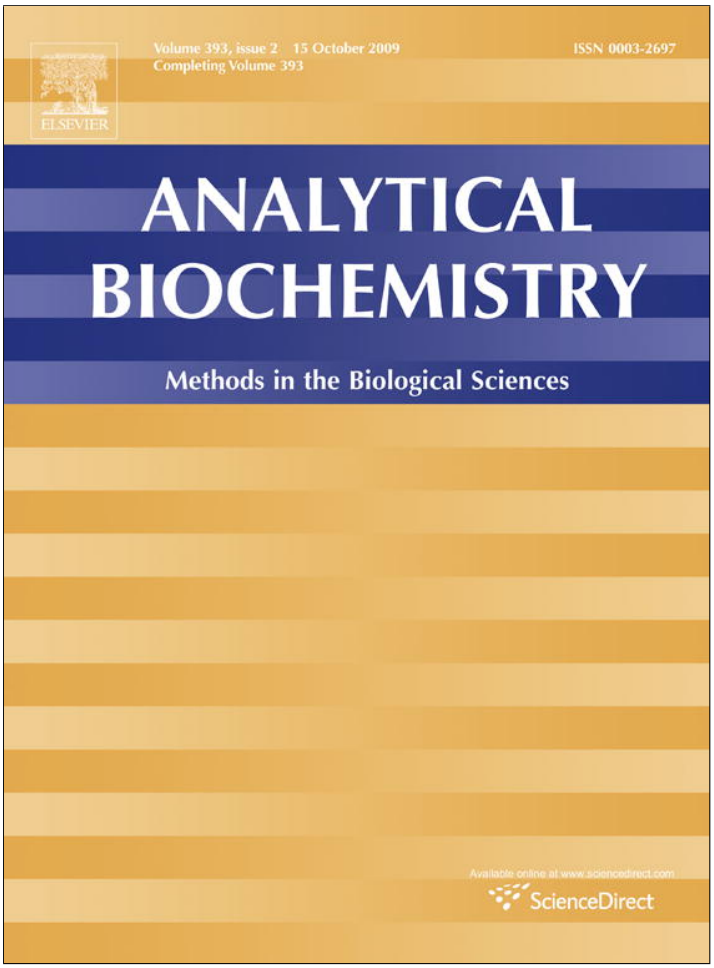

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Precursor ion scanning and sequencing of arginine-ADP-ribosylated peptide by mass spectrometry

Harumi Osago [a,*], Kazuo Yamada [a], Tomoko Shibata [b], Ken-ichi Yoshino [c,d], Nobumasa Hara [a], Mikako Tsuchiya [a]

[a] Department of Biochemistry, Shimane University, Faculty of Medicine, Izumo 693-8501, Japan
[b] Center for Integrated Research in Science, Shimane University, Faculty of Medicine, Izumo 693-8501, Japan
[c] Biosignal Research Center, Organization of Advanced Science and Technology, Kobe University, Kobe 657-8501, Japan
[d] Integrated Center for Mass Spectrometry, Graduate School of Medicine, Kobe University, Kobe 657-8501, Japan

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ABSTRACT

Arginine (Arg)-specific ADP-ribosylation is one of the posttranslational modifications of proteins and is thought to play an important role in reversibly regulating functions of the target proteins in eukaryotes. However, the physiological target protein has not been established. We examined the fragmentation pattern of both ADP-ribosyl-Arg (ADP-R-Arg) and Arg-ADP-ribosylated peptides by quadrupole tandem mass spectrometry and found a specific cleavage of ADP-R-Arg into *N*-(ADP-ribosyl)-carbodiimide (ADP-R-carbodiimide) and ornithine. Based on this specific fragmentation pattern, we successfully identified the modification site and sequence of Arg-ADP-ribosylated peptide using a two-step collision and showed that ADP-R-carbodiimide is an excellent marker ion for precursor ion scanning of Arg-ADP-ribosylated peptide. We propose that a combination of the precursor ion scanning with ADP-R-carbodiimide as a marker ion and two-step collision is useful in searching for physiological target proteins of Arg-ADP-ribosylation.

Arginine (Arg)[1]-specific ADP-ribosylation is a posttranslational modification of Arg residue of proteins with an ADP-ribose moiety of nicotinamide adenine dinucleotide (NAD) catalyzed by mono-ADP-ribosyltransferase (Art) (EC 2.4.2.31) [1]. The transferase, originally identified in the bacterial world, was also detected in eukaryotes and cloned from rabbit skeletal muscle [2], mouse lymphocytes [3], and chicken heterophils [4] as well as lung [5,6] and is classified into seven members (Art1–Art7) [1,5,7]. ADP-ribose attached to the guanidino group of Arg residue is reversibly removed by a ubiquitously distributed enzyme ADP-ribosylarginine hydrolase (AAH) (EC 3.2.2.19). The presence of both Art and AAH activities in eukaryotic cells implies that, as is the case with phosphorylation, the reversible protein Arg-ADP-ribosylation may act as a regulatory mechanism for the function of the target protein [8].

The physiological importance of Arg-ADP-ribosylation in animals, however, remains to be elucidated because physiological target proteins of the modification have not yet been identified. In eukaryotes, Arts have been reported to modify a number of proteins, including phosphorylase kinase [9], Ca^{2+}-ATPase [10], Mim-1 (Myb-induced myeloid protein-1) [11,12], actin [13], desmin [14], and integrins [15]; however, there is no direct evidence of their in vivo modification [16]. To detect endogenously ADP-ribosylated proteins, we previously developed a specific method using anti-ADP-R-Arg antibody (where ADP-R is ADP-ribose) combined with recombinant AAH enzyme and successfully detected possible target proteins in an Art-abundant tissue, rat skeletal muscle [17]. Thus, the next step to clarifying the significance of Arg-ADP-ribosylation in vivo is the identification of the target proteins and determination of the modified Arg residues in the proteins.

