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Biochemistry

Polypeptide composition of bacterial cyclic diguanylic acid-dependent cellulose synthase and the occurrence of immunologically crossreacting proteins in higher plants

(cellulose biogenesis/*Acetobacter xylinum*/affinity labeling)

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ABSTRACT **To comprehend the catalytic and regulatory mechanism of the cyclic diguanylic acid (c-di-GMP)-dependent cellulose synthase of *Acetobacter xylinum* and its relatedness to similar enzymes in other organisms, the structure of this enzyme was analyzed at the polypeptide level. The enzyme, purified 350-fold by enzyme-product entrapment, contains three major peptides (90, 67, and 54 kDa), which, based on direct photoaffinity and immunochemical labeling and amino acid sequence analysis, are constituents of the native cellulose synthase. Labeling of purified synthase with either [^{32}P]c-di-GMP or [α-^{32}P]UDP-glucose indicates that activator- and substrate-specific binding sites are most closely associated with the 67- and 54-kDa peptides, respectively, whereas marginal photolabeling is detected in the 90-kDa peptide. However, antibodies raised against a protein derived from the cellulose synthase structural gene (*bcsB*) specifically label all three peptides. Further, the N-terminal amino acid sequences determined for the 90- and 67-kDa peptides share a high degree of homology with the amino acid sequence deduced from the gene. We suggest that the structurally related 67- and 54-kDa peptides are fragments proteolytically derived from the 90-kDa peptide encoded by *bcsB*. The anti-cellulose synthase antibodies crossreact with a similar set of peptides derived from other cellulose-producing microorganisms and plants such as *Agrobacterium tumefaciens*, *Rhizobium leguminosarum*, mung bean, peas, barley, and cotton. The occurrence of such cellulose synthase-like structures in plant species suggests that a common enzymatic mechanism for cellulose biogenesis is employed throughout nature.**

The deposition of cellulose fibrils within the cell wall is an integral process of plant growth and development. Regrettably, a convincing demonstration of cellulose synthase activity in a cell-free system from plants has not yet been realized (1). The Gram-negative bacterium *Acetobacter xylinum*, which produces cellulose as an extracellular product, has long been regarded as an archetype for the study of cellulose biogenesis. The isolation of cellulose synthase from *A. xylinum* cells in a highly active state, combined with the discovery of a unique multicomponent regulatory system for the enzyme (2–5), has accentuated the potential of this bacterium as a model system. The cellulose synthase of *A. xylinum* is membrane-bound and utilizes UDP-glucose (UDP-Glc) as a sole exogenous substrate, forming β1,4-glucan chains and UDP. The enzyme is activated up to 200-fold by cyclic diguanylic acid (c-di-GMP). The intracellular concentration of c-di-GMP is controlled by the opposing action of two regulatory enzymes; diguanylate cyclase, which catalyzes its formation from two molecules of GTP, and Ca^{2+}-sensitive c-di-GMP phosphodiesterase A (PDE-A) which in conjunction with phosphodiesterase B catalyzes its degradation to 5′-GMP. An operon encoding four proteins required for bacterial cellulose synthesis (*bcs*) in *A. xylinum* has been isolated (6). The operon, 9217 base pairs in length, consists of four genes, *bcsA*, *bcsB*, *bcsC*, and *bcsD*, all of which are essential for maximal cellulose synthesis. The *bcsB* gene probably encodes the catalytic subunit of cellulose synthase, while the functions of the other three gene products are unknown. In this paper we report the biochemical and structural analysis of the cellulose synthase of *A. xylinum* in a highly purified form. The enzyme was found to contain distinct catalytic (UDP-Glc binding) and regulatory (c-di-GMP binding) domains, each residing in polypeptide structures that probably are proteolytically derived from the *bcsB* gene product. We also report the occurrence of similar immunologically related cellulose synthase-like peptide structures in plants and other cellulose-producing microorganisms.

