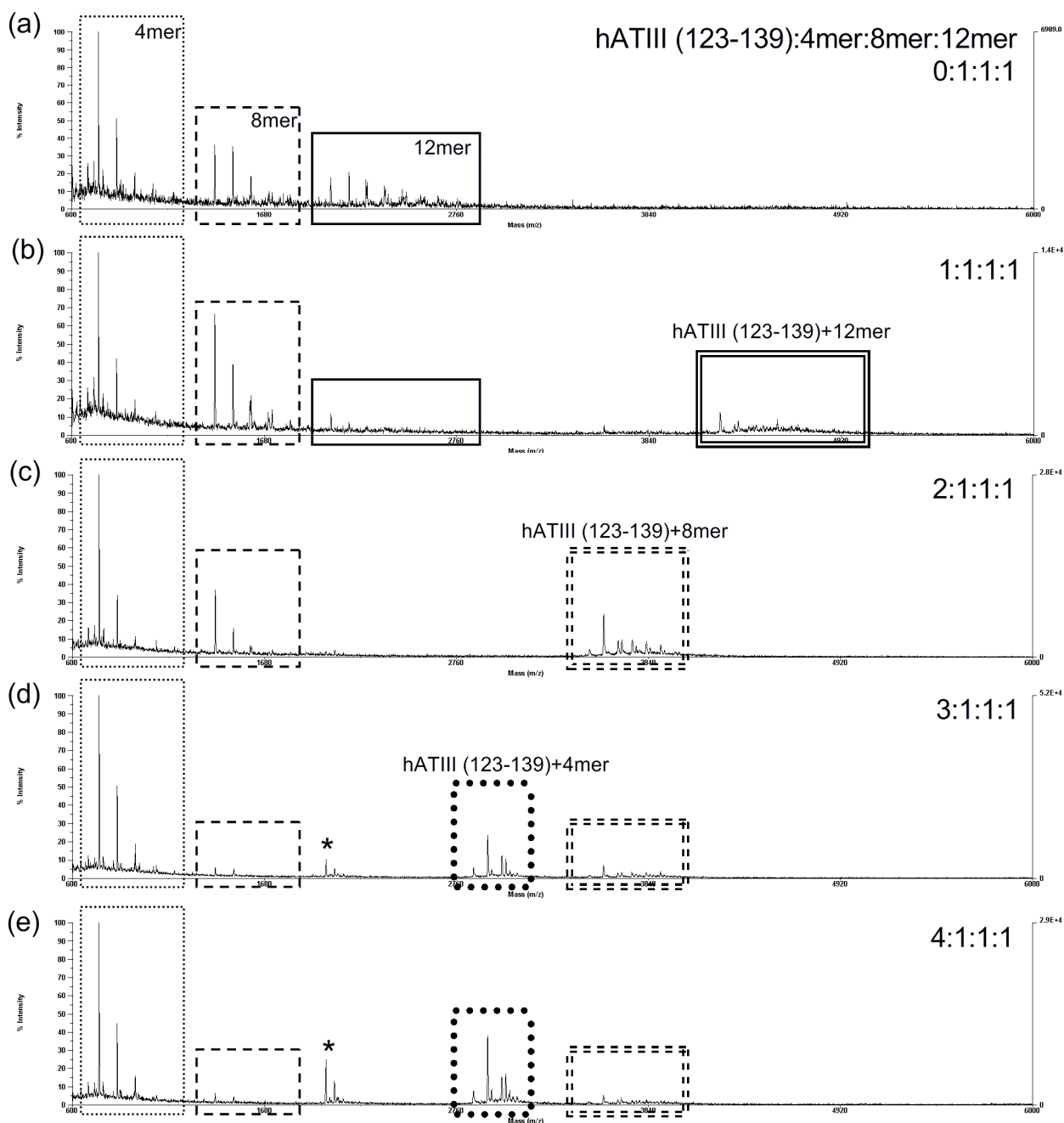


Fig. 2. MALDI-TOF-Mass spectra of mixtures of three oligo(tyrosine sulfate)s and hAT III (123-139). (a) hAT III (123-139):4mer:8mer:12mer = 0:1:1:1, (b) 1:1:1:1, (c) 2:1:1:1, (d) 3:1:1:1, (e) 4:1:1:1. * indicates a molecular ion of hAT III (123-139).

References

1. Ueki, M., Watanabe, S., Ishii, Y., Okunaka, O., Uchino, K., Saitoh, T., Higashi, K., Nakashima, H., Yamamoto, N., and Ogawara, H. (2001) *Bioorg. Med. Chem.*, **9**, 477–486.
2. Ueki, M., Watanabe, S., Saitoh, T., Nakashima, H., Yamamoto, N., and Ogawara, H. (2001) *Bioorg. Med. Chem.*, **9**, 487–492.
3. Ueki, M. and Yamaguchi, M. (2006) *Peptide Science 2006 (Proceedings of the International Conference of 43rd Japanese Peptide Symposium and 4th Peptide Engineering Meeting)*, eds. Ishida, H. and Mihara, H., The Japanese Peptide Society, Osaka, pp 188–189.
4. Yamaguchi, M. and Ueki, M. (2007) *Proceedings of the 20th American Peptide Symposium: Peptides for Youth* (eds. Escher, E., Lubell, W. D., and Valle, S. D., The American Peptide Society) in press.
5. Woods, A. S., Wang, H. J., and Jackson S. N. (2007) *J. Proteome Res.*, **6**, 1176–1182.
6. Onoue, S., Harada, S., Nemoto, Y., Yajima, T., and Kashimoto, K. (2003) *Peptides*, **24**, 821–826.