In the field of structural analysis of posttranslationally modified proteins, collision-induced fragmentation techniques of mass spectrometry (MS), such as tandem mass spectrometry (MS/MS) and MS^3, have been shown to have enormous potential, together with high accuracy and specificity, especially for identifying the modifications and determining the site and amino acid sequence of target proteins [18–23]. However, to apply this technique for identifying Arg-ADP-ribosylated proteins, basic knowledge about the MS/MS decay of ADP-R-Arg and practical procedures for selecting and identifying the Arg-ADP-ribosylated peptides are lacking. In this study, we describe for the first time the specific cleavage pattern

* Corresponding author. Fax: +81 853 20 2120.
E-mail address: biochem1@med.shimane-u.ac.jp (H. Osago).

[1] *Abbreviations used:* Arg, arginine; NAD, nicotinamide adenine dinucleotide; Art, ADP-ribosyltransferase; AAH, ADP-ribosylarginine hydrolase; Mim-1, Myb-induced myeloid protein-1; ADP-R, ADP-ribose; MS, mass spectrometry; MS/MS: tandem mass spectrometry; Lys-C, lysyl endopeptidase; RP-HPLC, reversed-phase high-performance liquid chromatography; UV, ultraviolet; LC, liquid chromatography; IS, ion spray voltage; DP, declustering potential; CE, collision energy; Orn, ornithine; FAB, fast atom bombardment.

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of Arg-ADP-ribosylation on MS/MS together with the procedures for identifying and specifically selecting Arg-ADP-ribosylated peptides, and we propose a combination of these procedures for detecting and sequencing Arg-ADP-ribosylated protein.

Materials and methods

Materials

Dithiothreitol, iodoacetic acid, lysyl endopeptidase (Lys-C), trifluoroacetic acid, ammonium formate, formic acid, acetonitrile, and methanol were purchased from Wako Pure Chemical Industries (Osaka, Japan). NAD was obtained from Oriental Yeast (Tokyo, Japan). ADP-R, Arg, and agmatine were obtained from Sigma (St. Louis, MO, USA). The peptides angiotensin I (DRVYIHPFHL), des-[Arg9]-bradykinin (RPPGFSPF), and [Arg8]-vasopressin (CYFQNCPRG) were designated as P1, P2, and P3, respectively. P1 was obtained from Sigma, and the other peptides were obtained from Peptide Institute (Osaka, Japan). A silica-based C_{18} column Atlantis dC_{18} (column size 2.1 × 150 mm, particle size 3 μm) was obtained from Millipore–Waters (Milford, MA, USA). Art (0.3 ng proteins/μl) and Mim-1 protein (2 mg/μl) were purified from chicken spleen according to the methods described previously [5,11].

Sample preparation

For preparing ADP-R-Arg or ADP-ribosyl agmatine, 100 mM Arg or agmatine was incubated for 3 h at 30 °C with purified Art enzyme (10 μl) and 5 mM NAD in 50 mM Tris–Cl (pH 9.0) in a total volume of 200 μl. For the modification of peptide, 100 μM P1, P2, or P3 was incubated with 1 mM NAD under the above-mentioned conditions. ADP-ribosylated materials were purified by reversed-phase high-performance liquid chromatography (RP-HPLC) (Gilson model 305 and 306 pumps, model 805 manometric module, and model 811B dynamic mixer) with ultraviolet (UV) monitoring at 254 and 215 nm on the C_{18} column with the mobile phases consisting of 0.1% trifluoroacetic acid in both water (solvent A) and acetonitrile (solvent B) at a flow rate of 0.2 ml/min. The percentage of solvent B was changed as follows: 0 min, 15%; 10 min, 30%; 35 min, 30%; 36 min, 15%; and 50 min, 15%. The fractions corresponding to the peak at 254 nm separated from that of NAD were collected. The ADP-ribosylated peptides P1, P2, and P3 were designated as AP1, AP2, and AP3, respectively. Mim-1 (200 μg) was reduced with 10 mM dithiothreitol in 8 M urea/40 mM sodium bicarbonate for 15 min at 50 °C in a total volume of 850 μl, alkylated with 50 mM iodoacetic acid for 30 min at room temperature in the dark, and then digested with 5 μg of Lys-C for 1 h at 37 °C in 100 μl of 25 mM sodium bicarbonate. The digest (~100 μM) was combined with the ADP-ribosylated peptides AP1, AP2, and AP3 (2–12 μM) to prepare the peptide mixture for precursor ion scanning.

Mass spectrometry

MS was performed on a quadrupole tandem mass spectrometer equipped with an electrospray ion source (Sciex API 3000, Applied Biosystems, Foster City, CA, USA). Liquid chromatography (LC) for LC/MS was performed on a C_{18} column by RP-HPLC (Shimadzu LC-10AD pumps, SIL-HTC autosampler, and DGU-14AM degasser) at a flow rate of 0.15 ml/min. For the analysis of ADP-R-Arg and ADP-ribosyl agmatine, the mobile phases consisted of 5 mM ammonium formate (solvent A) and methanol (solvent B). The percentage of solvent B was changed as follows: 0 min, 0%; 10 min, 70%; 15 min, 70%; 15.1 min, 0%; and 20 min, 0%. For the peptide analysis, the mobile phases consisted of 0.1% formic acid in water (solvent A) and acetonitrile (solvent B). The percentage of solvent B was changed as follows: 0 min, 15%; 0.5 min, 55%; 5.5 min, 55%; 5.6 min, 15%; and 10 min, 15%. Eluate was electrosprayed directly into the mass spectrometer. In the positive ion mode, the ion spray voltage (IS) and declustering potential (DP) were set to 3500 and 70 V, respectively. In the negative ion mode, the IS and DP were set to −3500 and −96 V, respectively. Nitrogen was used as the collision gas. The collision energy (CE) is provided in each figure legend. For precursor ion scanning, both Q1 and Q3 resolutions were set to low. For the pseudo-MS^3 experiment, only Q1 resolution was set to low.

