Unorthodox Intrasubunit Interactions in the Cellulosome of *Clostridium thermocellum*

Identification of Structural Transitions Induced in the S1 Subunit

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ABSTRACT

*Clostridium thermocellum,* an anaerobic thermophilic cellulolytic bacterium, produces an extremely cohesive, very high-molecular-mass, multienzyme-containing complex termed the *cellulosome.* One of its components, the S1 subunit, is a nonenzymatic, 210 kDa glycopolymer. Upon preconditioning of the intact cellulosome with low-ionic-strength or low-pH solutions, the S1 subunit separates in hot sodium dodecyl sulfate (SDS) solutions into a series of defined lower-molecular-mass subcomponents. Under the same conditions, the purified S1 subunit demonstrated the same behavior. Higher levels of glycosylation associated with the larger S1 subcomponents. The data support alterations in the conformational state of the S1 structure that lead to its disintegration induced by combined treatments with SDS and heating. Evidence is provided that this phenomenon may reflect a physiological response of the cellulosome, since similar alterations in S1 appear to accompany its binding to cellulose.

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INTRODUCTION

The degradation of cellulose by a variety of different cellulolytic bacteria appears to be mediated by remarkably similar types of cellulose-binding, multienzyme complexes termed cellulosomes (1-6). Characteristically, cellulosomes contain different enzymes (e.g., endoglucanases, exoglucanases, and xylanases) as well as other apparently nonenzymatic components, the most notable of which are glycopolypeptide components, which may have one of several functions, including proposed roles in cellulosomal organization, cellulose-binding, and association of the enzymatic components into the cohesive complex.

The first cellulosome to have been described belongs to the anaerobic, thermophilic, cellulolytic bacterium C. thermocellum (1, 7, 8). Its properties have been well documented since its initial description, and its in-depth functional and structural characterization continues to be subject to extensive study (4, 9, 10).

One of the most intriguing components of the cellulosome from C. thermocellum is the nonenzymatic 210 kDa glycopolypeptide, the S1 subunit (8, 11, 12). The oligosaccharide moiety(ies) of this very large cellulosomal subunit has recently been elucidated (13). However, study of the S1 subunit has been hampered throughout the years for two reasons: First, the structure of the cellulosome is remarkably stable to denaturation, and its separation into its component parts is experimentally difficult; only treatment with SDS at elevated temperatures has been shown to consistently dissociate the cellulosome complex into its collection of subunits (4, 5). Secondly, an unusual pH- and ionic strength-related anomaly in the SDS-PAGE mobility pattern of the S1 subunit has recently been described (14); this has been the source of a great deal of confusion regarding the precise identification of cellulosomal components.

In this work, we present additional insight into the anomalous mobility properties of the S1 glycopolypeptide subunit from C. thermocellum. For the purpose of this study, the S1 subunit was isolated from the intact cellulosome, and its response to pH and ionic strength was assessed. The phenomenon is shown to reflect an inherent consequence of S1 structure that appears to simulate its natural conformational transition upon binding to cellulose.