Projects: Re 665/4 „Aktuopaläontologie überlebender, kretazischer Porifera-Gemeinschaften in lichtarmen Zonen des Großen Barriere-Riffs nahe Lizard Island, Australien", Project Leader: J. Reitner (Göttingen)
Re 665/8 „Steuernde Funktionen und Erhaltungspotentiale moderner und fossiler Porifera", Project Leader: J. Reitner (Göttingen)

Characterization of Organic Matrix Proteins Enclosed in High Mg-Calcite Crystals of the Coralline Sponge *Spirastrella* (*Acanthochaetetes*) *wellsi*

Matthias Bergbauer, Robert Lange & Joachim Reitner

Area of Study: Great Barrier Reef, Lizard Island (Australia)
Environment: Cryptic habitats in Indo-Pacific coral reefs
Stratigraphy: Recent
Organisms: *Spirastrella* (*Acanthochaetetes*) *wellsi*
Depositional Setting:
Constructive Processes:
Destructive Processes:
Preservation:
Research Topic: Mineralization by sponge skeleton proteins

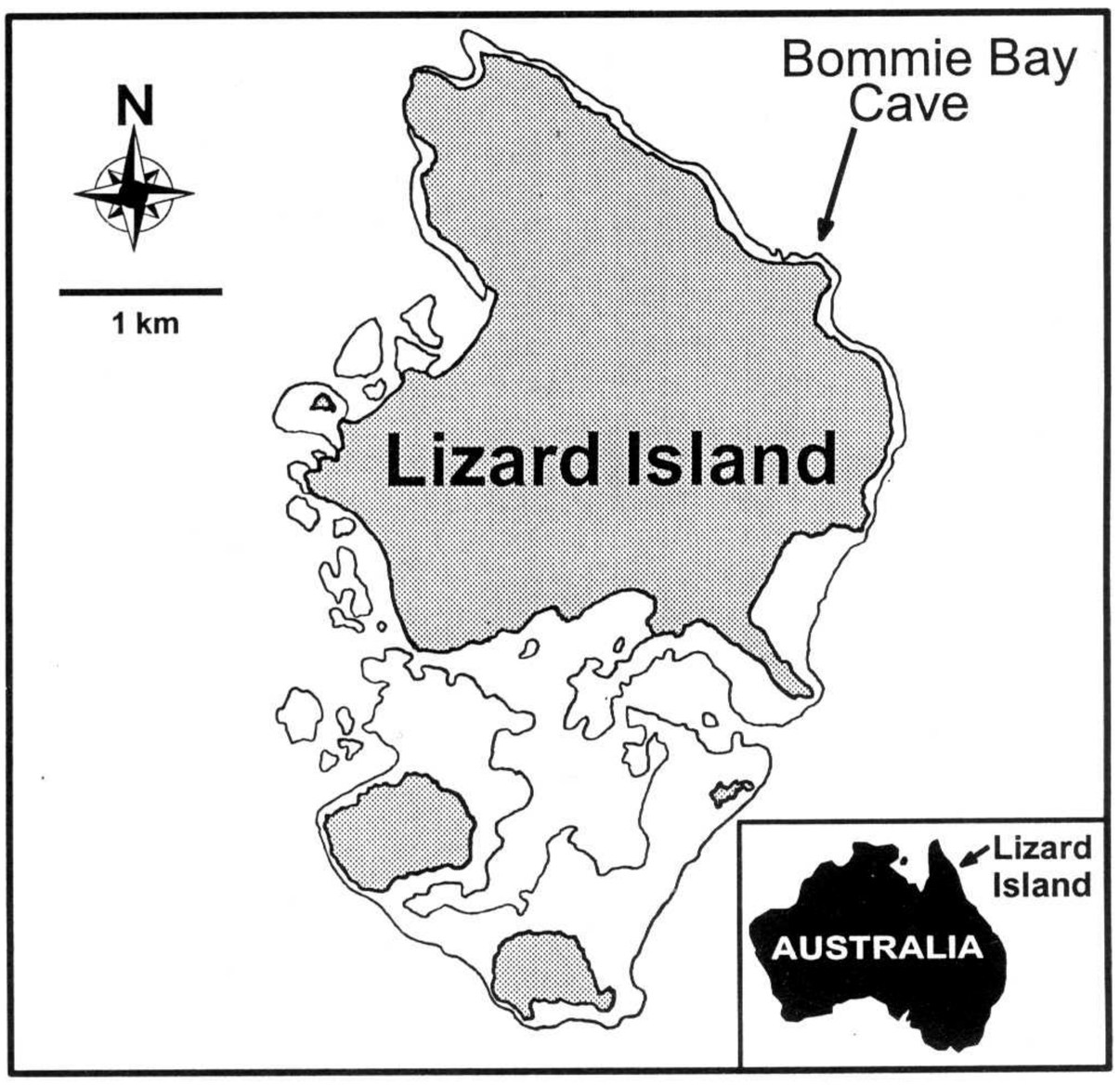

Fig. 1: Map showing the location of sampled cave at Lizard Island, Great Barrier Reef (modified from WÖRHEIDE et al. in press).

Abstract

Protein components found in freeze-dried specimens of the coralline sponge *Spirastrella* (*Acanthochaetetes*) *wellsi* were separated and characterized. Proteins extracted from skeleton crystals (matrix proteins) contained high concentrations of glycin (16 %) as well as enhanced amounts of asparagin/aspartic acid (11 %) and glutamin/glutamic acid (10 %). At least 10 proteins could be separated by SDS-PAGE. Six of them, with molecular weights between 30 and 45 kDa, may be considered as distinct matrix proteins. The bulk of total soluble proteins as well as all soluble matrix proteins are acidic with pH values below 5. Our results indicate that at least in one stage crystal growth is matrix mediated, i.e. controlled by the sponge.

1 Purpose of Studies and Methods

