CELLOBIOSE DEHYDROGENASE - AN UNSOLVED PROBLEM IN FUNGAL WOOD DEGRADATION

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Cellobiose dehydrogenase (CDH) is a 89 kD (1) extracellular enzyme produced by some wood degrading fungi in relatively large amounts (2-5). When the white rot fungus *Phanerochaete chrysosporium* is grown in a fermenter under cellulolytic conditions about 0.5 % of the total extracellular protein consists of CDH (5). The enzyme carries one flavin of FAD-type and one heme as prosthetic groups (2, 3). It appears to be a typical dehydrogenase with separated oxidative and reductive half reactions, with cellodextrins, mannodextrins and lactose as efficient electron donors (Figure 1) and a wide spectrum of electron acceptors including quinones, phenoxy radicals, complexed Fe (III) and cytochromes (5). Dioxygen is also reduced, but at a rather slow rate (2, 3, 6). Glucose is not a good electron donor. k_{cat}/K_m is as much as 87 000 times higher for cellobiose compared with its monomer (5).

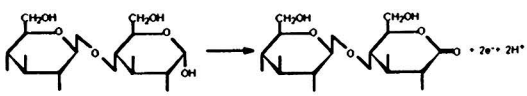

Figure 1. ***Reaction catalyzed by CDH***

The enzyme can be cleaved by papain into two fragments carrying heme and FAD respectively (7). We found that the FAD fragment retained all catalytic properties of the intact enzyme, but reduced one-electron acceptors, *i.e* complexed Fe (III), cytochromes and phenoxy radicals, at a slower rate than the intact enzyme, whereas two electron acceptors (quinones and triiodide ion) are reduced at the same rate by the fragment and the intact enzyme (6, 7). From this data the conclusion is drawn that the function of the heme domain is to stimulate reduction of one-electron acceptors, and that the natural electron acceptors probably belongs to this group.

CDH binds strongly and specifically to cellulose (7, 8). No binding was discovered to chitin, starch, or to the wood components xylan and mannan. The binding was not affected by NaCl, but was inhibited by ethylene glycol and stimulated by ammonium sulphate (5). Thus hydrophobic interaction and/or charge transfer probably play an important role in the binding. The bound protein is still active and therefore the cellulose binding appears to be separated from the catalytic site (7, 8). It has been suggested that the cellulose binding is due to a cellulose binding domain similar to the ones of fungal cellulases. However, no such domain is found in the amino acid sequence of CDH (9) and the cellulose binding mechanism of this enzyme also differs from the one of the fungal cellulase CBH 1 (5). The shape of CDH and its fragments have been determined by SAXS by Lehrner and Zipper. This molecule is long (180 Å) and thin with a "head" consisting of the FAD fragment and a "tail" corresponding to the heme fragment (10). A cDNA of CDH has been cloned and sequenced. The 5'end was obtained by PCR amplification. The cDNA contains 2310 translated bases excluding the poly (A) tail. The deduced mature protein contains 770 amino acid residues, procceded by a 18 residues long signal peptide. Data from protein characterization, *i.e.* sequencing, amino acid composition and molecular weight determinations are in good agreement with these finding. The regions of amino acid sequence corresponding to the heme and FAD domains were identified as well as the nucleotide binding motif. No homologous sequence were found for the heme domain, however the FAD domain appears to be related to a family of oxidoreductases including *Aspergillus niger* Glucose oxidase. The homologous sequences seems to be restricted to a flavin binding domain (5, 9).

Although rather much is known about CDH, its function is still an open question. There are three suggestions that are popular for the moment. First CDH may work as a generator of Fe^{2+} in a Fenton's

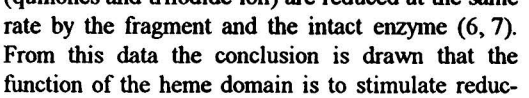

Figure 2. ***CDH as a generator in Fentons reaction.***

reaction (11) (Figure 2).