Peptide sequence identification by Mascot

A Mascot (Matrix Science) MS/MS ion search program was performed for "other mammalia" against Swiss–Prot 57.0 database (428,650 sequences and 154,416,236 residues). The parameter "fixed modification" of amino acid was set to "Arg replaced to Orn" (where Orn is ornithine). The search was conducted using "semiTrypsin" for enzyme specificity, with a maximum of "one missed cleavage" allowed and peptide and MS/MS tolerances set at 1.2 and 0.6 Da, respectively.

Results

Identification of fragmentation pattern of Arg-ADP-ribosylation

First, we examined the fragmentation pattern of the simplest form of Arg-ADP-ribosylation, ADP-R-Arg, on MS/MS. In the negative ion mode (Fig. 1A), the major product ions were detected at *m*/*z* 346.2 (AMP), 367.3 (ADP-R-Arg–AMP), 426.2 (ADP), 498.5, 540.0 (ADP-R–H_2O), and 582.4. Although the ions with an *m*/*z* value smaller than that of ADP-R (*m*/*z* 558.3) are ADP-R moiety-specific products, any product ion with an *m*/*z* value larger than that of ADP-R must consist of both ADP-R- and Arg-derived moieties. Actually, the ion at *m*/*z* 582.4 was assigned as deprotonated ADP-R-carbodiimide. Because this ion seems to result from cleavage of the guanidino group of ADP-R-Arg, the residual of ADP-R-Arg, namely Orn, could also be detected as a product ion. As shown in Fig. 1B, the ion at *m*/*z* 133.1 consistent with protonated Orn was detected in the positive ion mode. The product ion at *m*/*z* 498.5 is considered to result from cleavage of the ribose ring. When another guanidino compound agmatine was ADP-ribosylated and subjected to MS/MS in the positive ion mode, the ADP-R-carbodiimide ion was again detected together with the protonated residual from agmatine at *m*/*z* 89.2 (data not shown). These results suggest that the cleavage of carbodiimide from the guanidino moiety is a common fragmentation pattern for the ADP-ribosylated guanidino compounds.

To see whether this specific fragmentation pattern can be extended to Arg-ADP-ribosylated peptides, ADP-ribosylated angiotensin I (AP1) was subjected to MS/MS. As shown in Fig. 2B, in the negative ion mode, deprotonated ADP-R-carbodiimide at *m*/*z* 582.4 was detected together with the ions of ADP-R-derived fragments (at *m*/*z* 346.2, 426.2, and 498.5), quite similar to those of ADP-R-Arg (Fig. 1A). By MS/MS of AP1 in the positive ion mode (Fig. 2C), the ion at *m*/*z* 627.8 (AP1–ADP-R-carbodiimide) was also detected with protonated ADP-R-carbodiimide, indicating that the carbodiimide-deprived peptide contains Orn instead of Arg in the peptide. To confirm this, *m*/*z* 627.8 ion observed in the precursor ion spectrum of AP1 shown in Fig. 2A was subjected to MS/MS in the positive ion mode (see also next section below). The spectrum of both *b*- and *y*-type product ion series showed that the Arg residue of the peptide is replaced with Orn (Fig. 2D). Thus, Arg-ADP-ribosylated peptide also fragmented into ADP-R-carbodiimide

