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Sulfation, the Up-and-Coming Post-Translational Modification: Its Role and Mechanism in Protein–Protein Interaction

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Abstract

Tyrosine sulfation is a post-translational modification entailing covalent attachment of sulfate to tyrosine residues. It takes place in the trans-Golgi, is necessary for the bioactivity of some proteins, and improves their ability to interact with other proteins. In the present work, we show that a protein containing a sulfated tyrosine with a delocalized negative charge forms a salt bridge with another protein if it has two or more adjacent arginine residues containing positive delocalized charges. These noncovalent complexes are so stable that, when submitted to collision induced dissociation, the peptides forming the complex dissociate. Just one covalent bond fragments, the covalent bond between the tyrosine oxygen and the SO_3 sulfur, and is represented by the appearance of a new peak (basic peptide + SO_3), suggesting that in some instances covalent bonds will break down before the noncovalent bonds between the arginine guanidinium and SO_3 dissociate. The data implies that the dissociation pathway is preferred; however, fragmentation between tyrosine and the sulfate residue is a major pathway.

Keywords

sulfation; noncovalent interactions; salt bridges; guanidium group

Introduction

Tyrosine sulfation is a post-translational modification entailing covalent attachment of sulfate to tyrosine residues. It was first described by Bettelheim in 1954.[1] Proteins synthesized in the rough endoplasmic reticulum (RER) are modified in the trans-Golgi where tyrosine sulfation occurs and is catalyzed by the membrane-bound enzyme, tyrosylprotein sulfotransferase.[2] Tyrosine sulfation has been found in several secretory proteins,[3,4] plasma membrane proteins,[4] adhesion molecules,[6,7] coagulation factors, plasma proteins,[8,9] immune components,[10] and G-protein-coupled receptors such as the thyrotrophin receptor, which has a long N-terminal extracellular extension responsible for high-affinity hormone binding (sulfation of one of the two tyrosines in the motif is mandatory for high-affinity binding of TSH and activation of the receptor),[11] and the neuropeptide cholecystokinin (CCK), which regulates a diversity of emotions and motivations including negative affect and stress responses.[12] Tyrosine *O*-sulfate is a stable molecule and is excreted in urine. No enzymatic mechanism of tyrosine desulfation is known.

Sulfation consensus sites are predicted by the presence of acidic residues on the amino-terminal side of the targeted tyrosine. Bundgaard examined the role of residues neighboring the sulfation site of human progastrin *in vivo* and demonstrated that the charge of the residue in the amino-

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terminal position (−1) of the tyrosine is critical and can be neutral or acidic, whereas a basic residue abolishes sulfation, and the degree of sulfation is influenced by residues in positions −2 and −3.[13] Kehoe and Bertozzi observed that tyrosine sulfation improved protein–protein interaction[14] and may be necessary for the bioactivity of secreted proteins and peptides. In the present study, we show that, as in the case of a phosphorylated residue with its two delocalized negative charge, the improvement in interaction when sulfate is added is likely due to its delocalized negative charge on the sulfated residues in one of the proteins forming a salt bridge with another protein if it has two or more adjacent arginine residues containing positive delocalized charges.[15,16] ILyon et al.[17] showed that the putative heparin sulfate-binding site (s), as revealed by the recent elucidation of the crystal structure of Hep-2,[18] has two separate, positively charged clusters, involving up to 11 basic residues, mostly arginines, that have the ability to coordinate sulfate groups, probably forming a single extended binding site, while Sasaki showed that mutating arginines to alanines on mouse endostatin led to failure of the interaction of endostatin with heparin.[19]

Materials and Methods

Peptides

Peptides were purchased from Bachem (College Station PA): **sY**GGFL (sulfated leuenkephalin), DF**EE**IP**EEsY**LQ (hirudin), D**sY**MGWMDF-NH_2 (CCK 1–8), YGGFL**RR**IRPKLK-WNDQ (Dyn 1–17), YGGFL**RR**I (Dyn 1–8); **sY** denotes a sulfated tyrosine.

