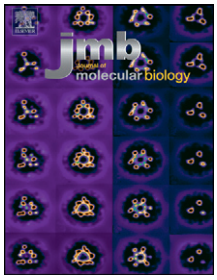

Structural and Functional Analysis of the GerD Spore Germination Protein of *Bacillus* Species

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http://dx.doi.org/10.1016/j.jmb.2014.02.004
Edited by J. H. Naismith

Abstract

Spore germination in *Bacillus* species represents an excellent model system with which to study the molecular mechanisms underlying the nutritional control of growth and development. Binding of specific chemical nutrients to their cognate receptors located in the spore inner membrane triggers the germination process that leads to a resumption of metabolism in spore outgrowth. Recent studies suggest that the inner membrane GerD lipoprotein plays a critical role in the receptor-mediated activation of downstream germination events. The 121-residue core polypeptide of GerD ($GerD^{60-180}$) from *Geobacillus stearothermophilus* forms a stable α-helical trimer in aqueous solution. The 2.3-Å-resolution crystal structure of the trimer reveals a neatly twisted superhelical rope, with unusual supercoiling induced by parallel triple-helix interactions. The overall geometry comprises three interleaved hydrophobic screws of interacting helices linked by short turns that have not been seen before. Using complementation analysis in a series of *Bacillus subtilis gerD* mutants, we demonstrated that alterations in the GerD trimer structure have profound effects on nutrient germination. This important structure–function relationship of trimeric GerD is supported by our identification of a dominant negative *gerD* mutation in *B. subtilis*. These results and those of others lead us to propose that GerD mediates clustering of germination proteins in the inner membrane of dormant spores and thus promotes the rapid and cooperative germination response to nutrients.

Introduction

One of the most characteristic features of the *Firmicute* phylum, which includes various *Bacillales* and *Clostridiales* species, is the ability of some of these species to form endospores in sporulation, a process induced in response to adverse growth conditions [1,2]. These spores are extremely resistant to all manner of environmental insults, properties that allow spores to exist in their metabolically dormant state indefinitely and remain viable for hundreds of years without water or nutrients [3,4]. However, during their long period of dormancy, spores are constantly sensing the environment, and when favorable conditions return, spores can return to active metabolism within minutes through the process of spore germination followed by outgrowth to generate growing cells [1,3,5,6]. As such, spores are ubiquitous throughout our environment, and pathogenic varieties have had a significant impact on human health and disease [6]. For example, *Clostridium botulinum*, *Clostridium perfringens* and *Bacillus cereus* are major agents of food spoilage and food-borne disease, while *Bacillus anthracis* spores cause anthrax in animals and man and can be used as a biological weapon. Thus, a detailed understanding of the mechanisms of sporulation and germination has both basic and applied interests.

A major signal that triggers spore germination is the presence of specific nutrients called germinants in spores' environments. These nutrient germinants are typically amino acids, purine nucleosides or sugars that are recognized in a stereospecific manner by cognate germinant receptors (GRs) located in the inner membrane of the spore. Three functional GRs