The subject of our study, the modern hadromerid coralline sponge *Spirastrella* (*Acanthochaetetes*) *wellsi* is an ultraconservative taxon first occurring in the Lower Cretaceous reefs. As an extant relic of a formerly important and widespread group of chaetetids, this sponge has a continuous fossil record with merely minor phylogenetic alterations. Despite the conservative character of coralline sponges, their calcareous skeletons show some advanced microstructural characteristics (REITNER 1992). *A. wellsi* exhibits a unique secondary high Mg-calcite basal skeleton (HARTMAN & GOREAU 1975). REITNER & GAUTRET (1996) reported the presence of calcium-binding macromolecules in intraskeletal matrices of *A. wellsi*, gave first data regarding some of their properties and suggested a matrix-mediated mode of mineralization. For more detailed insights in the mechanisms of mineralization by this sponge, chemical characterization of proteins of the skeleton organic matrix is essential. In this study, we were particularly interested in the:
– amino acid composition of matrix proteins
– quantity of proteins enclosed within the calcite crystals
– separation and characterization of these proteins.

Specimens of *A. wellsi* were collected by SCUBA diving in dark coral reef caves at Lizard Island (Great Barrier Reef, Australia), stored at -20°C and freeze-dried. In a first preparation, the skeleton of a single specimen was divided in 3 parts: the uppermost layer of approximately 1 mm thickness, the middle part consisting of easily visible chambered structures and the basal part built by dense material. In a second preparation, a whole, undivided specimen was used. All samples were ground to particles with a diameter of about 1 mm and decalcified with EDTA. To remove proteins not enclosed by crystals, the ground material was extracted with SDS prior to EDTA treatment in the second preparation. To get an overview about the total protein content of the freeze-dried sponge material, SDS-extraction was omitted in the first preparation. Insoluble components

