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Cellulase S_s (CelS) is Synonymous with the Major Cellobiohydrolase (Subunit S8) from the Cellulosome of *Clostridium Thermocellum*

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ABSTRACT

The controversy regarding the identity of a major cellulosomal component type from two different strains of *Clostridium thermocellum* has been resolved. The principal cellobiohydrolase, subunit S8, from the cellulosome of strain YS has been demonstrated to be synonymous with cellulase component S_s (CelS) from the cellulosome of ATCC strain 27405. This component is not related to any other cellulosomal subunit or cloned endoglucanase in this organism.

Index Entries: Cellulosome; *Clostridium thermocellum*; cellulase; cellobiohydrolase; multicomponent complex; multienzyme complex.

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INTRODUCTION

The cellulolytic anaerobic thermophile, *Clostridium thermocellum*, secretes numerous cellulases, the majority of which associate on the cell surface to form an ordered multienzyme complex, termed the cellulosome (*1*). Cellulosomes or cellulosome-like complexes have been described for each of the *C. thermocellum* strains examined (*2–4*). In addition, several unrelated cellulolytic bacteria have also been shown to produce similar multiprotein cellulase complexes (*5–8*).

Studies of the molecular biology and biochemistry of the cellulosome have been conducted by a number of research groups and have focused on several different strains of *C. thermocellum* (*2,8*). As a consequence, there is some uncertainty regarding the precise composition of the cellulosome and the identity of individual components. A case in point is the controversy surrounding the status of cellobiohydrolase as a cellulosome component. It is widely acknowledged that this enzyme is an important component of fungal cellulases, but it has only occasionally been described in bacteria (*8,9*).

Following proteinase treatment of the cellulosome from *C. thermocellum* strain YS, Morag et al. (*10*) purified a catalytically active truncated form of the major 75-kDa S8 subunit. The purified S8 exhibited cellobiohydrolase activity, and many of its enzymatic features coincided with those of the intact cellulosome. In a different study, Wu and Demain (*4*) considered that an 82-kDa cellulosomal subunit (designated S_s) from strain ATCC 27405 could well be synonymous with S8, but it was later concluded by Fauth et al. (*11*) that S_s is, in fact, an endoglucanase.

If the *C. thermocellum* cellulosome is to continue to be an accurate paradigm for the complex-forming bacterial cellulases, it is important that the status and role of the cellobiohydrolase component(s) be clarified. Recent publication of the sequence of the *celS* gene, purporting to code for subunit S_s (*12*), has enabled comparison of definitive data from two research groups, and we present here unequivocal evidence that the *celS* gene product is indeed synonymous with the cellobiohydrolase previously designated subunit S8.

MATERIALS AND METHODS

Cellulosome samples from *C. thermocellum* strains YS and ATCC 27405 were prepared by "affinity digestion" as described earlier (*13*). Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS-PAGE) was performed on 6% gels, and the separated proteins were transferred to polyvinylidene difluoride (PVDF) membranes by the method of Matsudaira (*14*). The blots were stained with Coomassie brilliant blue

R-250, and the S8 band was excised and its N-terminal amino acid sequence was determined by Edman degradation using an Applied Biosystems model 470A protein peptide sequencer. Peptide mapping of chymotryptic digests of the S8 and S_s bands was performed on 20% SDS-PAGE gels according to Cleveland et al. (*15*).

RESULTS AND DISCUSSION

Most of our knowledge about the cellulosome from *C. thermocellum* has accumulated from research performed on two different strains of this organism—strain YS (*2*) and strain ATCC 27405 (equivalent to NCIB 10682 (*9*). Although the characteristics of the cellulosome produced by these two strains are remarkably similar, there are differences both in the molecular properties of individual components and in their proportions within the complex. In this regard, the composition of the growth medium has been shown to alter the profile of the cellulosomal components (*16*). It can also be assumed that variation in experimental techniques and terminology used in different laboratories around the world will have contributed to discrepancies in the literature.

Within the cellulosome complex, multiple endoglucanases and xylanases interact with a noncatalytic multifunctional glycoprotein subunit that appears to mediate attachment of the cellulosome both to the bacterial cell surface and to the cellulosic substrate (*1,17–20*). This component has been termed S1 in strain YS (*21,22*) and S_L in strain 27405 (*23*), and its property of binding to the other (catalytic) components has been referred to as "organizing" (*16*), "scaffolding" (*24*), or "integrating" (*25*) activity.

The major subunit of the cellulosome, as determined by densitometry tracings of protein gels, has been termed S8 in strain YS (*22*) and S_s in strain 27405 (*23*). The S8 subunit was recently isolated from the cellulosome of strain YS (*10*) and was shown to be a distinctive cellobiohydrolase that displayed many of the biochemical characteristics of the intact cellulosomal complex (i.e., degradation of crystalline cellulose, inhibition by cellobiose, stabilization by Ca^{2+}, and similar substrate-product profiles). Purified S_s from the cellulosome of strain ATCC 27405 had a similar M_r to that of S8 and, in combination with the noncatalytic subunit (called S_L), was capable of hydrolyzing crystalline cellulose (*23*). However, in a further report (*11*), a different protein, identified to be S_s on the basis of immunological crossreactivity, was isolated directly from the growth medium of strain ATCC 27405. This protein was shown to be an endoglucanase and claimed to be synonymous with the J3 subunit of a "subcellulosome" purified by lectin affinity chromatography (*26*). The properties of this protein clearly differed from those of the cellobiohydrolase reported to be S8 (*10*).

Subunit S8	**G P T K A P T K D G T S Y K D L F X E**
CelS Protein	**G P T K A P T K D G T S Y K D L F V E**

Fig. 1. Comparison of the sequence of the first 19 amino acid residues in the N-terminus of the S8 subunit from the cellulosome of *C. thermocellum* YS and the deduced N-terminal sequence of the *celS* gene product.

In order to resolve this discrepancy, we have determined the N-terminal sequence of native S8, purified from the cellulosome of *C. thermocellum* strain YS. The sequence is shown in Fig. 1 and aligned with the deduced N-terminal sequence of recombinant S_s. The *celS* gene was isolated recently from the genome of strain ATCC 27405 using an oligonucleotide probe derived from the experimentally determined N-terminal sequence of purified native S_s (*12*). Comparison of the two sequences revealed a perfect match of over 19 residues, confirming that S8 and S_s are equivalent and suggesting that the *celS* gene, which failed to confer an identifiable phenotype on *E. coli* (unpublished results), does, in fact, code for a cellobiohydrolase. In support of these conclusions, we have shown that antiserum, prepared against S8, binds specifically to S_s (*10*). In addition, the two proteins comigrate when subjected to SDS-PAGE and produce identical peptide maps when digested with chymotrypsin.

The minor discrepancy between the reported M_r values for S8 and S_s (75,000 vs 82,000, respectively) should not be regarded as significant. Such variation between laboratories is quite common, and the value of 80,670, deduced from the nucleotide sequence of the *celS* gene, is likely to be the most accurate estimate.

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