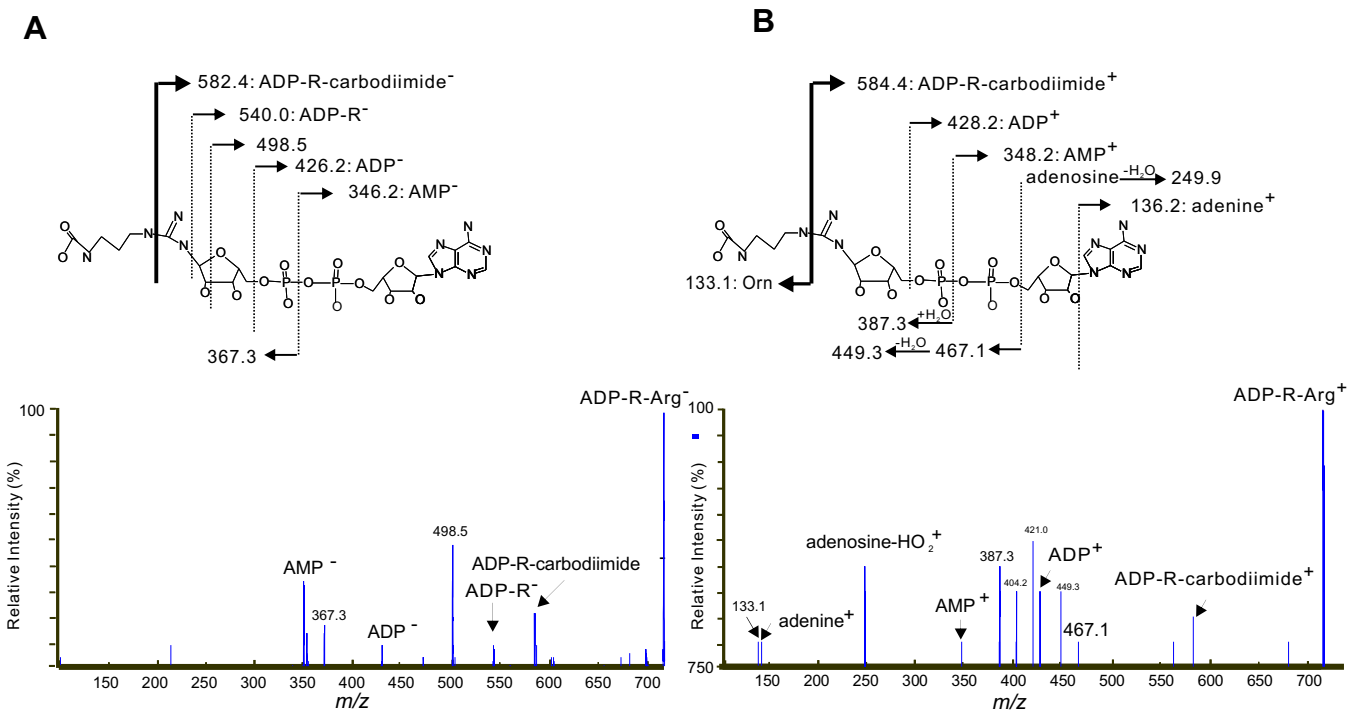

Fig. 1. Product ion spectra of ADP-R-Arg in the negative (A) and positive (B) ion modes with product ion assignments. CE was set at −35 and 30 V in (A) and (B), respectively.

and the residual peptide portion containing Orn. By MS/MS of the doubly charged protonated molecules of AP2 and AP3 (m/z 723.6 and 813.5, respectively), the ions of ADP-R-carbodiimide-deprived AP2 and AP3 (m/z 862.9 and 1043.0, respectively) were produced together with the protonated ADP-R-carbodiimide at m/z 584.4, again indicating the conversion of Arg to Orn. At least for AP2, MS/MS of doubly charged protonated ADP-R-carbodiimide-deprived AP2 at m/z 432.0 confirmed the conversion by detecting Orn instead of Arg. This conversion was never detected by MS/MS of the unmodified peptides P1, P2, and P3 (data not shown). These results indicate that the splitting into ADP-R-carbodiimide and Orn is a highly specific fragmentation pattern for Arg-ADP-ribosylation.

Determination of ADP-ribosylation site and amino acid sequence of Arg-ADP-ribosylated peptide

In general, the modified site and amino acid sequence of a possibly modified peptide can be determined by MS/MS. However, as described above, MS/MS of not the protonated molecule itself but rather the ion of ADP-R-carbodiimide-deprived peptide should be enough to determine the site and sequence of the peptide because the Orn residue itself represents the modification site. The nominal m/z (X) of the ions of ADP-R-carbodiimide-deprived peptide can be calculated as follows:

$$X = [n(a - 1.0) - 583.3 + m]/m,$$

where m is the charge of the ADP-R-carbodiimide-deprived peptide, 583.3 and 1.0 denote the nominal mass of ADP-R-carbodiimide and hydrogen, respectively, and a and n are the m/z and charge of the protonated molecule of the Arg-ADP-ribosylated peptide, respectively. As for AP1, the ion at m/z 627.8 (a = 919.4, m and n = 2) observed in the precursor ion spectrum of AP1 shown in Fig. 2A, probably generated by in-source decay [18], was subjected to MS/MS (Fig. 2D), and the peptide sequence [D-Orn-VYIHPFHL] was obtained. For AP2, the ion at m/z 432.0 (a = 723.6, m and n = 2) observed in the precursor ion spectrum of AP2 was subjected to MS/MS, and the peptide [Orn-PPGFSPF] was obtained (data not shown). The data set obtained by MS/MS of ADP-R-carbodiimide-deprived AP1 was enough to identify the original peptide as angiotensinogen by Mascot search.

However, on direct fragmentation of the protonated molecule itself at m/z 919.4 (doubly charged AP1), we were not able to determine the site or sequence because of the extreme complexity of the fragmentation pattern derived from the ADP-R moiety as well as the peptide portion in the product ion spectrum (Fig. 2C). As expected from the fragmentation pattern of ADP-R-Arg (Fig. 1B), during MS/MS of AP1, the ADP-R moiety did not remain intact but rather fragmented into multiple ions (m/z 136.2, 249.9, 348.2, and 428.2) together with their residual parts containing the peptide chain such as m/z 746.1 (AP1–AMP) and 795.3 (AP1–adenosine + H_2O). The breakdown of the peptide chain of these residues also occurred and again generated many different species in the peptide portion: m/z 737.7 (b_9 product ion of AP1–ADP) and m/z 745.5 (b_7 product ion of AP1–adenine). Similar results were obtained with MS/MS of AP2 and AP3 (data not shown). When the MS/MS data of the m/z 919.4 ion were analyzed by Mascot search, no peptide was identified.

These results indicate that MS/MS of the ADP-R-carbodiimide-deprived peptide, but not of the ADP-ribosylated peptide itself, by a two-step collision (in-source decay followed by MS/MS in this experiment) is not only sufficient but also necessary to determine the modification site and sequence of the modified peptide.