Sample Solutions

Solutions containing an equal volume of each of the sulfated peptides (10 pmol/μL) and/or YGGFL**RRI** or YGGFL**RR**IRPKLKWNDQ (10 pmol/μL) were prepared. A volume of 0.3 μL of sample was deposited on the sample plate followed by 0.3 μL of matrix and left to dry at room temperature.

Matrix

2,4,6-Trihydroxyacetophenone (THA) was purchased from Fluka (Milwaukee, WI). Matrix was prepared fresh daily as a saturated solution in 50% ethanol.

Instruments

A MALDI-TOF/TOF (4700 Proteomics Analyzer, Applied Biosystems, Framingham, MA) with an Nd:YAG laser (355 nm) at a repetition rate of 200 Hz was used in this study for both MS and MS/MS analysis in positive ion mode. For MS analysis, mass spectra were acquired in reflectron mode in positive ion mode and were the sum of 400 laser shots. For MS/MS analysis, mass spectra were the sum of 1000 laser shots, and a collision energy of 1 keV with air as the collision gas was used to induce fragmentation.

Results and Discussion

Sulfation is a post-translational modification that is more ubiquitous than currently perceived. Niehrs demonstrated that sulfation is the most abundant post-translational modification of tyrosine residues and occurs in many soluble and membrane proteins.[20] Burlingham recently showed that, in addition to sulfated tyrosine, on rare occasions, both serine and threonine could be sulfated.[21] The modification makes proteins and peptides far more interactive as exemplified by several cases.[17–19] Whether the location of the sulfation is on an internal residue or on the amino terminus does not seem to make a difference as to the ability of the sulfated peptide to interact with epitopes containing two or more adjacent arginines (Arg). The interaction between

sulfated leuenkephalin **sY**GGFL (MH^+ 636.30 amu) and Dyn 1–8 (MH^+ 981.64 amu) or Dyn 1–17 (MH^+ 2147.34 amu) gave stable noncovalent complexes at *m*/*z* 1616.85 (Figure 1A) and 2782.56 (Figure 1B) with a relative abundance (RA) of 75 and 40%, respectively. If one would consider the sodiated complexes and the ones with a carboxyl group loss, the population of complexes is even more abundant than at first look. The interaction between D**sY**MGWMDF-NH_2 (MH^+ 1143.35 amu), a fragment of CCK where the sulfate is at position two, and Dyn 1–8 or Dyn 1–17 gave stable noncovalent complexes at *m*/*z* 2124.07 (Figure 2A) and 3290.73 (Figure 2B) with an RA of 90 and 45%, respectively. In addition, in Figure 2B, the dimer of D**sY**MGWMDF-NH_2 at amu 2285.34 (RA 10%) formed an NCX with Dyn 1–17 at amu 4431.96 (RA 8%). In the case of the hirudin fragment DF**EE**IP**EEsY**LQ where the sulfated residue is third from the carboxyl terminus and two pairs of adjacent glutamates are also present in the sequence, it formed noncovalent complexes with both Dyn 1–8 (Figure 3A) and 1–17 (Figure 3B) with an RA of 72 and 57%, respectively, at *m*/*z* 2473.21 and 3638.72. The hirudin peptide fragment was chosen because, in addition to the sulfated tyrosine, it contains another motif, mainly, two sets of adjacent glutamate that are capable of forming noncovalent complexes with adjacent arginines as previously demonstrated.[16,22–24] In Figure 3A, in addition to the NCX of DF**EE**IP**EEsY**LQ + YGGFL**RR**I (at amu 2473.21, RA 72%), the following peaks are seen: DF**EE**IP**EEsY**LQ + 2 [YGGFL**RR**I] (at amu 3453.88, RA 15%), suggesting that complex formation took place with both the sulfate and the glutamates. The following fragments show the loss of the sulfate group at amu 1412.66 (Hrn-HSO_3, RA 38%), 1434.69 [(Hrn-HSO_3) + Na^+, RA 63%], and 1537.76 [DF**EE**IP**EEsY**LQ + 2 Na^+, RA 23%]; nevertheless, we still see a NCX at 2414.21 [(Hrn-HSO_3) + Na^+ + YGGFL**RR**I (RA 25%)] due to the two pair of Glu, although the RA of the NCX is significantly lower. Thus, although the RA of the sulfated Hrn was 23% and that of the nonsulfated was 63%, the RA of the complex containing the sulfate is 72%, while the RA of the nonsulfated peptide which contains two pairs of adjacent glutamate at amu 2415.21 had a much lower RA (25%). This seems to infer that the sulfate group is much more likely to form a stable complex than the adjacent glutamates would or that the increased electronegativity it imparts to the peptide makes it three times as likely to form a NCX as the two pairs of glutamates present in the nonsulfated one. Gilson et al. have shown that salt bridges involving phosphotyrosine are about twice as strong as those involving monoanionic side chains.

