Review of
Solution Structure of the Pore-Forming
Protein of *Entamoeba histolytica*

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*O. Hecht, et.al., 2004, J. Biol. Chem. 279, 17834-17841*

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*Entamoeba histolytica* and Amoebiasis

- *E. histolytica* is a protozoan parasite
- Responsible for Amoebiasis (intestinal and extraintestinal infections)
- Second (after malaria) cause of death due to parasitic disease
- 1 in 10 people become sick from an infection
- 50 million people worldwide suffer from infection
- 100,000 people per year die as a result

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http://www.biosci.ohio-state.edu/~parasite/ehistolytica.html
http://www.dpd.cdc.gov/dpdx/HTML/Amebiasis.htm & WHO Weekly Epidemiological Record
Biochemistry of Amoebiasis

- Many factors are involved in expressing Amoebiasis
- Membrane polypeptides (Amoebapore A,B,C) form pores in cell membrane and thereby destroy host cells
- When Amoebapore A is disactivated, E. histolytica stops causing disease

http://sun.menloschool.org/~cweaver/cells/c/cell_membrane/

Scope of the Paper

- Isolate pore-forming protein Amoebapore A
- Determine protein structure (NMR spectroscopy, etc.)
- Understand pore-forming mechanism
Outline

- Medical importance of *Entamoeba histolytica* and Amoebapore A
- Structure determination using NMR
- Other useful techniques
  - Circular Dichroism Spectroscopy
  - Size Exclusion Chromatography
- Pore-forming activity of Amoebapore A
  - pH
  - Dimerization
  - Histidine residue
- Pore-formation mechanism
- Summary

NMR Spectroscopy

- Purified protein is obtained frozen
- NMR spectroscopy
- Dilute protein in 93% H₂O, 7% D₂O solution
  - 2D TOCSY
  - NOESY
  - Natural abundance HSQC
- Obtain total 1570 distance restraints
**NOE Short/Medium Range Limits**

- 20 calculated versions of the protein
- Rms deviations for backbone atoms in helices around 0.25 Angstroms
- 75% of backbone angles are in ‘most favored’ region of a Ramachandran plot, 0% in disallowed regions

**Structure of Amoebapore A – 1/2**
Structure of Amoebapore A – 2/2

- 77 residues in 5 $\alpha$-helices (I, II, III, IV, V)
- Single disulfide bridge between helices II and III
- Double disulfide bridges between helices I and V
- Helices I and II connected by 8 residue loop
- Helices II and II connected by 7 residue loop
- Helices III, IV, and V virtually merge

Electrostatic Potentials

- Electrostatic potentials of Amoebapore A, NK-Lysin, and Granulysin (Red = negative, Blue = positive potential)
- Amoebapore A is largely hydrophobic, NK-Lysin and Granulysin are hydrophilic
- Pore-forming mechanisms are different

CD Spectroscopy – 1/2

- Most biological molecules are optically active
- Plane polarized light = right + left handed polarizations
- Optically active medium preferentially absorbs either left or right handed polarizations, giving elliptical polarization
- Circular Dichroism spectroscopy measures ellipticity


CD Spectroscopy – 2/2

- Circular Dichroism Spectroscopy is a quick, low-resolution technique for determining protein structure
- CD spectroscopy is sensitive to structural changes in secondary structure

Size Exclusion Chromatography

- Also called gel-permeation/filtration chromatography
- Solution is passed through a porous filtration column
- Large molecules take a short time to pass
- Small molecules take a long time to pass


Fun Calculation

- Assume mass of Amoebapore A is 7.7kg/mol
- Assume diameter of *Entamoeba histolytica* is 20µm
- What do you get if you put $5.3 \times 10^{44}$ molecules of Amoebapore A in a cell?
a Black Hole!

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Pore-forming activity and pH

- Measure pore-forming activity in various pH solutions
- Amoebapore A is most active at pH 5.2
- Activity decreases with higher pH
- Other saposin-like proteins are active over broad range of pH

Hypothesis 1: pH changes protein conformation


pH and CD Spectroscopy

Circular Dichroism (CD) spectroscopy is sensitive to structural changes in secondary structure

- CD spectra of Amoebapore A look similar at all pH
  - pH 3 (solid line)
  - pH 4 (dotted line)
  - pH 5, 6, 7
- Structure of protein does not change with pH

Hypothesis 2: pH changes ’protein size’
**pH and Size Exclusion Chrom.**

- Elution time depends on pH
  - pH 3.5 (black)
  - pH 5.2 (green)
  - pH 8.0 (red)
  - Ignore blue
- Protein exists as monomer at pH 3.5, 8.0
- Protein forms dimers at pH 5.2

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**Dimerization**

- High pore-forming activity is linked with dimerization
- Possibility that dimerization is a prerequisite for pore formation
- What causes dimerization?
- Authors exclude electrostatic interactions
- Instead, they examine role of Histidine residue

Histidine, Wikipedia Image
Histidine Residue – 1/2

- Single Histidine residue in Amoebapore A is exposed to the solvent (no long range NOE were observed)
- Histidine side chain has a lot of freedom
- Modification of this residue with DEPC stops pore-forming activity
- Modification of this residue also stops oligomerization

Histidine Residue – 2/2

- Compare structures of Amoebapore A with and without DEPC treatment using CD spectroscopy
- Removal of Histidine residue does not change large scale structure of protein
Dimer Orientation

- In the antiparallel setup, hydrophobic areas point in the same direction (outward)
- There is an attractive electrostatic interaction between the two molecules
- Hydrophobic areas can interact with the hydrophobic environment of cell membranes

Barrel Stave Model

- Amoebapore A forms heximers and higher oligomers in solution
- Pores are hydrophobic outside and hydrophilic inside
- Modelled pore has inner diameter of 2nm, compared with measured diameters between 1.3nm and 2.2 nm
- Barrell Stave Model of pore formation suits the properties of Amoebapore A

Summary

- Studied pore-forming protein of *Entamoeba histolytica*, cause of Amoebiasis
- Obtained high resolution maps of Amoebapore A structure
- Tracked protein in various pH environments
- Linked pore-forming activity with dimerization
- Identified Histidine Residue as source of dimerization
- Proposed and defended a mechanism for pore-formation