Study of structural and functional proteins in the sea anemone *Actinia fragacea* (Cnidaria) and potential biomedical interest

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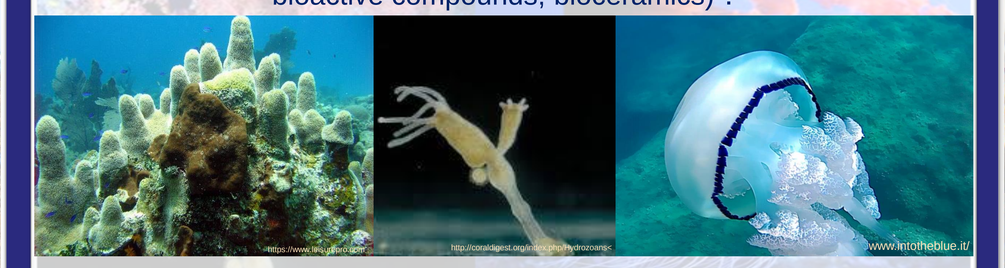
INTRODUCTION

Marine invertebrates provide a diverse source of proteins with several applications due to their broad structural and biological properties[1].

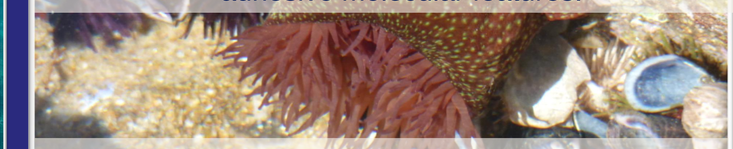
Cnidarians' basic features, ecology and diversity make them interesting models in different biotechnological fields (e.g. regenerative capacity; bioactive compounds; bioceramics)[2].



Other biotechnology interests emerged in the biomedical field, including collagens from jellyfish and adhesives proteins of hydrozoans[3,4].

AIM

✓ Perform molecular biology, proteomic tools and other protein characterization techniques in *Actinia fragacea* to analyse the collagen and protein adhesive molecular features.



✓ Provide information to the biomedical field, with focus on the development of biomaterials for tissue engineering, wound healing and drug delivery.

MATERIAL AND METHODS

Collagen acidic extraction[3]

Collection: Praia do Aterro, NW Portugal

Washing and cutting (Water) → Alkaline pre-treatment (0.1 M NaOH; Water pH 7.0) → Extraction (0.5 M Acetic acid) → Precipitation (2.6 M NaCl solution in 0.05M Tris-HCl (pH 7.5)) → Re-precipitation (0.7 M NaCl) → Freeze-drying

All phases were performed at 4 °C to avoid collagen denaturation.

Adhesive protein extraction from the pedal disk[2]

Washing and cutting (Water) → Freeze-drying → Extraction of soluble protein fraction (Buffer: 40nM Tris-HCl, 5mM $MgCl_2$ 1mM DTT, protease inhibitors, at pH 8.0; Vortex; Centrifugation 16,000g, 20 min, 4ºC; Pellet: Soluble fraction) → Extraction of insoluble protein fraction (Buffer: 7M urea, 2M thiourea, 4% CHAPS (w/v), 65 mM DTT, 0.8% ampholytes (v/v), at pH 4–7; Vortex; Incubation overnight, 4ºC; Centrifugation16,000g, 20 min, 4ºC)

Characterization

- ✓ Extraction yield: dry mass of the extract/initial wet mass
- ✓ SIRCOL assay
- ✓ Bradford Assay
- ✓ SDS-PAGE
- ✓ Fourier Transform Infrared Spectroscopy (FTIR)
- ✓ Circular Dichroism (CD)
- ✓ Aminoacid analysis
- ✓ Mass Spectrometry
- ✓ BLAST

PRELIMINARY RESULTS: COLLAGEN CHARACTERIZATION

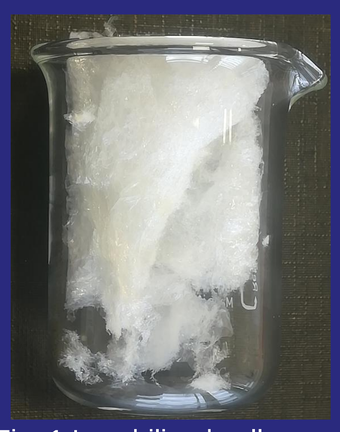


Fig. 1 Lyophilized collagen

Extraction yield: < 1%

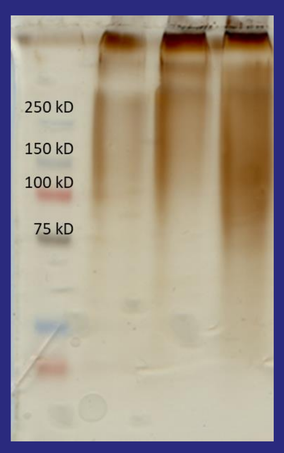


Fig. 3 Electrophoresis analysis of collagen of *A. fragacea.*

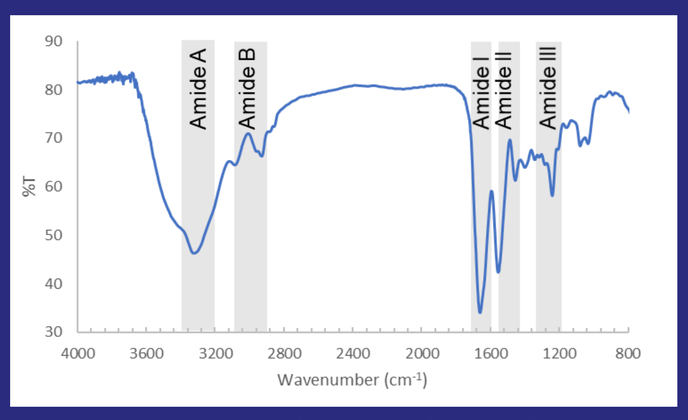


Fig. 2 FTIR spectra of collagen from *A. fragacea*.

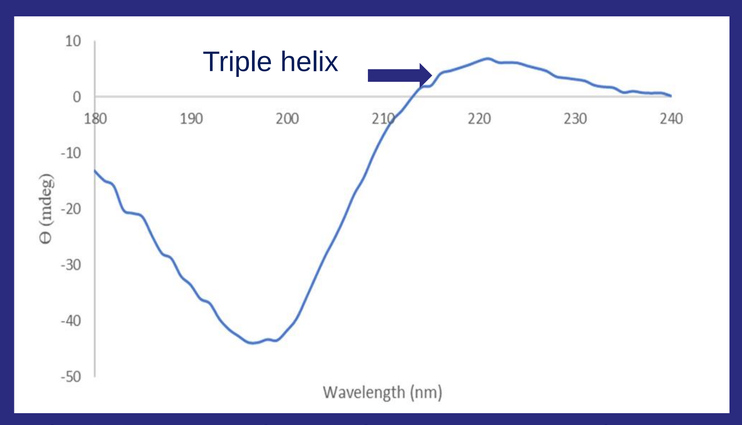


Fig. 3 CD spectra from A. *fragacea* measured at 4 ºC.

OBSERVATIONS

- Very few protocols for sea anemone collagen extraction are available in literature[6]. Extraction yields could be increased with optimization of collagen isolation procedure (enzymatic treatment; modification of precipitation step).
- No bands were observed, suggesting the possible presence of other compounds that are not sensitive to the staining procedures employed[7].
- FTIR and CD results are in accordance with previously described for marine collagen obtained from other marine sources[7,8].

Further investigation will allow to evaluate the potential of the sea anemone A*. fragacea* as a source of compounds of biomedical interest.

References:

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