In the context of an (*i*, *i* + 4) interaction, they also implied that an arginine partner will yield greater stability than a lysine residue will. Although phosphorus and sulfur belong to the same row of the periodic table but are not in the same column, they still have some similar properties, mainly, their electronegativity and ionization potentials (Table 1 in ref [15]). In Figure 3B, again the RAs of the NCX of Dyn 1–17 and the sulfated and nonsulfated hirudin fragment are 57% (at amu 3638.92) versus 7% (at amu 3538.02), showing again that the sulfated peptide is markedly more effective in forming NCXs.

MS/MS of the Noncovalent Complexes

As previously seen with noncovalent complexes formed through electrostatic attraction between peptides containing adjacent glutamates or adjacent aspartates or a phosphorylated threonine, serine, or tyrosine on one peptide and adjacent arginines on the other, the peptides forming the complex dissociate rather than fragment.[26–28] Figure 4 illustrates that the collision-induced dissociation of the complexes (amu 1616.85, 2124.07, 2473.21) of each of the sulfated peptides and Dyn 1–8 shows peaks at amu 981.69 (Dyn 1–8), 1061.60 (Dyn 1–8 + SO_3), and sometimes a peak at 80.05 ($SO_3$2-). Figure 5C shows that CID of the complexes (amu 2782.56, 3290.73, 3638.72) resulted in peaks at amu 2147.30 (Dyn 1–17), 2227.71(Dyn 1–17 + SO_3), and sometimes a peak at 120.06 (HSO_3 + Na^+). The sulfated peptides are very acidic and hence not seen in positive ion mode, probably due to suppression by the positive charge of Dyn 1–8 and 1–17. The peaks at 1061.60 and 2227.71 are 80 amu larger than those of Dyn 1–8 and 1–

17, suggesting that similarly to phosphorylated residues where the covalent bond between the oxygen contributed by the amino acid residue and the phosphorus breaks down rather than the electrostatic bond between the phosphate and the arginine residue, the bond between the oxygen contributed by tyrosine and the sulfur breaks down before the sulfate–guanidinium salt bridge. However, the relative abundance of the peaks at 1061.60 and 2227.71 does not exceed 10%, while in the case of the phosphate, the yield was much higher. This is probably due to the fact that the phosphorylated residues we used were serine and threonine.[26–28] Tyrosine, an aromatic amino acid residue, possesses a delocalized pair of electrons because of its benzene ring, making phosphorylated tyrosine and sulfated tyrosine more electronegative than phosphorylated serine or threonine and, thus, better able to withstand the electrostatic pull from the powerful positive charge of the arginines guanidinium group.

Although the interest in protein–protein interaction is on the rise,[29,30] the biological role of tyrosine "sulfation" has not been brought to the fore and is not always clear. The present work reinforces the suggestion that the presence of the negatively charged sulfate groups "sulfation" facilitates protein–protein interactions, thus, increasing the stability of proteins and inducing correct protein folding.