MATERIALS AND METHODS

Cell Growth and Preparation of Enzyme Fractions. *A. xylinum* 1306-21 was grown in R-20 medium in the presence of 0.1% cellulase for 24 hr (6). Cells were ruptured in a French pressure cell and the membrane fraction was isolated, treated with trypsin, and then solubilized in digitonin (6, 7). The cellulose synthase was further purified from the digitonin-soluble fraction by enzyme-product entrapment essentially as described for chitin synthase (8), except that the glycerol cushion contained 12% (vol/vol) glycerol, 1 mM UDP-Glc, and 15 μM c-di-GMP in TME buffer (50 mM Tris·HCl, pH 8.5/10 mM $MgCl_2$/1 mM EDTA) and the reaction mixture contained 10 μM c-di-GMP and 1 mM UDP-Glc in 140 mM Tris·HCl, pH 8.5/5.5 mM $CaCl_2$/20 mM $MgCl_2$. The resultant pellet containing the entrapped enzyme was washed twice and finally suspended in TME buffer. The enzyme exhibits a specific activity of 230 nmol of glucose incorporated into cellulosic product per minute per milligram of protein, which represents an overall purification of 350-fold with respect to the crude extract and an average recovery of activity of 20%. The product has been characterized to be exclusively β1,4-D-glucan as in ref. 2.

The synthase was released from the product-entrapped state by a two-stage digestion with cellulase. Entrapped enzyme (500 μg of protein) was shaken for 15 min at 4°C with highly purified cellulase (20 μg) in 50 mM acetate (pH 5.0).

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Abbreviations: c-di-GMP, cyclic diguanylic acid; PDE-A, phosphodiesterase A.

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Unbound cellulase (>80% of the protein added) was removed by centrifugation and the pellet, containing actively bound cellulase and retaining all original synthase activity, was resuspended in acetate buffer, reshaken at 4°C for 4 hr, and centrifuged. Up to 50% of the original synthase activity was released into the supernatant. *Agrobacterium tumefaciens* C58 was grown on YEB medium (9) and *Rhizobium leguminosarum* 248 on TY medium (10). Stem tissue from etiolated mung bean, pea, and barley seedlings (3–5 days old) was homogenized and washed. Membranes were prepared as described (11). Cotton fiber membranes were prepared from locules (19–22 days post-anasthesis) as described (12).

Polyacrylamide Gel Electrophoresis. SDS/PAGE was carried out according to Laemmli (13). Cellulase-released cellulose synthase was electrophoresed in nondenaturing gels and c-di-GMP-dependent synthase activity regions on the gel were detected by fluorography (12). These regions were extracted for SDS/PAGE analysis as described (8).

Photolabeling. Direct photoaffinity labeling was carried out essentially as described (14). Purified cellulose synthase was mixed with either [^{32}P]c-di-GMP (1220 MBq/μmol) or [α-^{32}P]UDP-Glc (74 MBq/μmol) in a final volume of 30 μl containing 2 mM $CaCl_2$ in TME buffer (pH 7.5). The mixtures, in open tubes, were irradiated with a 254-nm UV lamp at a distance of 4 cm for 20 min at 4°C. To the mixtures was then added SDS sample buffer and, respectively, 7 nmol of unlabeled c-di-GMP or 500 nmol of unlabeled UDP-Glc. The mixtures were heated at 90°C for 5 min before SDS/PAGE. The gels were stained with Coomassie brilliant blue, destained, dried, and autoradiographed (4 hr to 1 day). The intensity of radiolabeled bands was determined by densitometric scanning.

Western Immunoblotting. A 561-amino acid peptide encoded by a 1683-base-pair DNA region between the *Sst* I and *Bam*HI restriction sites of the cellulose synthase gene (*bcsB*) (6) was expressed as a tripartite peptide in the vector pTac-Neo1.1 (ATCC 37686) in *Escherichia coli*. The peptide was purified by SDS/PAGE and injected into rabbits. Western blotting was performed (15) with the primary antiserum diluted 1:1000 in 0.3% Tween 20/phosphate-buffered saline. The immunological reaction was visualized by the alkaline phosphatase technique (15). For Western blot analysis of whole-cell extracts, exponentially growing cells were harvested and suspended in 50 mM Tris·HCl (pH 7.0) containing 10 mM EDTA and, where indicated, a combination of protease inhibitors: 0.5 mM phenylmethylsulfonyl fluoride, 10 μM leupeptin, 0.1 mM 7-amino-1-chloro-3-tosylamido-2-heptanone ("tosyllysine chloromethyl ketone"), and 0.1 mM L-1-tosylamido-2-phenylethyl chloromethyl ketone. The cells were sonicated for 2 min and immediately mixed with SDS sample buffer, boiled for 5 min, and subjected to SDS/PAGE and immunoblotting.

