To the Editor-in Chief,

Sir

Proposal for a Common Nomenclature for Peptide Fragment Ions Generated Following Sequence Scrambling During Collision-Induced Dissociation

Tandem mass spectrometry is often used in the analysis of peptides, generating sequence specific fragment ions. The characteristic fragment ions produced by a particular peptide ion can then be used to elucidate the original amino acid sequence. A nomenclature for the labelling of these fragments was originally devised by Roepstorff and Fohlman [1] and then later modified by Biemann [2]. Collision Induced Dissociation (CID) results in cleavage of the amide bond, producing either b- or y-ions, where the charge is retained on the amino or carboxy terminus respectively [3]. Subsequent loss of carbon monoxide from b-ions can also yield a corresponding a-ion series.

The structure of y-ions has been shown by the work of Mueller et al. [4] and Cordero et al. [5] to be that of a protonated amino acid (y₁) or a protonated truncated peptide (yn). However, b-ion dissociation products are often observed in a form that cannot be explained by simple bond cleavage. Work has been undertaken by various groups to provide a greater understanding of the mechanisms causing production of such species. Early work by Yalcin et al. [6] showed b-ions to consist of a linear peptide backbone terminating in a cyclic oxazolone ring. This ring structure is of particular interest as it hinders the formation of b₁ fragment ions under standard conditions. Later work by Vazquez et al. [7] showed formation of a macrocyclic intermediate from the initial oxazolone terminating b-ion, thought to be generated through attack of the N-terminal amine on the carbonyl carbon within the oxazolone ring. This macrocycle intermediate can undergo further fragmentation following ring opening at various positions, to give non-sequence product ions via rearrangement. The generation of these non-native sequence product ions (sequence scrambling) has been supported by the work of other groups [8-10] who have demonstrated that bN-ions (where N≥5) can form fully cyclic structures. Harrison [11] has shown that the abundance of non-sequence ions produced following macrocyclic ring opening increases with elevated CID collision energies. Interestingly, they demonstrated that peptide chain N-acetylation prevents such sequence scrambling, supporting the proposed mechanism of macrocycle formation. The presence of the acetyl group at the N-terminus is thought to prevent nucleophilic attack of the oxazolone ring, meaning macrocycle formation does not occur and subsequent rearrangement is avoided. Recent work by Maitre et al. [12] has demonstrated that a-ions can also undergo scrambling processes, whereas it was previously thought that such fragmentation pathways were exclusive to b-ions.

Elucidation of the mechanisms that govern scrambling of peptide ions [13-16] and the resultant consequences for large scale tandem mass spectrometry analyses [17-19] are research areas of growing interest. However, description of the origins of these non-native peptide sequence ions is fraught with confusion which is further complicated by the absence of a uniform approach toward naming the scrambled products. Consequently, we propose an extension to the currently used peptide fragmentation nomenclature (1, 2) so that fragment ions deriving from macrocycle formation can be easily assigned. The proposed nomenclature can be applied to the products of scrambled b-ions and enables both the fragment ion in question and the pathway from which it was derived to be assigned (Figure 1). The nomenclature can also be extended to the scrambling of a-
ions. Importantly, this notation makes no assumption regarding the precise mechanism of scrambling.

To facilitate use of the proposed nomenclature for scrambled product ions, the peptide bonds in the initial sequence are numbered in accordance with standard nomenclature. A square bracket is used to contain the series of fragmentation processes that result in formation of the initial scrambled product. Inside this bracket, the point at which initial fragmentation has taken place, and hence where attack of the N-terminal residue forms the macrocycle is specified, followed by the amide bond number at which macrocycle ring opening occurs. Finally, (outside of the square bracket) any ion resulting from further fragmentation of the scrambled product ion is assigned, again according to traditional nomenclature.

For example, using the peptide YAGFL that has been shown to exhibit sequence scrambling by Gaskell et al. [20], initial fragmentation to generate the b4 ion (YAGF) with the oxazolone structure is then followed by N-terminal attack of the oxazolone ring and macrocycle formation. If ring opening were subsequently to occur at bond 2 (giving the scrambled sequence GFYA), the process would be described inside the square bracket as [b22]. In the absence of further fragmentation, this scrambled product is isobaric with the initial b4 ion and thus the two ions cannot be distinguished. However, subsequent fragmentation of this scrambled product (either during MS/MS in a Q-TOF where the products of multiple dissociation events are often observed, or at the level of MS3 in an ion trap) will yield a sequence determinable scrambled fragment ion; in the example above, fragmentation of the scrambled product to generate a b3 ion (GFY) would yield a product ion with the nomenclature [b42]b3. Should scrambling arise from an a-ion, this can be described as [a22] if the precursor ion has been isolated prior to subsequent fragmentation. Alternatively, where there is ambiguity in the mechanism of final product formation, potentially arising either via b-ion rearrangement and subsequent a-ion formation or via an a-ion intermediate, this can be written as [b22]-28.

Further fragmentation/rearrangement events can be easily described by extension of this proposed nomenclature.

Sincerely,

Ross Chawner1, Simon J. Gaskell2 and Claire E. Eyers1

1Michael Barber Centre for Mass Spectrometry, Manchester Interdisciplinary Biocentre, School of Chemistry, University of Manchester, Manchester M1 7DN, UK. 2Queen Mary University of London, London, E1 4NS, UK

* Correspondence to: C. Eyers, Michael Barber Centre for Mass Spectrometry, Manchester Interdisciplinary Biocentre, School of Chemistry, University of Manchester, Manchester M1 7DN, UK (Claire.Eyers@manchester.ac.uk)
References


Figure 1. (A) The $b_5$ ion is generated by Collision Induced Dissociation. This is followed by formation of the oxazolone ring through attack of the oxygen lone pair on the newly formed terminal carbonyl group. (B) N-terminal nucleophilic attack of the oxazolone ring to form the macrocycle. (C) The resultant macrocyclic intermediate undergoes ring opening to give sequence scrambled $b_5$ ions. N.B. Numbering of the peptide bonds is performed for the initial sequence and the order maintained throughout the scrambling pathway. This numbering system enables not only the scrambled product but also the fragmentation pathway from which it was derived to be assigned. Bond 0 is that formed by N-terminal nucleophilic attack of the oxazolone ring. Ring opening at this position results in reformation of the original sequence.
Figure 1. (A) The b5 ion is generated by Collision Induced Dissociation. This is followed by formation of the oxazolone ring through attack of the oxygen lone pair on the newly formed terminal carbonyl group. (B) N-terminal nucleophilic attack of the oxazolone ring to form the macrocycle. (C) The resultant macrocyclic intermediate undergoes ring opening to give sequence scrambled b5 ions. N.B. Numbering of the peptide bonds is performed for the initial sequence and the order maintained throughout the scrambling pathway. This numbering system enables not only the scrambled product but also the fragmentation pathway from which it was derived to be assigned. Bond 0 is that formed by N-terminal nucleophilic attack of the oxazolone ring. Ring opening at this position results in reformation of the original sequence.

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