Acknowledgments

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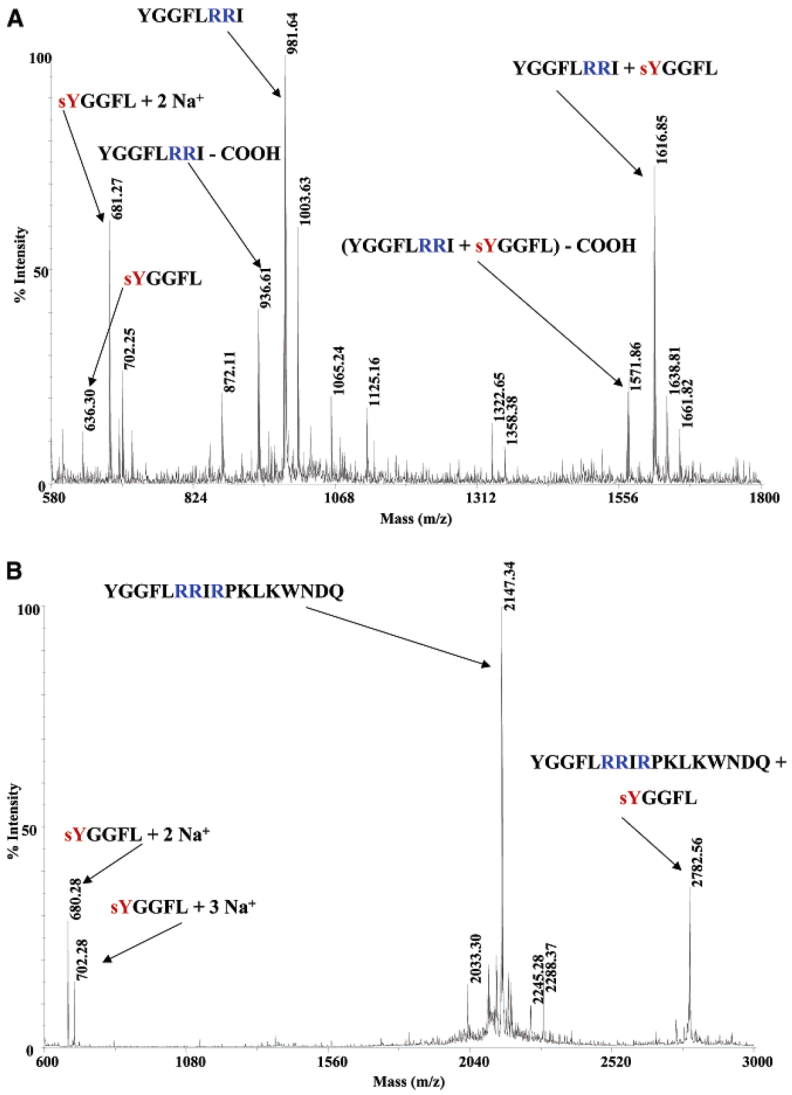

Figure 1.
(A) Spectrum showing a noncovalent complex of sulfated leuenkephalin and Dyn 1–8. (B) Spectrum showing a noncovalent complex of sulfated leuenkephalin and Dyn 1–17.