Gel Filtration. The molecular weight of native cellulose synthase was estimated by gel filtration on a calibrated Sepharose CL-4B column (1.5 × 90 cm).

Enzyme and Protein Assays. Cellulose synthase was assayed (4) in the presence of 20 μM UDP-Glc and 1 μM c-di-GMP. Diguanylate cyclase and PDE-A activities were determined as described (5). Protein was determined according to Bradford (16). c-di-GMP and [^{32}P]c-di-GMP were purified by HPLC (5). [α-^{32}P]UDP-Glc was prepared from [α-^{32}P]UTP and glucose 1-phosphate essentially as described (17). The labeled product was purified by HPLC.

RESULTS

Native Cellulose Synthase: Purification, Stability, Kinetics, and Molecular Weight. The enzyme purified 350-fold as described in *Methods and Materials* is retrieved in a pellet, presumably associated with its insoluble glucan product. Synthase activity cannot be resolubilized by extensive washing at either low or high ionic strength, by changes in pH, or by sonication. However, the enzyme may be effectively released from the product-entrapped state by treatment with cellulase. The highly purified synthase is entirely devoid of PDE-A and diguanylate cyclase activities.

The enzyme in the product-entrapped state is much more stable than the cruder membrane-bound and digitonin-solubilized forms (2, 7) and retains full activity at 30°C for up to 60 min. This enhanced thermal stability may be due to the tight association of the purified enzyme with its reaction product. Experiments with the digitonin-solubilized system further support this notion (Fig. 1). Inclusion of either activator, c-di-GMP, or substrate, UDP-Glc, has no significant effect on enzyme stability; however, in the presence of both of these, enzyme activity remains stable for up to 60 min at 30°C. Since these conditions are optimal for enzyme catalysis, the stability may indeed be related to the strong affinity of the synthase for newly formed glucan product that has not yet dissociated from the enzyme site. An alternative explanation is that the active form of the synthase (i.e., binding both its activator and substrate) is a particularly stable conformation.

The pH optimum of the purified enzyme (pH 7.9–8.6 at 30°C) does not differ from that of cruder preparations. The apparent K_m of the enzyme for UDP-Glc (125 μM), which is independent of c-di-GMP concentration, also conforms to earlier values (2, 7). The synthase reaction has an absolute requirement for Mg^{2+} (K_m = 3.3 mM). Unlike PDE-A, which is strongly inhibited by Ca^{2+}, the synthase reaction is insensitive to this cation. Finally, the kinetics of c-di-GMP activation, which in crude preparations and at extremely low effector concentrations display partially cooperative effects (18) (most probably due to residual PDE-A activity), obey a Michaelis–Menten relationship in the case of the purified enzyme. The apparent K_{act} value calculated for c-di-GMP (0.35 μM) is independent of UDP-Glc concentration. Gel filtration of the digitonin-soluble enzyme yielded one distinct peak of enzyme activity, corresponding to an apparent molecular mass of 420 kDa.

Structural and Functional Relatedness of Peptide Components of Cellulose Synthesis. SDS/PAGE of the highly purified enzyme gave rise to two major bands at 90 and 67 kDa and a less prominent band at 54 kDa, after staining with either