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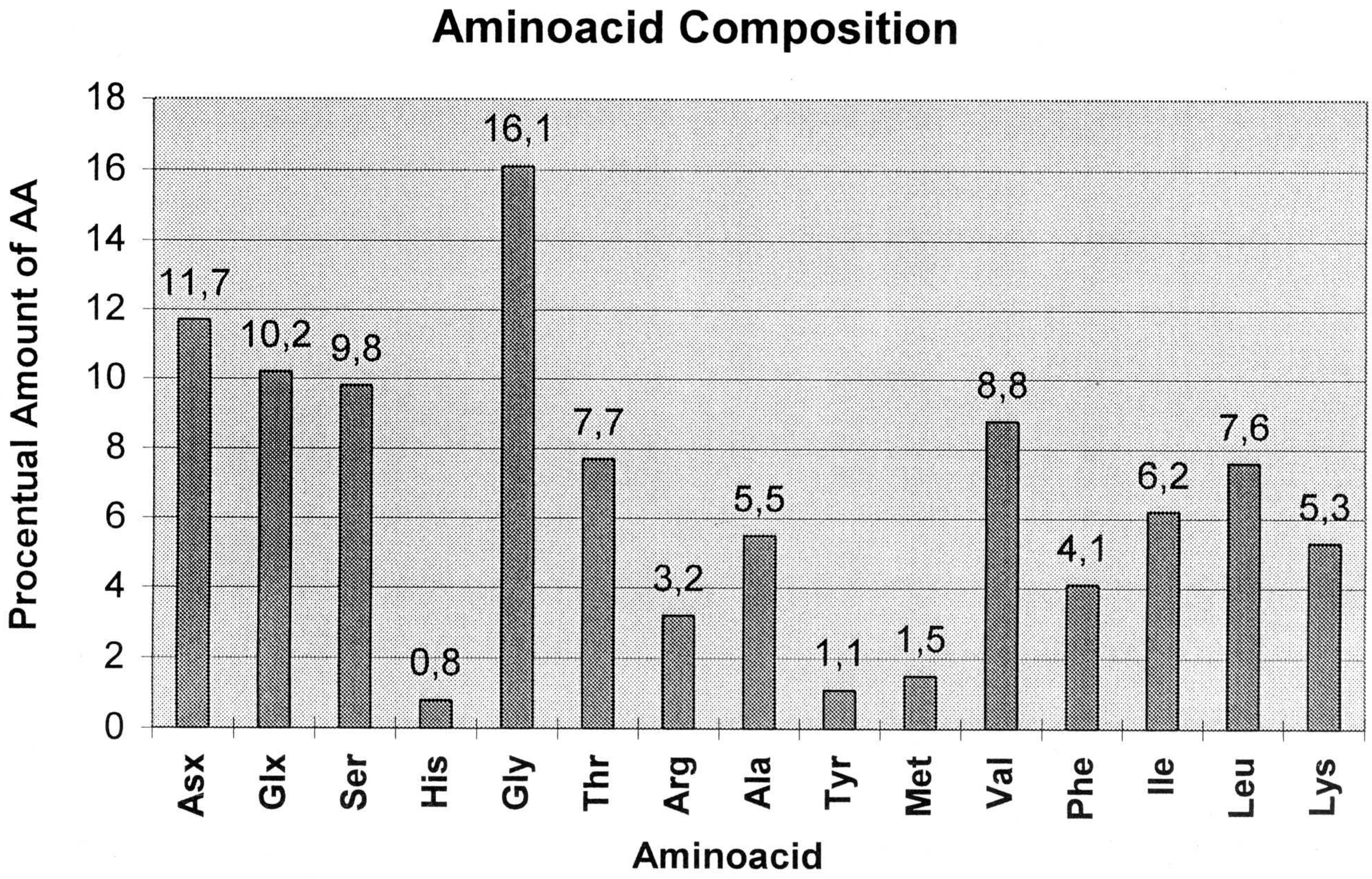

Fig. 2: Amino acid analysis of *A. wellsi* skeleton proteins. Quantities are based on three independent amino acid analyses.

were removed by centrifugation. Soluble components were desalted by gelchromatography, and subsequently concentrated increasing the protein content 100 fold. Protein concentrations were determined by the nanoOrange protein quantification assay (Molecular Probes). Samples were electrophoresed on a 10 % SDS gel (LAEMMLI 1970) and silver stained (HEUKESHOVEN 1985). Isoelectrical focusing of proteins was done with precast gels (pH-range 3-10, SERVA). Amino acid composition was determined after hydrolysis of the proteins (1 h with 6 M HCl under vacuum) and subsequent OPA-derivatization. Separation of amino acids was performed by HPLC equipped with a RP-column.