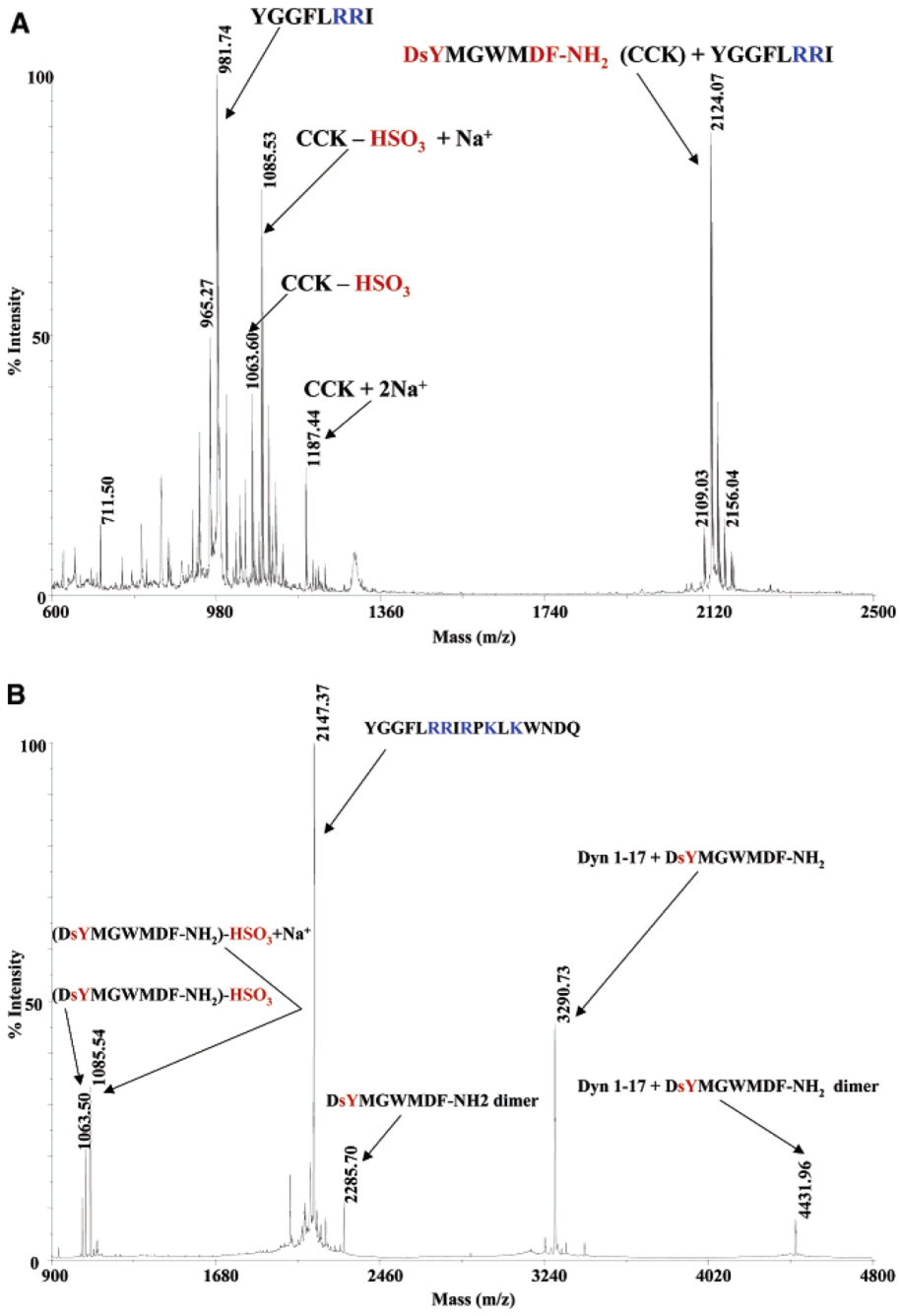

Figure 2.

(A) Spectrum showing a noncovalent complex of sulfated cholecystokinin fragment and Dyn 1–8. (B) Spectrum showing a noncovalent complex of sulfated cholecystokinin fragment and Dyn 1–17.