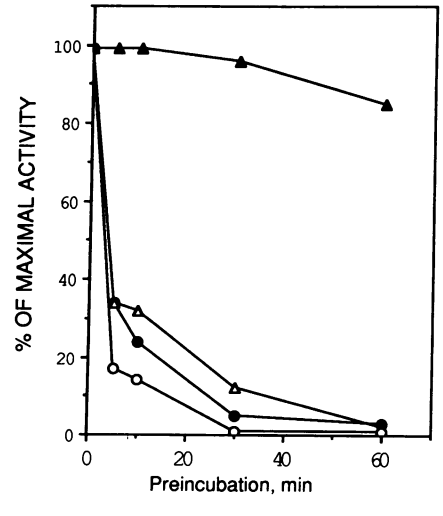

FIG. 1. Effects of substrate and activator on stability of cellulose synthase. Preincubation mixtures (at 30°C) contained digitonin-solubilized membranes (0.4 mg of protein) in TME buffer alone (○) or supplemented with 10 μM c-di-GMP (△), 1 mM UDP-Glc (●), or 10 μM c-di-GMP plus 1 mM UDP-Glc (▲). At the times indicated, aliquots were assayed for cellulose synthase activity.

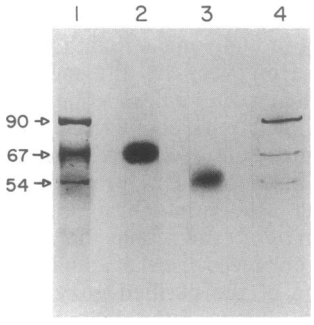

FIG. 2. SDS/PAGE, direct photoaffinity labeling, and immunoblotting of product-entrapped cellulose synthase. Lane 1, Coomassie blue-stained gel (10 μg of protein); lane 2, enzyme (2 μg) photolabeled with [^{32}P]c-di-GMP (10 μM); lane 3, enzyme (10 μg) photolabeled with [α-^{32}P]UDP-Glc (20 μM); lane 4, enzyme (9 μg) immunoblotted with anti-cellulose synthase antiserum. Molecular sizes were determined from protein standards run concurrently and are shown in kilodaltons at left.

Coomassie blue or silver nitrate (Fig. 2). So far, attempts at additional purification by subjecting the cellulase-resolubilized enzyme to a second entrapment step or to gel electrophoresis in nondenaturing gels have not yielded an appreciably different peptide pattern than may be seen here. It appears therefore that each of these three polypeptides is in some way related to the protein structure of native cellulose synthase. To test this hypothesis, a series of direct photoaffinity labeling experiments have been conducted.

Irradiation of purified cellulose synthase in the presence of [^{32}P]c-di-GMP followed by SDS/PAGE, Coomassie blue staining, and autoradiography results in the exclusive incorporation of label into a peptide at 67 kDa (Fig. 2, lane 2). The extent of radiolabeling is linear with irradiation time up to 20 min, and with the amount of enzyme up to 5 μg, and does not occur with a heat-inactivated enzyme or in the presence of excess EDTA. It appears from this experiment that the 67-kDa polypeptide actually contains all or part of the amino acid sequence comprising the c-di-GMP binding site of cellulose synthase. To establish further that this labeling reaction displays the properties expected for such a site, both the kinetics and specificity of the photoaffinity labeling were examined. Radiolabel incorporation as a function of ligand concentration yields a typical saturation curve (Fig. 3*A*). The concentration of c-di-GMP at which half-maximal binding occurs, 0.5 μM, is similar to the apparent K_{act} value measured for this compound as an activator of the cellulose synthase (see above). The photolabeling is also highly specific for c-di-GMP. Thus, other cyclic dinucleotides such as c-di-AMP or c-di-XMP, which neither activate the synthase nor inhibit its activation by c-di-GMP, similarly do not interfere with the labeling effect. On the other hand, unlabeled c-di-GMP or c-di-IMP, which is also a potent activator of the synthase (18), blocks the radiolabeling of the 67-kDa polypeptide by 90% and 70%, respectively, when present in 10-fold excess.