The created hydroxyl radical should then cause damage to various wood components and thereby stimulate enzymatic degradation. We have demonstrated that CDH in presence of hydrogen peroxide and complexed Fe (III)-ions can degrade cellulose, hemicellulose and lignin (12). Other suggestions are that CDH cooperates with the lignolytic enzymes lignin peroxidase and manganese peroxidase. In the case of lignin peroxidase the function should be to

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inhibit repolymerization of phenoxy radicals on naked cellulose (6, 13). Cooperation with manganese peroxidase should be based on the fact that CDH can solubilise insoluble Mn (IV) and provide cellobionic acid as chelating agent for Manganese ions (14). CDH can indeed work in both this ways, but the fact that CDH is produced by fungi that lacks these peroxidases talks however against this suggestion (5). A third attractive hypothesis is that CDH is the first member in an electron transport chain with the function to capture the easily available reducing energy of cellobiose, which is the major product of cellulose degradation (15). It is however not likely that this could happen without the involvement of a membrane system. In eukaryotic organisms such systems are located in mitochondria. Thus, such an electron transport chain must involve a transmembrane transport. Alternatively, an ATP generating system could exist in the cytoplasmic membrane.

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INMOVILIZACIÓN DE ENZIMAS DE POSIBLE USO INDUSTRIAL, SELECCIÓN DE SOPORTES A PARTIR DE MATERIAS PRIMAS DE BAJO COSTO

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La quitina es uno de los polisacáridos más abundantes de la naturaleza, componente principal de varias formas de vida incluyendo insectos, crustáceos y paredes de hongos unicelulares. Es dura, relativamente inerte, biodegradable y biocompatible. Esto hace de la quitina y el quitosán (quitina desacetilada) un material con excelentes propiedades para la inmovilización de enzimas de posible uso industrial (1).

La α-quimotripsina, según la bibliografía, tanto en estado libre como inmovilizada, presenta actividad catalítica en medios parcial o totalmente orgánicos (2), lo que incrementa su actividad sintetásica, que a su vez implica la posibilidad de obtención de péptidos vía enzimática.

La quitina obtenida de diferentes fuentes naturales (langosta, langostinos, cigalas, galeras, cangrejos español y cangrejo americano) de acuerdo al protocolo puesto a punto en este trabajo; se caracterizó por espectroscopía de IR, microscopia electrónica de barrido, grado de desacetilación y medidas de acuofilia. La caracterización físico-química de las muestra obtenidas, diferencia a cada una de ellas, encontrándose posteriormente una relación entre la actividad enzimática de los derivados inmovilizados y sus propiedades.

En todas las quitinas obtenidas se inmovilizó α-quimotripsina vía glutaraldehído, realizándose el estudio de sus actividades hidrolíticas y sintetásicas en medios acuosos y acuoso-orgánicos respectivamente.

Los resultados obtenidos muestran un rendimiento de obtención similar para las diversas fuentes utilizadas (15-23 %), así como el rendimiento de inmovilización (90 %). El derivado de α-quimotripsina inmovilizado en quitina obtenida de langostinos es el más activo, tanto hidrolíticamente, nueve veces más que la enzima libre y sintetásicamente, 90 % de rendimiento del péptido Bz-Tyr-Leu-NH_2 (medio orgánico, 70 % butanodiol en tampón carbonato pH 9); interpretándose este hecho como consecuencia de la mayor acuofilia, estructura superficial del soporte y grado de desacetilación de esta muestra concreta. El estudio calorimétrico llevado a cabo con este derivado indica que las interacciones medio parcialmente orgánico-enzima inmovilizada son irreversibles. Además, existe una relación entre sus constantes cinéticas hidrolíticas y las diferentes estructuras, siendo el derivado inmovilizado en quitina de langostinos el mejor respecto a su mecanismo cinético.

Se puede concluir por tanto que una aplicación de las quitinas es su utilización como soporte en la inmovilización de α-quimotripsina, siendo de gran relevancia la síntesis peptídica en medios parcialmente orgánicos.

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