Selection of Arg-ADP-ribosylated peptides by precursor ion scanning

The ADP-R-carbodiimide may be a good marker for a precursor ion scanning to detect any Arg-ADP-ribosylated peptides in crude protein digest in searching for target proteins of ADP-ribosylation. Thus, we tried to specifically select ADP-ribosylated peptides (AP1, AP2, and AP3) mixed with Lys-C digest of Mim-1 by a precursor ion scanning with the ADP-R-carbodiimide ion as the marker ion. The

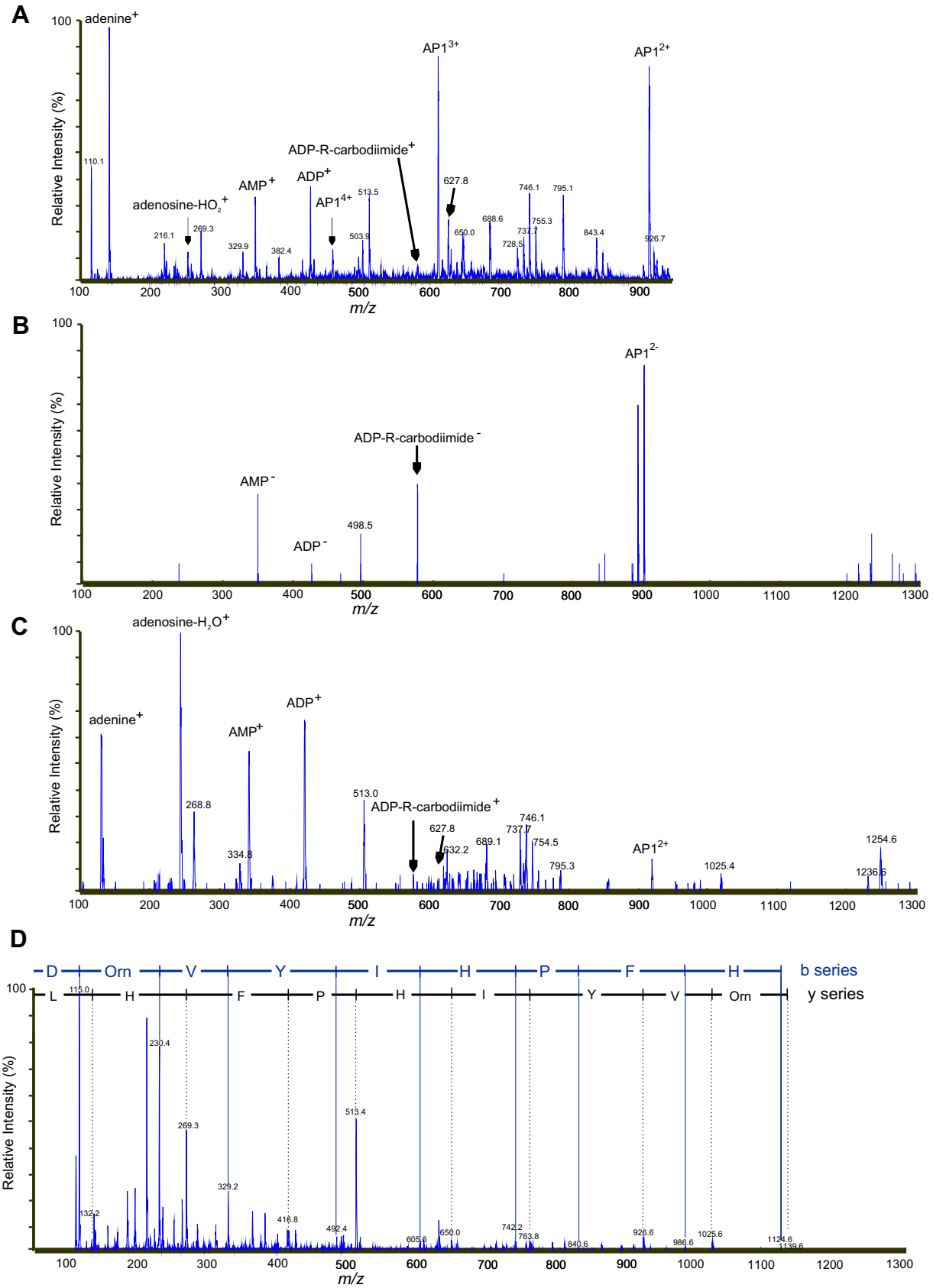

Fig. 2. MS/MS and peptide sequencing of AP1. (A) Precursor ion spectrum of AP1 in the positive ion mode. Product ion spectra of doubly charged AP1 shown in (A) were obtained in the negative (B) and positive (C) ion modes with CE set at −35 and 50 V, respectively. The peaks of differently charged molecules and the product ions are labeled. (D) Product ion spectrum of the doubly charged ADP-R-carbodiimide-deprived AP1 at *m*/*z* 627.8 in the precursor ion spectrum of AP1 shown in (A) in the positive ion mode. IS, DP, and CE were set to 4500, 100, and 40 V, respectively. The peptide amino acid sequence [D-Orn-VYIHPFHL] was determined from both *b*- and *y*-type product ion series.

precursor ion spectra of the mixture in the positive and negative ion modes are shown in Fig. 3A and B, respectively. Precursor ion scanning of the mixture for *m*/*z* 582.4 of deprotonated ADP-R-carbodiimide in the negative ion mode not only detected all ADP-ribosylated peptides (AP1, AP2, and AP3) but also greatly simplified the signals of the spectrum (Fig. 3C) compared with the precursor ion spectrum of the mixture in the negative ion mode (Fig. 3B). These results indicate the potency of precursor ion scanning for