A similar set of direct photoaffinity labeling experiments were carried out employing the substrate itself, UDP-Glc (Fig. 2). As shown in lane 3, irradiation of purified enzyme in the presence of [α-^{32}P]UDP-Glc results in the labeling of a different peptide, which migrates in SDS/PAGE at 54 kDa. In this case too, the extent of labeling is linear with the time of irradiation up to 20 min and with protein concentration up to 40 μg. Similarly, UDP-Glc binding to the 54-kDa polypeptide as a function of ligand concentration yields a saturation curve with a K_d value of 100 μM (Fig. 3*B*), which compares favorably with the apparent K_m for UDP-Glc as a substrate in the synthase reaction. The labeling reaction is inhibited by 90% in the presence of 1 mM UDP-Glc and by 85% in the presence of 1 mM UDP, a competitive inhibitor of the synthase reaction (2). Under these conditions, compounds which have no effect on the synthase reaction, such as 1 mM UDP-xylose, GDP-glucose, glucose 1-phosphate, glucose 6-phosphate, and glucose 1,6-bisphosphate, do not interfere with the labeling effect. It thus seems likely that this peptide is closely associated with the substrate-binding or catalytic site of the enzyme. Notably, neither UDP-Glc (1 mM) nor c-di-GMP (20 μM) affects the labeling pattern of the other, indicating that activator and substrate bind to the enzyme in an independent fashion.

To verify that our conclusions regarding the identification of specific sites by affinity labeling were indeed relevant to the regulatory or catalytic activity of the enzyme, we compared the sensitivities of the cellulose synthase reaction and the direct photoaffinity binding reactions to the sulfhydryl reagent *p*-hydroxymercuribenzoate. As expected, preexposure of the enzyme to 1 μM of this reagent completely blocked the photolabeling of the enzyme with c-di-GMP or UDP-Glc as well as the cellulose-producing reaction.

A structural relationship between the 90-kDa polypeptide and the two smaller peptide components of cellulose synthase was indicated by immunochemical analysis with rabbit anti-cellulose synthase antiserum. This antiserum was raised against a protein translation product derived from the cloned cellulose synthase structural gene, *bcsB* (6). In Western blot analysis of highly purified enzyme preparations the antiserum strongly labeled the 90-kDa as well as the 67- and 54-kDa peptides (Fig. 2, lane 4). Indeed, with direct photoaffinity

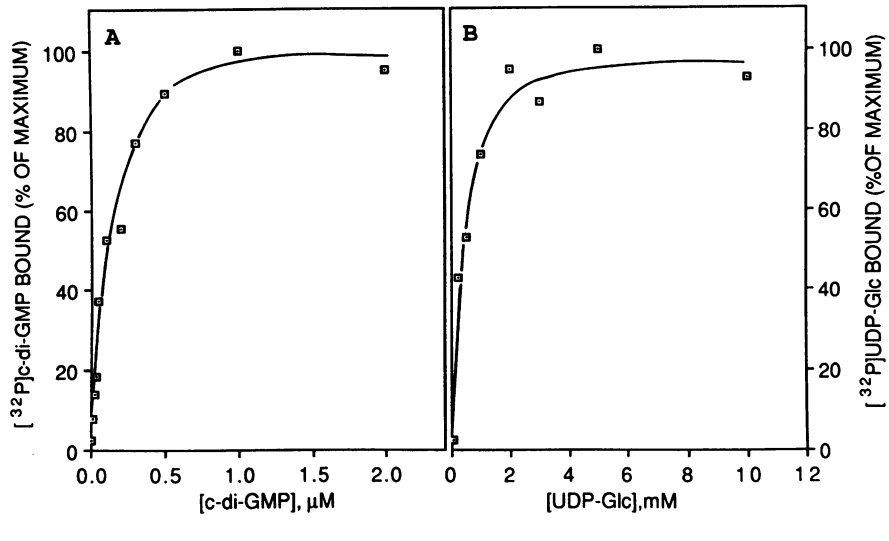

FIG. 3. Effect of ligand concentrations on photolabeling of product-entrapped cellulose synthase. (*A*) Enzyme (1 μg) photolabeled with various concentrations of [^{32}P]c-di-GMP. (*B*) Enzyme (9 μg) photolabeled with various concentrations of [α-^{32}P]UDP-Glc.

labeling as above, some labeling with either [^{32}P]c-di-GMP or [^{32}P]UDP-Glc could be detected in the 90-kDa region after long periods of autoradiography (14 days). Notably, the immunolabeling method demonstrated exclusively these three peptides for whole-cell extracts in either the presence or the absence of protease inhibitors. Thus, while the three major peptides of purified cellulose synthase appear to be distinct in their binding capacities, they are sufficiently related to display immunological crossreactivity. The three may all be derived directly from the *bcsB* gene product or arise from one or more structurally related genes.

