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Minireview

The Bacterial Cellulosome

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Abstract

Aspects of the structural and functional properties of the bacterial cellulosome, an enzyme complex combining several modes of degradation of cellulose, are described. Findings obtained by classical approaches, especially electron microscopy and studies of the degradation processes, are compared and substantially broadened by results of the application of recent investigation approaches and techniques. For a better understanding, this Mini review contains citations taken from a dissertation (s. below, section „New approaches“).

Keywords: bacterial enzyme complex, cellulolytic enzymes, structural organization, functional organization, classical and recent investigation approaches and results

The Classical View of Structure and Function of the Cellulosome

The existence of a bacterial enzyme complex containing cellulolytic enzymes, later called Cellulosome, is known for more than three decades [1-3]. Since then, considerable experimental efforts led to a huge number of publications regarding the organization of this complex (Figures 1 and 2) [4-14]. Besides functional properties of the enzymes involved in cellulolytic functions, the structural organization of the complex was of major interest. After all, functional properties of such a huge enzyme complex can only be understood on the basis of a detailed knowledge of the macromolecluar structure and the interaction of its components. Early electron microscopic investigations revealed, as the structural basis of the complex, a huge central elongated protein (scaffoldin, 12) to which rows of individual cellulolytic enzymes are attached by dockerins, i.e. not catalytically active domains of the enzyme particles (Figures 2-4). Binding of the catalytically active sites to the substrate (cellulose) happens by contact. One of the investigations allowed further insight into characteristic features of the scaffoldin [9]. It revealed details indicating substructures along the scaffoldin: sites to which the enzyme proteins might have been attached (probably coiled domains), and fibrillar helical stretches between these sites (Figure 3). The existence of the rows of enzyme proteins mentioned above, attached, by dockerin domains of the enzymes, to the scaffoldin could actually be visualized. The approach for such an investigation was the application of a very special electron microscopic mounting and staining technique („stretching“ of the scaffoldin molecules on the support film, followed by negative staining). In later experiments, this basic structural investigation was confirmed. However, none of the new techniques (s. below) did allow such an advantage of a direct visualization.

New Approaches

A few years ago, a research group in Munich, especially a doctoral student in her doctoral thesis [21], took up a series of experimental approaches with the goal to extend and broaden knowledge of structural and functional properties of the cellulosome by application of recently developped investigation techniques [15 – 24].

Regarding broadened knowledge of structural and functional properties of cellulosomes, the author of this doctoral thesis wrote (citation): „These complex protein systems responsible for lignocellulose decomposition by bacteria rely on serial and synergistig modes of action performed by a variety of enzymes with divergent activities. Those enzymes are arranged on extracellular scaffolds by means of non-covalent receptor – ligand interactions. The Lego-like arrangement of subunits in

