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INFLUENCE OF BASIC RESIDUES ON THE C-TERMINAL REARRANGEMENT OF PEPTIDES IN GAS PHASE

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INTRODUCTION

The combination of mass spectrometry and soft ionization techniques have demonstrated its usefulness in protein sequencing due to its capability to handle complex mixture and sequence unknown and chemically modified peptides.

The interpretation of the Collision Activated Decomposition (CAD) spectra often tend to be complex, specially when intrinsic interactions regulate the fragmentation of peptides in gaseous phase. When basic amino acids are located within the peptide sequence or at the N-terminus, due to the C-terminal rearrangement signals appear in the CAD spectra originated by the interaction of the C-terminal hydroxyl group with the carbonyl group of the nearest amino acid (2). The peaks corresponding to this rearrangement has 18 mass units more than its correspondent b_n serie and sometimes they are the most intense daughter ion in the spectrum such that specific sequences at the C-terminus could be misassigned.

We designed and synthesized a set of peptides in order to study some factors that could affect the appearance of this fragmentation such as the position of the basic residue within the sequence, the nature of the amino acids involved in the rearrangement and the way in wich the different basicity of two isobaric amino acids such as lysine and glutamine could be helpful to differentiate them taking into account the intensities ratio of $b_{n-1}/b_{n-1}+18$.

MATERIALS AND METHODS

Mass spectra were obtained with a JEOL JMS-HX110HF two sector mass spectrometer operated with a JEOL JMA DA-5000 data system, ionization with a 4 KV Xenon beam produced positive ions that were analyzed at 10 kV accelerating voltage and resolution 1000. In the B/E linked-scan measurement Argon was used as a collision gas to decrease the intensity of the precursor ion to 50%.

All synthetic peptides were obtained using the multiple solid phase peptide synthesis. Dried and protected peptide-resin were treated with HF containing the appropriate scavenger mixture according to the **Low-High** procedure.

RESULTS AND DISCUSSIONS

The CAD spectra of peptides labeled with ^{18}O at their C-terminus shows signals with different isotopic ion distribution (3). The C-terminal ions show doublet signals, the N-terminal ions their natural isotopic distribution and the rearrangement ions a distribution different form the two pools previously mentioned allowing us an easier identification of this phenomenon and thus avoiding misassignments of the sequence.

Two set of pentapeptides were synthesized, one of them has a common amino acid at the C-terminus (Phe) and the unique change is at the penultimate amino acid, in the other set the penultimate amino acid was fixed (Ala) and the C-terminal was changed.

To facilitate the comparison between peptides we evaluated the intensity ratio b_4+18/b_4 because it shows the probability for both fragmentation to be formed. In the first set of peptides this parameter was similar for all peptides suggesting that the nature and the bulkiness of the side chain of the amino acid involved in the rearrangement do not have appreciable influences in this phenomenon with the exception of Pro as judge by the

highest value of b_4+18/b_4. In the other set we observed light differences between peptides except when Lys and Arg are the amino acids lost during rearrangement wich suggest that the stability of the neutral molecules formed for this two amino acids are superior than the other.

We also synthesized five pentapeptides with the same amino acid composition, but the argine residue was located at different positions along the sequence and the results showed that this amino acid facilitated the rearrangement when it was located at position n-1.

Our results confirmed that this phenomenon is strongly influenced by the basicity of the amino acids within the sequence. In the CAD spectra of a peptide with the sequence KGIEF the ions of b_4+18 and b_4 has similar intensities, although for the peptide with Gln instead of Lys the rearrangement was almost insignificant. It was found that the appearance of this fragmentation allow to differentiate this two isobaric amino acids.

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ALGUNAS CARACTERISTICAS DE STICHOLYSINA, UNA NUEVA CITOLISINA DE *Stichodactyla helianthus*

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INTRODUCCION

Sticholysina es un polipéptido básico (pI = 9,8), purificado de la anémona *Stichodactyla helianthus* de alrededor 18 kDa de masa molecular. Se caracteriza por una potente actividad hemolítica (H_2C_{50}= 25-30 ng/mL) la cual es activada (Ca^{2+}, Mg^{2+}) o inhibida (Co^{2+}, Mn^{2+}) por diferentes iones divalentes y presenta una moderada actividad fosfolipásica y actividad anticoagulante (1, 2, 3). Otro factor que modula la actividad hemolítica (AH) es el pH del medio.

A partir de los estudios de pH se pudo demostrar que la AH no depende de manera significativa, de su actividad fosfolipásica (4). Resultados preliminares habían sugerido que el incremento de la fuerza iónica del medio provocaba un aumento de la AH (1). En el presente trabajo se muestran otras características moleculares y funcionales de esta citolisina y el estudio de la influencia de la fuerza iónica sobre el mecanismo hemolítico de Sticholysina.

MATERIALES Y METODOS

La AH fue estimada como ha sido previamente informada (1). El perfil de hidropatía se trazó a partir de los estimados de Kyte y Doolittle (5). El curso temporal de la hemólisis fue determinado según (6), a partir de la caída de la turbidez de una suspensión eritrocitaria.

RESULTADOS Y DISCUSION

A partir de la secuencia aminoacídica de Sticholysina (8) se procedió a realizar el perfil de hidropatía del polipéptido. El análisis del perfil de hidropatía de la citolisina revela la presencia de una zona de Indice medio de hidrofobicidad positiva extendida a partir del aminoterminal, la cual se encuentra seguida de zonas alternas negativas y positivas para concluir en una zona negativa relativamente importante cercana al carboxilo terminal. Estos resultados indican la posible importancia de la regiòn aminoterminal de la proteína en su inserción a la membrana, lo cual permitiría justificar molecularmente su elevada capacidad membranotrópica.

Se determinaron los parámetros cinéticos que caracterizan la actividad fosfolipásica de este polipéptido (Km = 19 mM; Vm = 0 .06 mU/mg y Kcat = 1.1 min^{-1}).

Por otra parte, en un sistema eritrocitos-liposomas se comprobó la capacidad fusogénica de esta citolisina mediante la incorporación de la radioactividad asociada a los liposomas al sistema eritrocitario.

La fuerza iónica del medio provocó la agregación de Sticholysina en solución en estructuras oligoméricas de mayor efectividad hemolítica. Tal agregación tiene lugar de manera inmediata. Este proceso se favorece con la