As already reported (6), further evidence for the structural relationship between the two major cellulose synthase-related peptides (90 and 67 kDa) and the *bcsB* gene product was provided by the results of N-terminal amino acid sequence analysis. The sequence of the first 18 amino acids of the 90-kDa peptide is identical with the deduced amino acid sequence of *bcsB* starting at codon 25 (Fig. 4). The N-terminal amino acid sequence obtained for the 67-kDa peptide also shows a good match with the predicted sequence from codons 196–205 of *bcsB*.

Presence of Related Peptides in Other Cellulose-Producing Organisms. Cellulose biogenesis is a universal phenomenon, and the cellulose synthase may well be the only enzyme unique to the pathway leading to cellulose. To examine the possible occurrence of cellulose synthase-like peptides in other organisms, whole-cell extracts from other Gram-negative bacteria and from some species of plants were probed with the anti-cellulose synthase antiserum (Fig. 5). Western blot analysis demonstrated the presence of immunologically related peptides only in other cellulose-producing microorganisms such as *Agrobacterium tumefaciens* and *Rhizobium leguminosarum* and not in more closely related, but non-cellulose-forming, species such as *Acetobacter suboxidans*. Extracts derived from mung bean (*Phaseolus aureus*), peas (*Pisum sativum*), barley (*Hordeum*), and cotton (*Gossypium*) gave a number of discrete peptide bands, the most prominent being in the molecular mass region of 90 and 54 kDa. No such material was detectable in various mammalian extracts, which therefore do not harbor such a set of cellulose synthase-like antigens.

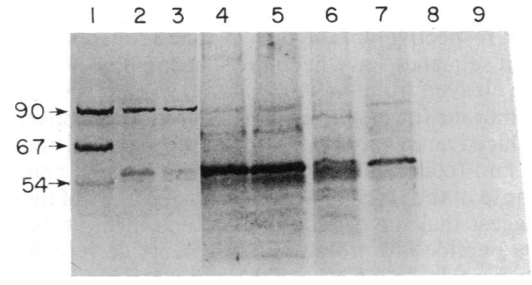

FIG. 5. Western blot analysis of bacterial and plant cell extracts with anti-cellulose synthase antiserum. Whole-cell extracts of *Acetobacter xylinum* (lane 1), *Agrobacterium tumefaciens* (lane 2), *Rhizobium leguminosarum* (lane 3), *Bacillus subtilis* (lane 8), *Acetobacter suboxidans* (lane 9) and membranous preparations of mung bean (lane 4), peas (lane 5), cotton (lane 6), and barley (lane 7) were subjected to SDS/PAGE and the separated proteins were transferred to nitro-cellulose paper and probed with the antiserum. Bacterial extracts were derived from 30 μg of cells and plant membranes from 60 μg of tissue. Additional extracts analyzed with negative results were from *E. coli*, beef liver, beef brain, and *Drosophila*.

DISCUSSION

In catalyzing the "committed" step in cellulose formation—a metabolic dead-end with regard to carbon utilization—the cellulose synthase would logically be a prime candidate for strict regulation. Our results on the protein structure of this enzyme and its mechanism, together with the recent genetic analysis of the overall process (6), provide the basis for a deeper understanding of the mechanisms of cellulose biogenesis throughout the cellulose-producing species.

From these results it appears that c-di-GMP binds directly to the enzyme in a reversible manner at a regulatory site distinct from the catalytic, or substrate-binding, site. These sites have been shown to be mutually independent in the sense that activator does not affect substrate binding and vice versa. Cellulose synthase in either a highly purified state or immobilized within polyacrylamide gel (12) retains sensitivity to the activator, indicating that the site through which the c-di-GMP effect is exerted is either integral to or tightly associated with the catalytically active structure of the enzyme. A different result would be expected if the cyclic dinucleotide, instead of binding directly, acted via an accessory agent such as a protein kinase (21) or a protease (22), which would probably be lost during purification or require a soluble phase in which to manifest its effect. Furthermore, since essentially no PDE-A or other structurally modifying activities could be detected in the course of the synthase reaction in highly purified synthase preparations, it appears that covalent modification of the activator is not an obligatory event in the glucan chain-forming reaction.