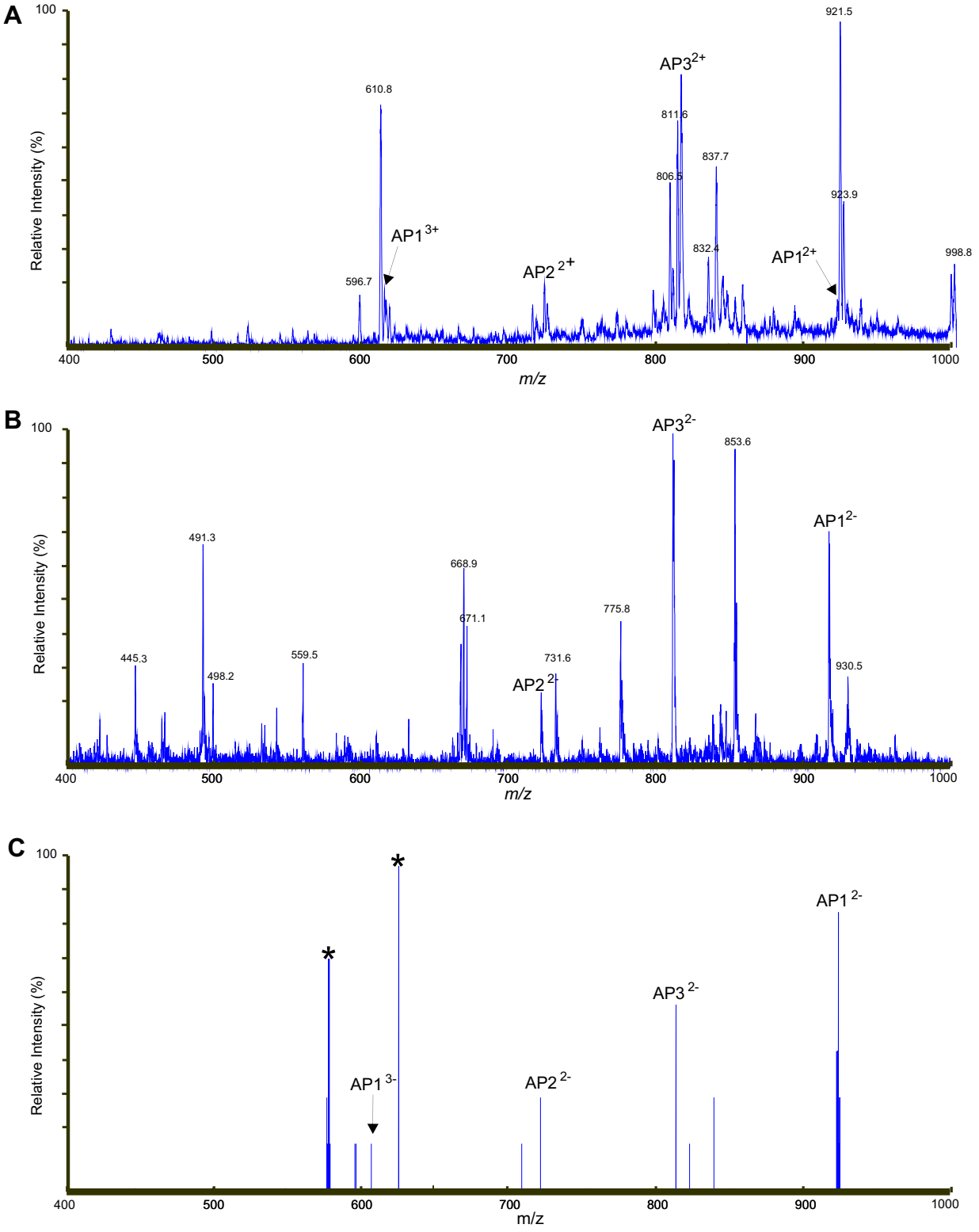

Fig. 3. Precursor ion scanning of protein digest containing Arg-ADP-ribosylated peptides. Arg-ADP-ribosylated peptides AP1, AP2, and AP3 were mixed with a Lys-C digest of Mim-1 protein. (A and B) Precursor ion spectra of the mixture in the positive (A) and negative (B) ion modes. (C) Precursor ion scanning of the mixture in the negative ion mode for *m*/*z* 582.4 with CE set at −35 V. The differently charged molecules are labeled. Asterisks represent nonspecific noise. All peptides were coeluted by a stepwise gradient. Total data acquisition times of these three spectra were identical.

ADP-R-carbodiimide in the negative ion mode for a specific selection of Arg-ADP-ribosylated peptides in crude peptide mixtures.

Discussion

In this study, we have described for the first time the specific cleavage pattern of Arg-ADP-ribosylation to generate ADP-R-carbodiimide and Orn using MS/MS. This finding supports the detection of ADP-R-carbodiimide, or imidocarbonylamine group linked to ADP-R, on fast atom bombardment (FAB)–MS of an ADP-ribosylated iron protein nitrogenase [24]. Other than in the previous study, all structural analyses of ADP-ribosylation by MS have only described a mass increment of 541.0 Da corresponding to the addition of ADP-R without further fragmentation [14,25,26] or have only determined the peptide sequence by MS/MS of the candidate protein selected with conventional methods such as affinity chromatography [27] or radiolabeling [28], both of which lack the absolute and direct evidence of the presence or site of ADP-ribosylation. Thus, procedures for determining the modification site and sequence of the modified peptide together with specific selection of the modified peptide should be developed. Here we were able to select Arg-ADP-ribosylated peptide, and directly identify and localize the modification of peptides, taking advantage of the specific cleavage pattern of the Arg-ADP-R adduct. The application of the procedure used in this study should be useful for identifying the target proteins of Arg-ADP-ribosylation.