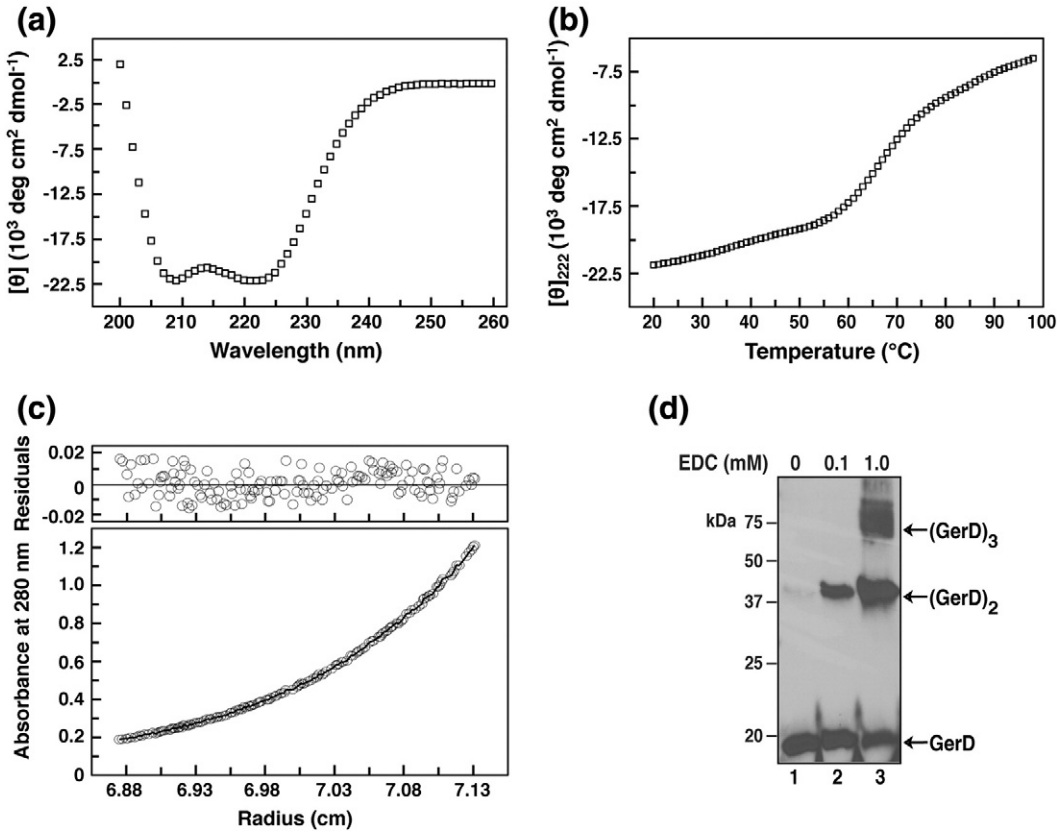

Fig. 1. GerD forms a stable α-helical trimer in solution. (a) CD spectrum of *G. stearothermophilus* GerD$^{60\text{-}180}$ (25 μM) at 20 °C in TBS (pH 8.0). (b) Thermal melt monitored by CD at 222 nm with 35 μM protein. (c) Representative sedimentation equilibrium data for GerD$^{60\text{-}180}$ (35 μM) at 20 °C and 14,000 rpm in TBS (pH 7.6). The data fit closely to a trimeric complex. (Upper) The deviation in the data from the linear fit for a trimeric model is plotted. (d) Cross-linking of total lysates of the wild-type *B. subtilis* PS533 spores by varying concentrations of EDC, which cross-links adjacent carboxyl groups to primary amines. Equal amounts of the resulting lysates were analyzed by SDS-PAGE\Western blotting using anti-*B. subtilis* GerD serum. Similar results were obtained when a lysate from spores lacking the GerA, GerB and GerK GRs were cross-linked with EDC (data not shown). The black arrows on the right side of the blot indicate the predicted molecular weights for GerD monomer, dimer and trimer.

are found in *Bacillus subtilis* spores, each encoded by the homologous tricistronic *gerA*, *gerB* and *gerK* operons [4,7]. The GerA GR responds to L-alanine or L-valine, while the GerB and GerK GRs cooperate to respond to an amino acid and sugar combination of L-asparagine, D-glucose, D-fructose and potassium ions (AGFK). Specific germinant–GR interaction results in transduction of a signal that leads to the release of the large depot (~10% of the spores dry weight) of pyridine-2,6-dicarboxylic acid [dipicolinic acid (DPA)] and associated cations, predominantly Ca^{2+} (CaDPA) from the spore core, likely via a channel composed at least in part of SpoVA proteins [8,9]. DPA release then triggers degradation of the spore's peptidoglycan cortex by cortex-lytic enzyme, eventually leading to resumption of metabolism, macromolecular synthesis and vegetative growth.

While a number of the physical changes accompanying spore germination and the proteins involved in this process have been identified, there is as yet no understanding of how signals are transduced from GRs to other spore components to initiate the physiological route to germination. The fact that spores can integrate and amplify signals from multiple GRs in determining rates of commitment and germination [10] suggests that there is an additional protein involved in GR-dependent signal transduction. In *Bacillus* species, an obvious candidate for an intermediate role in this signal transduction pathway is the GerD protein. The *gerD*-null mutation greatly decreases rates of GR-dependent germination in response to nutrient germinants with spores of *B. subtilis* and *Bacillus megaterium* but does not affect spore germination induced by agents that do not act through GRs (Gupta, S. and Christie, G., personal communication) [11]. Moreover, recent work demonstrates that, in *B. subtilis* spores, GRs and GerD colocalize in a small cluster termed the germinosome in the spore's inner membrane and that GerD is essential for this GR clustering [12].