temperatura, al menos hasta 37°C, lo cual apoya la hipótesis de que la formación del oligómero está mediada por interacciones hidrofóbicas. La preincubación de Sticholysina en un medio desprovisto de sales origina la pérdida de su AH y ésta se recupera por la adición de fuerza iónica al medio de incubación.

Estos resultados aportan nuevas evidencias sobre el mecanismo de acción de Sticholysina, en particular, sobre el importante papel de la fuerza iónica del medio sobre su actividad biológica, lo cual pudiera estar relacionado con los mecanismos de regulación de su actividad en el entorno actual en que ejerce su función.

REFERENCIAS

1. TEJUCA, M. *et al.* (1994). *Biología* **8 (1)**.
2. VEITÍA, R. et al. (1994). *Biología* (en prensa).
3. DÍAZ, A. *et al*. (1992). *Rev. Iberoamer Tromb Homeost.* **5: 8-11**
4. TEJUCA *et al* . (1994) *Biología* (en prensa).
5. KYTE, J. and R. F.DOOLITTLE (1982) *J. Mol. Biol.* **157**:105-132
6. HARSHAMAN, S. and N. SUGG (1985) *Infect and Immunity* **25**:37-40.
7. KEM, W. and B. DUNN (1988). *Toxicon* **26 (11)** 997-1008
8. MORERA, V. *et al.* (1994). *Biotecnología Habana 94. Libro de Resúmenes.*

PRIMARY STRUCTURE ANALYSIS OF THE HAEMOLYTIC POLYPEPTIDE Sticholysin ISOLATED FROM A SEA ANEMONE

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INTRODUCTION

Sticholysin (STIC), a new discovered polypeptide isolated from the caribean sea anemone *Stichodactyla helianthus* has been shown to have a potent haemolytic activity (HC_{50}=25-30 ng/mL) and moderate fosfolipase A_2 activity (15-20 mU/mg). Here we report the verification of 95% of the primary structure obtained by automated Edman degradation, amino acid analysis and mass spectrometry. Sequence alignment with protein databases revealed very high homology with other natural proteins.

MATERIALS AND METHODS

The last purification step of the protein was HPLC in a reversed phase (rp) column C8 using an acetonitrile gradient (in 0.1% TFA).

Peptides from trypsin digestion were isolated by using a rp-C18 column. Amino acid analysis of the hydrolyzed protein was performed by the automatic analyzer Alpha Plus 4151 (LKB, Sweden). Protein was previously oxidized by performic acid. Intact protein and isolated peptides were sequenced in a dual-phase automatic sequencer 810 (KNAUER, Germany), released amino acids were detected on line by rp-HPLC. Fast atom bombardment (FAB) mass spectra of the tryptic peptides were obtained in a double sector mass spectrometer HX-110HF (JEOL, Japan) at 10 kV accelerating voltage. Electrospray (ESI) mass spectrum was adquired in the first stage of a tandem HX-110HF spectrometer. Similarity between sequences were found by searching in SWISSPROT database with FASTA program. CLUSTALV program was used for multiple sequence alignments.

RESULTS AND DISCUSSION

The protein was finally obtained with high purity by rp-HPLC and then verified by SDS-PAGE, migrating as a single band close to the 18 kDa molecular weight marker. Amino acid analysis of the totally hydrolyzed protein was performed after performic acidic oxidation of cysteines and methionines. Cysteic acid was not found and therefore our protein is cystein free. The calculated molecular mass considering the amino acid analysis and excluding triptofan and proline was 17 899.0 and the experimental molecular mass obtained by ESI mass spectrometry of the intact protein was 19 401 ± 11 Da.

Amino terminal sequencing of the purified protein exhibits exactly the same amino acids per cycles (up to cycle 29) than the already reported Cytolysin-III, which

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EQU2  SADVAGAVIDGASLSFDILKTVLEALGNVKRKIAVGVDNESGKTWTALNTYFRSGTSDIV
CYT3  A--LAGTIIAGASLTFQVLDKVLEELGKVSR----------------------SGTTDVI
STIC  A--LAGTIIAGASLTFQVLDKVLEELGKVSRKIAVGTDNESGGTWTALNAYFRSGTTDVI
      .--.**..*-****.*..*--***-**.*-*----------------------***.*..
EQU2  LPHKVPHGKALLYNGQKDRGPVATGAVGVLAYLMSDGNTLAVLFSVPYDYNWYSNWWNVR
CYT3  LPEFVPNTKALLYSGRKDTGPVATGAVAAFQYYMSSGNTLGVMFSVPFDYNWYSNWWDVK
STIC  LPEFVPNTKALLYSGRKDTGPVATGAVAAFQYYMSSGNTLGVMFSVPFDYNWYSNWWDVK
      **.-**.-*****.*.**-********.-.-*-**-****.*.****.*********.*.
EQU2  IYKGKRRADQRMYEELYYNLSPFRGDNG-WHTRNLGYGLKSRGFMNSSGHAILEIHVSKA
CYT3  IYSGKRRADQGMYEDLYYG-NPYRGDNGHWE-KNLGYGLRMKGIMTSAGEAKMQIKISR-
STIC  IYSGKRRADQGMYEDLYYG-NPYRGDNGHWE-KNLGYGLRMKGIMTSAGEAKMQIKISR-
      *********-***.***-.*.*****-*.-.******.-.*.*-*.*.*-..*-.*.
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has also been isolated from *St. helianthus* (1) and with similar biological function. Unexpectedly, from cycle 30 to 51 amino acids detected did not match at all with Cytolysin-III, but coincide again after cycle 51 to 67.

In order to verify the primary structure the protein was digested with trypsin. Isolated peptides were sequenced and their mass were confirmed by FAB mass spectrometry. 95% of the protein sequence was verified and 87% coincides with Cytolysin-III (CYT3). On the other hand, sequence alignment with protein database also revealed more than 65% homology with Equinatoxin-II (EQU2) isolated from the australian sea anemone *Actinia tenebrosa* and the european *Actinia equina,* which also contains the 22 amino acids insertion fragment.

The amino acid composition match very well with the theoretical composition considering also complete similarity with Cytolysin-III in the still non-verified 5% of the molecule sequence, however the experimental molecular mass differ from that calculated (19341 Da) thus we suppose the existance of minor differences within the non-verified amino acid sequences.

REFERENCES

BLUMENTHAL, K. M. and W. R. KEM (1983). *J. Biol. Chem.* **258** (9): 5574-5580.

A PROGRAM TO AID THE INTERPRETATION OF THE MS/MS SPECTRA OF PEPTIDES

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INTRODUCTION

The combination of Collision Activated Decomposition (CAD) and linked-scan measurement has demonstrated its utility for sequencing unknown and chemically modified peptides. When a peptide pass through the collision cell yield many daughter ions that can be pooled in two groups, the N- and C-terminal ions.

Both ion series contain complementary information on the peptide sequence, the N-terminal ions (**a,b,c"**) provide us the sequence from the N- to the C-terminus and the C-terminal ions (**x, y", z**) from the C- to the N-terminus. In the manual interpretation of the CAD spectra it is not obvious the assignment of the daughter ions as an **N-** or **C-**terminal ion unless the peptide be partially labeled with ^{18}O at their C-terminus (1,2). Even so, for an skillful specialist the interpretation of a complicated CAD spectra often takes long time and the results can be ambiguosly assigned to several sequences.

Various computer algorithms has been described in order to aid the peptide sequencing but few of them behave well for peptides with molecular weight over 1 000. Our algorithm based on the graphos theory (3)