We specifically selected the Arg-ADP-ribosylated peptide from the protein digest by precursor ion scanning in the negative ion mode with ADP-R-carbodiimide ion (m/z 582.4) used as the marker ion. This marker ion, containing both the modification group ADP-R and a part of the modified amino acid Arg, is able to determine the modification itself as well as the modified site. Precursor ion scanning techniques have been successfully used to detect other modifications of proteins such as phosphorylation (m/z 79.0) [19] and glycosylation (m/z 204.1) [20]. In terms of identifying amino acid-specific modifications, our marker ion (i.e., ADP-R-carbodiimide) is similar to the marker ion immonium at m/z 216.0, which is used to identify tyrosine-phosphorylated proteins [21].

Although the modification site and sequence of phosphopeptides can be determined by MS/MS [19,21], in the case of glycopeptide, which has a tendency for the modification group to be fragmented on the cleavage of the main peptide, determination of the modified site and sequence requires a two-step collision or fragmentation. The first step involves removal of the complex and labile modification group while leaving a small portion of the group to mark the modified amino acid, and the second step involves fragmentation of the peptide chain with the small marker moiety to localize the site and determine the amino acid sequence. For this purpose, MS^3 [22] or pseudo-MS^3 [23] has been used. Because the ADP-R moiety is also easily fragmented on MS or MS/MS, pseudo-MS^3 (as shown in the current study) or MS^3 is required to obtain the fragmentation pattern of the main peptide chain after the removal of the modification group (i.e., the ADP-R-carbodiimide moiety). To remove this moiety, the first collision can be performed by MS/MS using an ion trap mass spectrometer or may be archived by in-source decay with a tandem mass spectrometer.

A combination of the above-described methods, namely selection of candidate peptides by the precursor ion scanning with ADP-R-carbodiimide as the marker ion and further fragmentation of the ion of the ADP-R-carbodiimide-deprived peptide, would provide highly specific and reliable data applicable for MS/MS database searching of protein. Recently, sequencing ADP-ribosylated kemptide by electron capture dissociation, as well as selecting the modified peptide with AMP produced by collision-induced dissociation as a diagnostic ion, was reported [29]. We suggest that ADP-R-carbodiimide instead of AMP as a diagnostic ion would greatly improve the specificity of searching for Arg-ADP-ribosylated peptides.

In this study, we found the specific cleavage of ADP-R-Arg into ADP-R-carbodiimide and Orn by collision-induced dissociation. Using this feature, we developed the precursor ion scanning with ADP-R-carbodiimide as the marker ion and two-step collision for the selection and identification of Arg-ADP-ribosylated peptide, respectively. We propose that a combination of these two novel procedures would be greatly helpful to explore the target proteins for Arg-specific ADP-ribosylation in vivo and lead to a better understanding of the physiological significance of this modification in living cells.

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References

[1] I.J. Okazaki, J. Moss, Glycosylphosphatidylinositol-anchored and secretory isoforms of mono-ADP-ribosyltransferases, J. Biol. Chem. 273 (1998) 23617–23620.
[2] A. Zolkiewska, M.S. Nightingale, J. Moss, Molecular characterization of NAD:arginine ADP-ribosyltransferase from rabbit skeletal muscle, Proc. Natl. Acad. Sci. USA 89 (1992) 11352–11356.
[3] M. Seman, S. Adriouch, F. Scheuplein, C. Krebs, D. Freese, G. Glowacki, P. Deterre, F. Haag, F. Koch-Nolte, NAD-induced T cell death: ADP-ribosylation of cell surface proteins by ART2 activates the cytolytic P2X7 purinoceptor, Immunity 19 (2003) 571–582.
[4] M. Terashima, K. Mishima, K. Yamada, M. Tsuchiya, T. Wakutani, M. Shimoyama, ADP-ribosylation of actins by arginine-specific ADP-ribosyltransferase purified from chicken heterophils, Eur. J. Biochem. 204 (1992) 305–311.
[5] M. Terashima, H. Osago, N. Hara, Y. Tanigawa, M. Shimoyama, M. Tsuchiya, Purification, characterization, and molecular cloning of glycosylphosphatidylinositol-anchored arginine-specific ADP-ribosyltransferases in chicken, Biochem. J. 389 (2005) 853–861.
[6] M. Terashima, M. Takahashi, M. Shimoyama, Y. Tanigawa, Y. Urano, M. Tsuchiya, Glycosylphosphatidylinositol-anchored arginine-specific ADP-ribosyltransferase7.1 (Art7.1) on chicken B cells: the possible role of Art7 in B cell receptor signalling and proliferation, Mol. Cell Biochem. 320 (2009) 93–100.
[7] D. Corda, M. Di Girolamo, Functional aspects of protein mono-ADP-ribosylation, EMBO J. 22 (2003) 1953–1958.
[8] I.J. Okazaki, A. Zolkiewska, T. Takada, J. Moss, Characterization of mammalian ADP-ribosylation cycles, Biochimie 77 (1995) 319–325.
[9] M. Tsuchiya, Y. Tanigawa, T. Ushiroyama, R. Matsuura, M. Shimoyama, ADP-ribosylation of phosphorylase kinase and block of phosphate incorporation into the enzyme, Eur. J. Biochem. 147 (1985) 3–40.
[10] N. Hara, M. Tsuchiya, K. Mishima, Y. Tanigawa, M. Shimoyama, ADP-ribosylation of Ca^{2+}-dependent ATPase in vitro suppresses the enzyme activity, Biochem. Biophys. Res. Commun. 148 (1987) 989–994.
[11] K. Mishima, M. Terashima, S. Obara, K. Yamada, K. Imai, M. Shimoyama, Arginine-specific ADP-ribosyltransferase and its acceptor protein p33 in chicken polymorphonuclear cells: co-localization in the cell granules, partial characterization, and in situ mono(ADP-ribosyl)ation, J. Biochem. 110 (1991) 388–394.
[12] K. Yamada, M. Tsuchiya, K. Mishima, M. Shimoyama, p33, an endogenous target protein for arginine-specific ADP-ribosyltransferase in chicken polymorphonuclear leukocytes, is highly homologous to mim-1 protein (myb-induced myeloid protein-1), FEBS Lett. 311 (1992) 203–205.
[13] M. Terashima, C. Yamamori, M. Shimoyama, ADP-ribosylation of Arg28 and Arg206 on the actin molecule by chicken arginine-specific ADP-ribosyltransferase, Eur. J. Biochem. 231 (1995) 242–249.
[14] H. Zhou, T.W. Huiatt, R.M. Robson, S.W. Sernett, D.J. Graves, Characterization of ADP-ribosylation sites on desmin and restoration of desmin intermediate filament assembly by de-ADP-ribosylation, Arch. Biochem. Biophys. 334 (1996) 214–222.
[15] A. Zolkiewska, J. Moss, Integrin α7 as substrate for a glycosylphosphatidylinositol-anchored ADP-ribosyltransferase on the surface of skeletal muscle cell, J. Biol. Chem. 268 (1993) 25273–25276.
[16] M. Di Girolamo, N. Dani, A. Stilla, D. Corda, Physiological relevance of the endogenous mono(ADP-ribosyl)ation of cellular proteins, FEBS J. 272 (2005) 4565–4575.