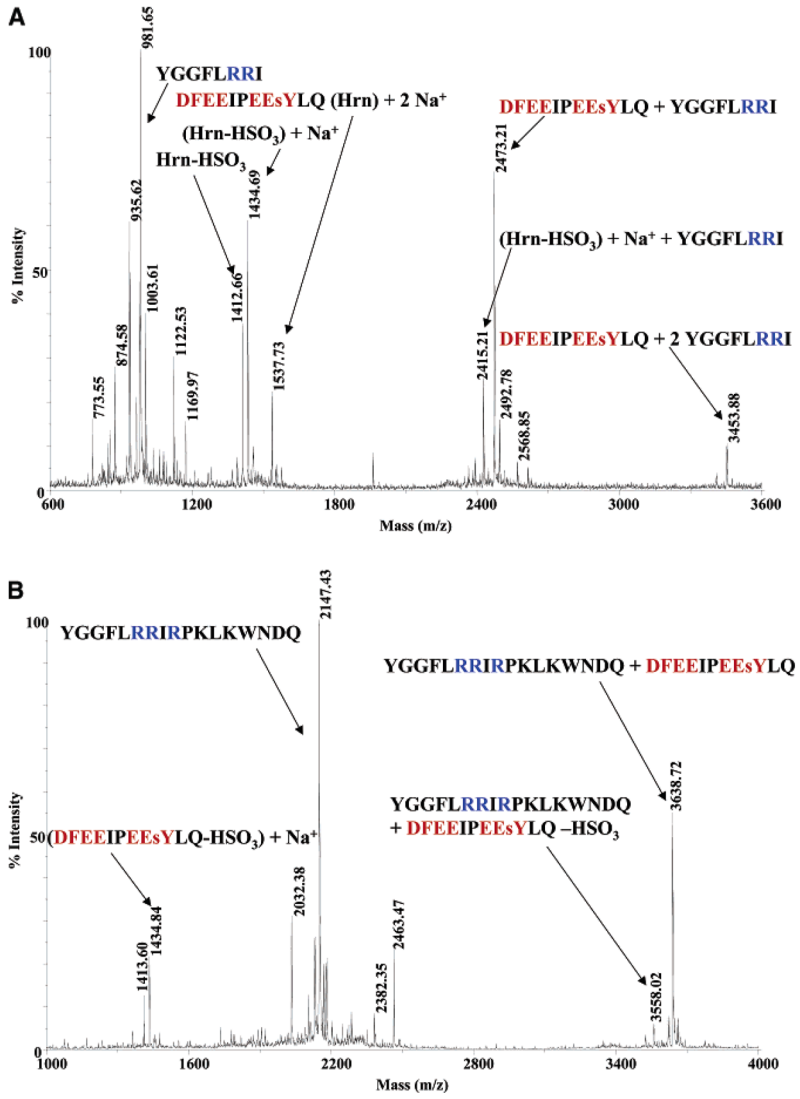

Figure 3.

(A) Spectrum showing a noncovalent complex of sulfated hirudin fragment and Dyn 1–8. (B) Spectrum showing a noncovalent complex of sulfated hirudin fragment and Dyn 1–17.