2 Results and Discussion

In the extracted soluble matrix enclosed within skeleton crystals, protein concentration was 35 µg/100 mg freeze dried skeleton material as determined by the nanoOrange method. Analysis of amino acid composition (Fig. 2) revealed high concentrations of glycin (16.1 %) and enhanced amounts of asparagin/aspartic acid (Asx, 11.7 %) and glutamin/glutamic acid (Glx, 10.2 %). Due to method inherent limitations, it could not be distinguished between aspartic acid and glutamic acid on the one hand and asparagin and glutamin on the other hand. This is because glutamin and asparagin as amides hydrolyze in acid medium to release the free acids aspartic acid and glutamic acid, respectively. In comparison, a distinct fraction of shell proteins of the bivalve *Mytilus californicus* was shown to have Asx and Glx concentrations of 32 % and 17 %, respectively (ADDADI & WEINER 1985). In this regard, matrix proteins of *A. wellsi* differ significantly in their amount of acidic amino acids. Even though nearly all Asx and Glx are formed by the corresponding acids, they would contribute to no more than 22 % of total amino acids, compared with up to 49 % in case of the shell proteins mentioned above. Nevertheless, the tendency to an increased acidity of the matrix proteins is obvious.

The skeleton of one specimen was divided in 3 parts as described in the methods section. In this preparation protein analyses were done without SDS extraction prior to EDTA treatment. At least 10 proteins could be separated by SDS-PAGE from the upper layer (Pl. 1/1). In contrast, the middle and the basal part contained significant fewer proteins. In particular, the distinct 90 kDa protein present in the upper part was absent in the middle as well as in the basal part. Also, the array of proteins below 31 kDa found in the upper part was not detectable in the two lower parts. However, some proteins with molecular weights between 30 and 45 kDa were present in all three preparations. The upper part of the skeleton is the active zone of the sponge, enclosing nearly all of its living soft tissues. Therefore, rests or even traces of these living tissues may contribute to the

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Plate 1

Fig. 1: SDS gel electrophoresis of *A. wellsi* proteins from 3 distinct parts of the skeleton. The material was not SDS-treated prior to EDTA treatment, giving the total soluble protein content. The left lane contains molecular mass markers.

Fig. 2: Isoelectrical focusing of *A. wellsi* proteins from 3 distinct parts of the skeleton. Isoelectrical point (pI) markers are shown on the right.

Fig. 3: SDS-PAGE of *A. wellsi* material. Lane 1: not SDS-extractable material ("matrix proteins"), lane 2: total proteins (not SDS-treated material), lane 3: SDS-extract (proteins extractable by SDS). Molecular mass markers are shown on the left.