[17] H. Osago, M. Terashima, N. Hara, K. Yamada, M. Tsuchiya, A new detection method for arginine-specific ADP-ribosylation of protein: a combinational use of anti-ADP-ribosylarginine antibody and ADP-ribosylarginine hydrolase, J. Biochem. Biophys. Methods 70 (2008) 1014–1019.
[18] W.D. Lehmann, Single series peptide fragment ion spectra generated by two-stage collision-induced dissociation in a triple quadrupole, J. Am. Soc. Mass Spectrom. 9 (1998) 606–611.
[19] S.A. Carr, M.J. Huddleston, R.S. Annan, Selective detection and sequencing of phosphopeptides at the femtomole level by mass spectrometry, Anal. Biochem. 239 (1996) 180–192.
[20] S.A. Carr, M.J. Huddleston, M.F. Bean, Selective identification and differentiation of N- and O-linked oligosaccharides in glycoproteins by liquid chromatography–mass spectrometry, Protein Sci. 2 (1993) 183–196.
[21] H. Steen, B. Kuster, M. Fernandez, A. Pandey, M. Mann, Detection of tyrosine phosphorylated peptides by precursor ion scanning quadrupole TOF mass spectrometry in positive ion mode, Anal. Chem. 73 (2001) 1440–1448.
[22] U.M. Demelbauer, M. Zehl, A. Plematl, G. Allmaier, A. Rizzi, Determination of glycopeptide structures by multistage mass spectrometry with low-energy collision-induced dissociation: comparison of electrospray ionization quadrupole ion trap and matrix-assisted laser desorption/ionization quadrupole ion trap reflectron time-of-flight approaches, Rapid Commun. Mass Spectrom. 18 (2004) 1575–1582.
[23] N. Takemori, N. Komori, H. Matsumoto, Highly sensitive multistage mass spectrometry enables small-scale analysis of protein glycosylation from two-dimensional polyacrylamide gels, Electrophoresis 27 (2006) 1394–1406.
[24] M.R. Pope, S.A. Murrell, P.W. Ludden, Covalent modification of the iron protein of nitrogenase from *Rhodospirillum rubrum* by adenosine diphosphoribosylation of a specific arginine residue, Proc. Natl. Acad. Sci. USA 82 (1985) 3173–3177.
[25] R.E. West, J. Moss, M. Vaughan, T. Liu, T.Y. Liu, Pertussis toxin-catalyzed ADP-ribosylation of transducin: cysteine 347 is the ADP-ribose acceptor site, J. Biol. Chem. 260 (1985) 14428–14430.
[26] G. Paone, A. Wada, L.A. Stevens, A. Matin, T. Hirayama, R.L. Levine, J. Moss, ADP ribosylation of human neutrophil peptide-1 regulates its biological properties, Proc. Natl. Acad. Sci. USA 99 (2002) 8231–8235.
[27] N. Dani, A. Stilla, A. Marchegiani, A. Tamburro, S. Till, A.G. Ladurner, D. Corda, M. Di Girolamo, Combining affinity purification by ADP-ribose-binding macro domains with mass spectrometry to define the mammalian ADP-ribosyl proteome, Proc. Natl. Acad. Sci. USA 106 (2009) 4243–4248.
[28] L.H. Coye, C.M. Collins, Identification of SpyA, a novel ADP-ribosyltransferase of *Streptococcus pyogenes*, Mol. Microbiol. 54 (2004) 89–98.
[29] S.M. Hengel, S.A. Shaffer, B.L. Nunn, D.R. Goodlett, Tandem mass spectrometry investigation of ADP-ribosylated kemptide, J. Am. Soc. Mass Spectrom. 20 (2009) 477–483.