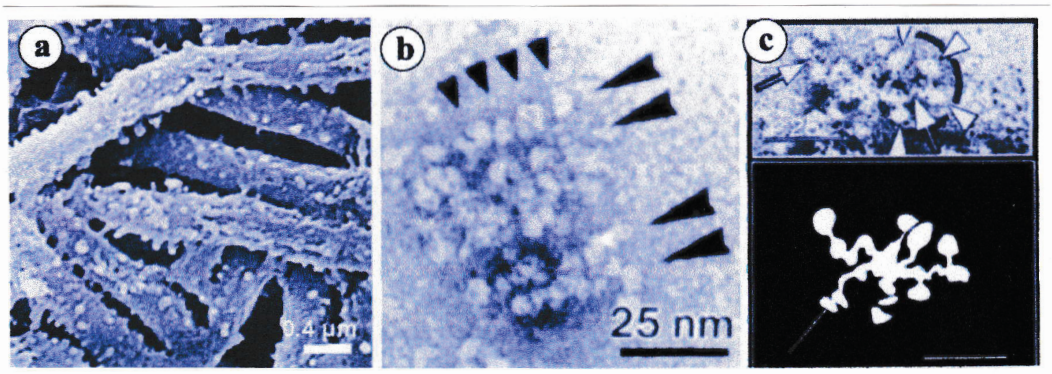

Figure 1. Cellular and macromolecular architecture of the cellulosome of the bacterium Clostridium thermocellum. a) C. thermocellum cells exhibiting „protuberances" representing cellulosomes and polycellulosomes. Scanning electron micrograph. From Bayer and Lamed 1986 b) Electron microscopic projection of a partially decomposed and flattened cellulosome. Deformation was caused by the preparation procedure for electron microscopy. Note parallel rows of individual proteins interpreted to be enzymatic subunits, indicating that several cellulosomal structural/functional units (scaffoldin, s. Figure 1c), with adhering enzymatic subunits were making up this cellulosome. Negative staining. From Mayer et al. 1987 c) Electron microscopic projection (top) and explaining drawing (bottom) of an artificially flattened protein complex. Detached from an isolated cellulosome, interpreted to be composed of the fibrillar scaffoldin (CipA) and adhering enzymatic subunits. The faint fibrillar linkers that connect the catalytically active main masses (domains) of the enzymatic subunits are, in fact, „dockerins" (catalytically inactive domains of the enzyme subunits) (s. Figure 4). Top part of Figure 1c from Mayer et al. 1987; bottom part of Figure 1c from Madkour and Mayer, 2003.

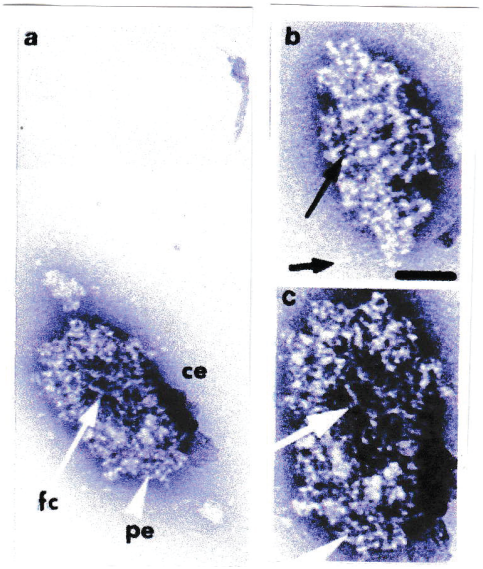

Figure 2. Intact cellulosomes depicted by transmission electron microscopy of negatively stained samples. a) An intact cellulosome. Intact cellulosomes often have a shape similar to an olive. fc, a cavity crossed by fibrillar structures interpreted as the scaffoldin. The cavity can be seen due to penetration of the negaive staining solution. b) as a) but without a visible cavity c)Higher magnification of the cellulosome depicted in a) Magnification bar: in b: 1 micrometer. From Madkour and Mayer, 2003.

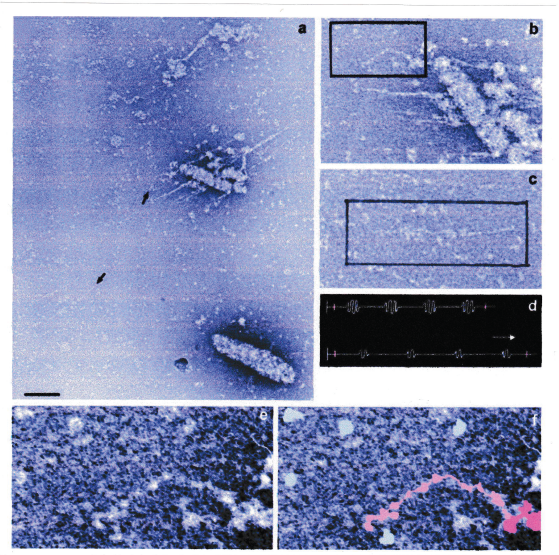

Figure 3. Partial disruption of cellulosomes combined with „stretching" of the scaffoldin caused during the mounting procedure for electron microscopy. This effect did occur due to unidirectional streaming of the water in the sample on the surface of the electron micrscopic grid and subsequent unidirectional blotting of the water prior to application of the negative staining solution a) Various states of disruption and stretching. Magnification bar: 0.25 micrometer b) and c) Obvious stretching of the fibrillar scaffoldin. Alternating coiled and fibrillar substructures can be seen along the scaffoldin molecule (much better visible in Figs.3 e) and f) d)Top and bottom: schematic presentation of the states of the scaffoldin, created by a low (top) and a higher (bottom) degree of „stretching".Higher degrees of „stretching" cause lower size of the coiled domains by artificial mechanical stress e) and f) A scaffoldin molecule in a stretched state, with adhering additional protein masses at one of its ends (compare with Fig.3b; probably the site that makes the initial contact with the bacterial cell). Alternating coiled and fibrillar stretches can be seen along the scaffoldin molecule. It is postulated that catalytically active enzyme proteins (seen in the background) had been detached from the coiled domains of the scaffoldin, their original site of location. From Madkour and Mayer, 2003