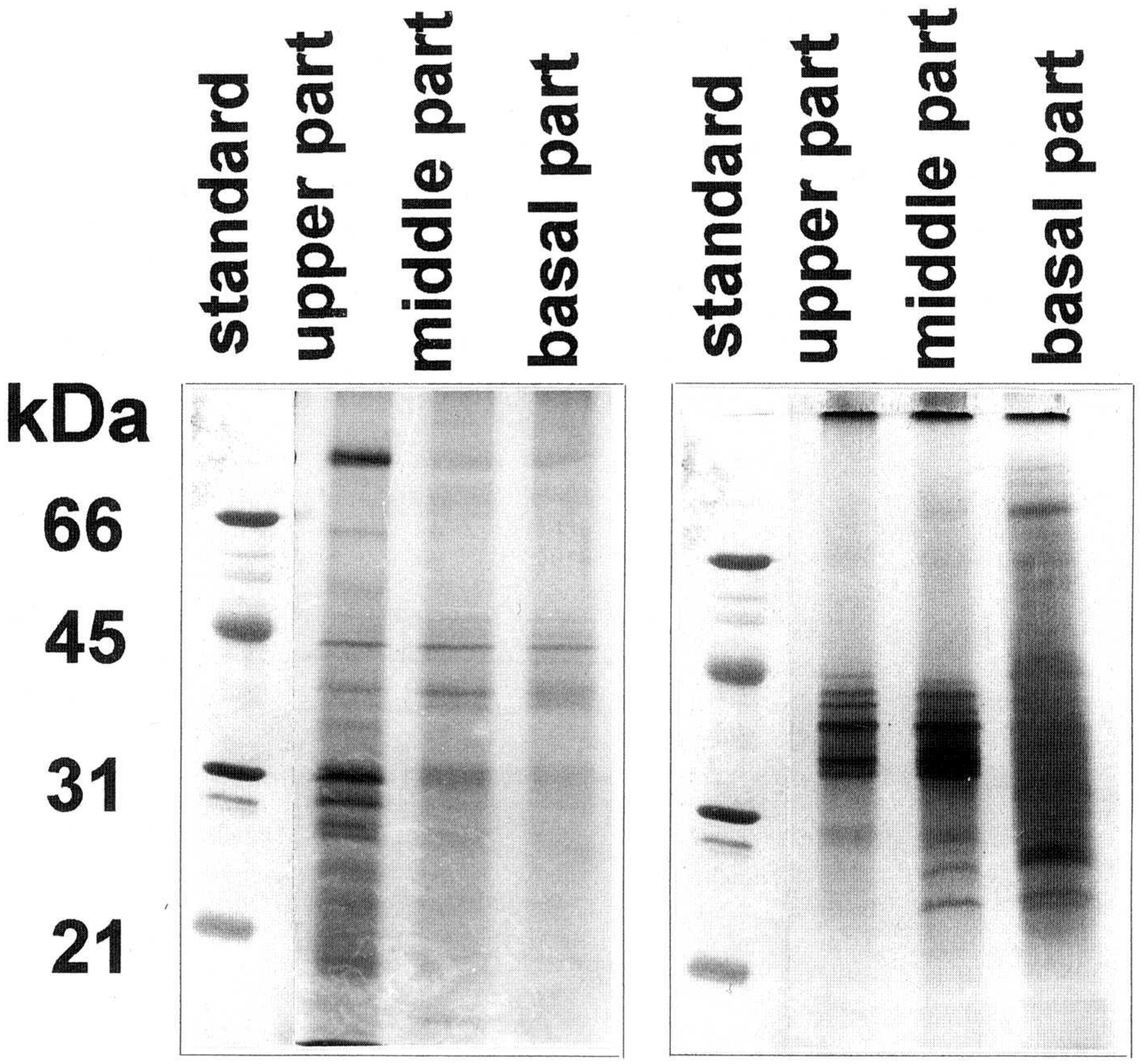

Fig.1

Fig.3

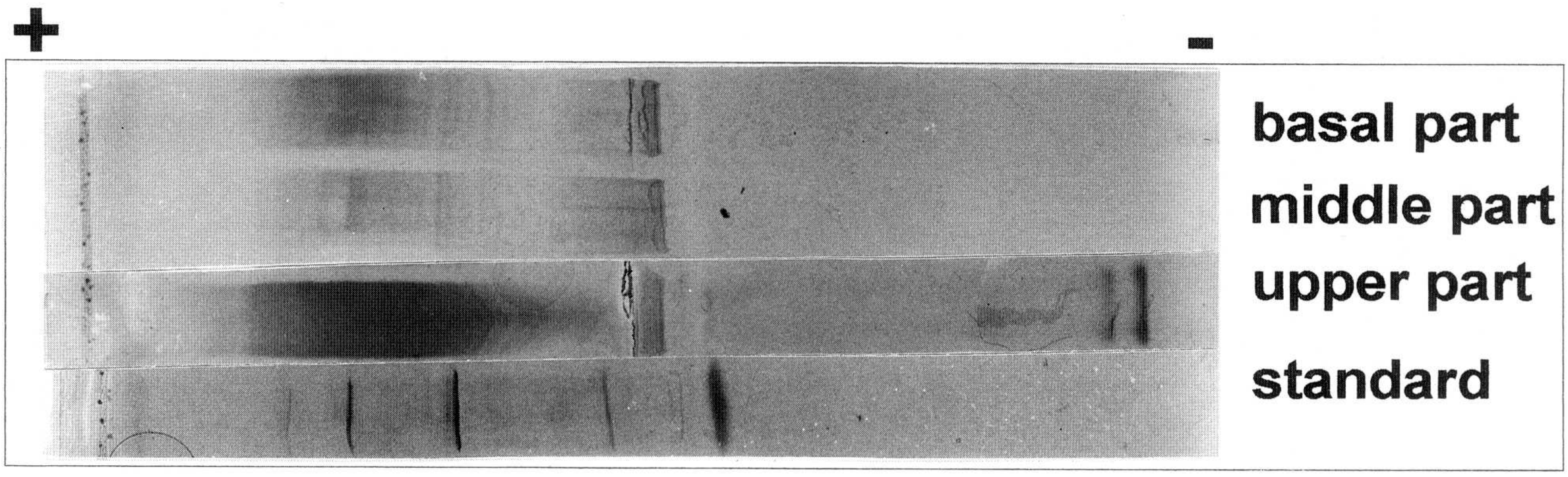

Fig.2

proteins found in the upper part but not in the two lower parts. Taking this into account, only proteins present in all three skeleton parts should come into consideration as matrix proteins.

The acidity of sponge proteins was further confirmed by isoelectrical focusing (Pl. 1/2). Total soluble proteins (not SDS treated) of the three skeleton parts were investigated. All but two proteins from the upper part of sponge skeleton were shown to be acidic with pI values below 5 as compared with ß-lactoglobulin (pI 5.20) as marker protein. In contrast, proteins from the middle and basal part were acidic without exception.

Proteins detected in the second preparation, not using different parts of a sponge but a whole specimen, are shown in Pl. 1/3. This assay also demonstrated the effect of SDS extraction in removing non-skeleton proteins prior to EDTA treatment. Total protein content of the sponge can be compared with skeleton ("matrix") proteins, i.e. not SDS extractable material. In both preparations, approximately 6 distinct proteins clustering between 30 and 45 kDa could be found. In the skeleton material remaining after SDS extraction, these 6 proteins were the only ones detectable (Pl. 1/3, lane 1). In contrast, material which was not SDS-treated contained additional proteins, including one 90 kDa protein and some proteins with molecular weights below 30 kDa (Pl. 1/3, lane 2). Probably, these proteins were identical with those described above for the upper part of sponge skeleton. These additional proteins were found to be extractable with SDS (Pl. 1/3, lane 3). In contrast, the 6 proteins with molecular weights between 30 and 45 kDa revealed not extractable with SDS. Clearly, these proteins were protected from SDS extraction by surrounding carbonate crystals. Therefore, only these "matrix proteins", or at least some of them, should be associated with crystal growth.

As it could be shown here, skeletal crystals of *A. wellsi* harbor a suite of distinct soluble proteins. They all are highly acidic, have molecular weights between 30 and 45 kDa and can be separated by SDS-PAGE. However, it should be noticed that some of the thick bands may not represent distinct proteins but degradational products of them. Further studies of our group will focus on the isolation of these proteins in a native form to investigate their attributes regarding mineralization in vitro.

Acknowledgements

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