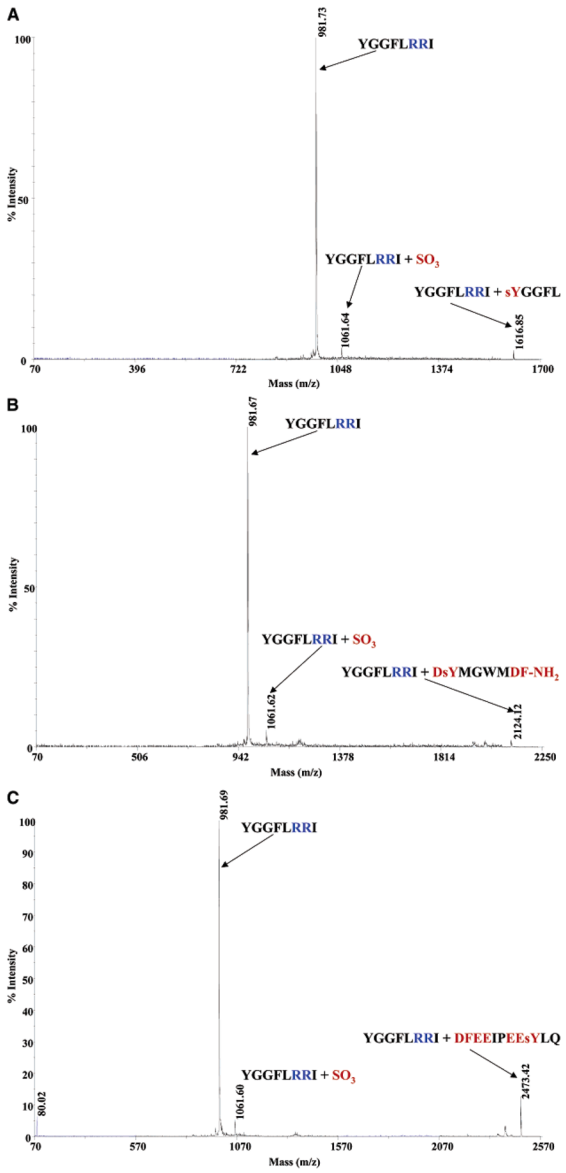

Figure 4.

MS/MS spectrum of the complexes of Dyn 1–8 and (A) sulfated leuenkephalin, (B) sulfated cholecystokinin fragment, (C) sulfated hirudin fragment. All spectra show that CID lead to a dissociation of the complex and the appearance of an additional peak at amu 1061 (Dyn 1–8 + SO_3), demonstrating that the noncovalent bond between the arginine guanidinium and the sulfate could be more stable than the covalent bond between the tyrosine oxygen and the sulfate S.

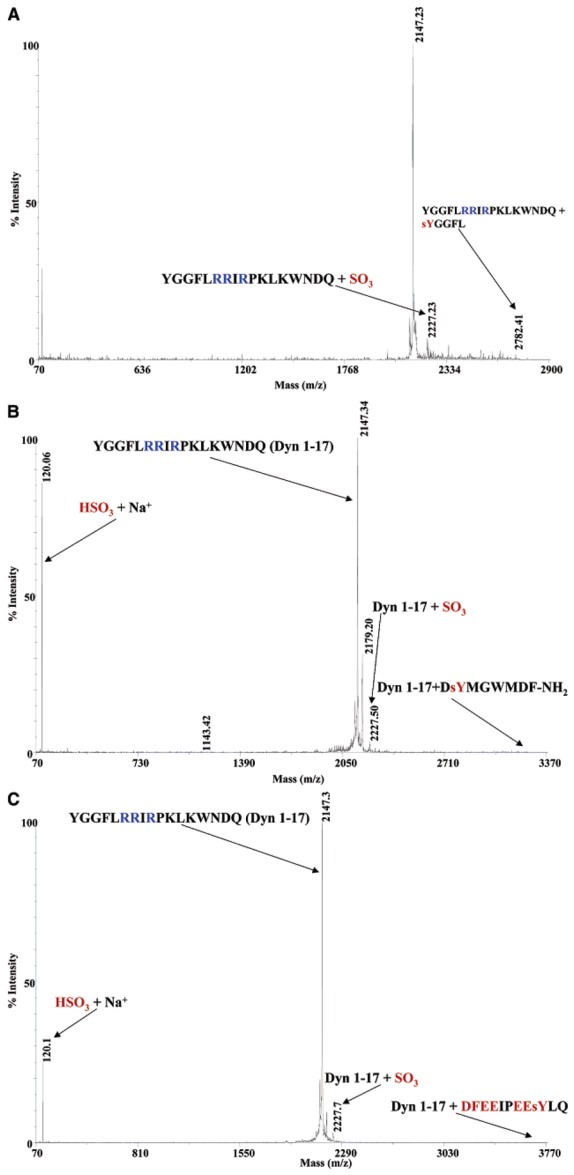

Figure 5.

MS/MS spectrum of the complexes of Dyn 1–17 and (A) sulfated leuenkephalin, (B) sulfated cholecystokinin fragment, (C) sulfated hirudin fragment. All spectra show that CID leads to a dissociation of the complex and the appearance of an additional peak at amu 2227 (Dyn 1–17 + SO_3), demonstrating that the noncovalent bond between the arginine guanidinium and the sulfate could be more stable than the covalent bond between the tyrosine oxygen and the sulfate S.