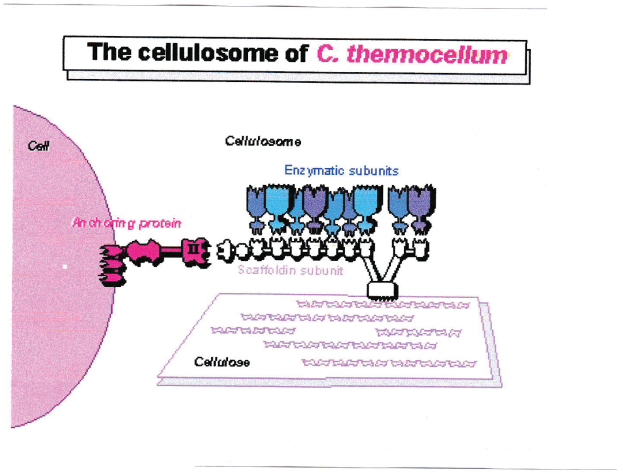

Figure 4. Model drawing of the structural/functional unit of a cellulosome. Several such units are usually making up a cellulosome. The componets of such a unit are depicted in the drawing: a scaffoldin is attached to the bacterial cell by an anchoring protein. This protein makes contact with the scaffoldin to which the catalytically active enzyme subunits are attached via their (catalytically inactive) dockerins. The model drawing does not explicitely show the contact of the catalytically active enzyme subunits with the substrate. In Fig.2 the situation is more evident; it can be assumed that the scaffoldin makes the initial contact with the bacterial cell, the catalytic sites of the enzymes are exposed, on the surface of the cellulosomes, so that contact with the substrate is easily possible. From Madkour and Mayer 2003, modified.

cellulosomes enables the microbe to engineer designer complexes targeted to specific biomass types or for use at different stages of biomass deconstruction. Precise control of enzyme arrangement and modularity lead to excellent hydrolytic efficiency of cellulosomes that is interesting from the point of view of biofuel production for environmentally sustainable energy. One goal of this thesis was to develop a novel assay for studying the effectiveness of multi-component enzyme mixtures on complex lignocellulose substrates. In nature, cellulosomes function in conditions where hydrodynamic shear forces mechanically stress cells adhered to biomass. This evolutionary pressure led to unique mechanical properties of cellulosomal protein domains and extreme stability of involved receptor – ligand interactions under external force. Investigation of the mechanostability of non-covalent protein – protein interaction that hold cellulosomal components together, namely cohesin – dockerin complexes (20) is also a topic discussed in this thesis.This part of the work concentrates on the unique mechanical properties of cellulosomal components. Particularly, highly specific protein – protein complexes responsible for the assembly of cellulosomes are investigated. These cohesin – dockerin non-covalent links bridge bacterial host cell and cellulosic carbon sources in turbulent environments, and therefore are subject to mechanical forces in vivo. The complex is characterized using single molecule force spectroscopy. To this end, an improved experimental protocol was developed and implemented. Next, the mechanisms behind the exceptional mechanostability of the interaction were elucidated employing full-atom steered molecular dynamic simulations. A new network-based analysis of simulation trajectories is developed to visualize the force propagation paths through the protein complexes“.

It is evident that applications of new techniques substantially broaden our knowledge of stuctural and functional properties of cellulosomes and also of applications in Biotechnology (25 - 32).

Conclusion

As expected, application of recently developed approaches and techniques lead to a substantially extended knowledge on structural and functional details of the cellulosome as an example for a huge bacterial enzyme complex. Nevertheless, a comparison with a model of the „classical“ view on its structural and funcional organization (s. Fig.4) reveals that this classical view was a solid basis for extended studies. All available data taken together might also be a valuable starting point for further improvements in the application of cellulose-degrading enzymes in technical processes (s. above, biofuel as an example).

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