The cellulose synthase contains three major peptides, at 90, 67, and 54 kDa as resolved by SDS/PAGE. Direct photoaffinity labeling with either [^{32}P]c-di-GMP or [α-^{32}P]UDP-Glc indicates a differential relationship for the three peptides with respect to the structure of the active enzyme; activator- and substrate-specific binding sites are most closely aligned with the 67- and 54-kDa peptides, respectively, while the presence of such binding sites could hardly be demonstrated in the 90-kDa peptide.

Despite the presumed differential delegation of functions, all three of the peptide components of native cellulose synthase appear to be structurally related to the protein encoded by the cellulose synthase structural gene (*bcsB*). This conclusion is based on the immunological interaction of these peptides with anti-cellulose synthase antiserum and is further strongly supported, in at least the case of the 90- and 67-kDa peptides, by the high homology found between their N-terminal amino acid sequences and the amino acid se-

90 kDa peptide

Codon no.	25															42
Deduced amino acids	Ala	Pro	Ala	Pro	Gln	Pro	Ala	Gly	Ser	Asp	Leu	Pro	Pro	Leu	Pro	Ala	Ala	Ala
Sequenced amino acids	Ala	Pro	Ala	Pro	Gln	Pro	Ala	Gly	Ser	Asp	Leu	Pro	Pro	Leu	Pro	Ala	Ala	Ala

67 kDa peptide

Codon no.	195										205
Deduced amino acids	Lys	Gly	Cys	Thr	Asp	Pro	Ser	Asn	Gly	Leu	Leu
Sequenced amino acids		Gly	Gly	Val	Asp	Pro	Ser	Asn	Gly	Leu	Leu

FIG. 4. Comparison of the N-terminal amino acid sequences of the 90- and 67-kDa peptides with the sequences predicted by the cellulose synthase gene (*bcsB*). Bands resolved by SDS/PAGE were cut from the gels and electroeluted (19). N-terminal amino acid sequences were determined by Edman degradation (20). Deduced sequences are as referred to in ref. 6.

quences deduced from the *bcsB* gene starting at codons 25 and 196, respectively. [The first 25 amino acid residues of the deduced sequence have been proposed to comprise a leader peptide cleaved from the *bcsB* protein when it is deployed in the membrane (6).] Peptides starting from these positions on the deduced amino acid sequence of the gene and continuing to the end (codon 802) have molecular masses compatible with those of the isolated peptides. On the basis of these data we suggest that the 67-kDa peptide and most probably the 54-kDa peptide are proteolytic fragments derived from the 90-kDa peptide. In the case of the c-di-GMP-binding peptide, this proposal may be supported by the presence of lysine, a common proteolysis target, as the last amino acid prior to the initiation of the derived 67-kDa peptide sequence. The relative inability of the 90-kDa peptide to exhibit the two binding activities may be attributed to a conformational restraint within its tertiary structure that is relieved upon its cleavage. The precise function of this largest peptide is not clear, but it may represent an inactive precursor that undergoes post-translational processing to generate the 67- and 54-kDa peptides, both of which appear to participate directly in the regulatory and catalytic functioning of the enzyme. The native form of the cellulose synthase molecule (apparent molecular mass, 420 kDa) would then be that of a hetero-oligomeric protein complex containing both catalytically active (67- and 54-kDa) and inactive (90-kDa) components. An alternative hypothesis is that the 90-kDa peptide represents the active form of the cellulose synthase as encoded by the *bcsB* gene and the occurrence of the other two peptides is due to proteolysis during enzyme isolation, especially since this procedure involves a trypsinization step. However, it must be noted that similar affinity-labeled peptides were found in purified preparations where trypsin had not been introduced, leaving open the possibility that cleavage in these preparations may have occurred by some endogenous proteolytic activity. Although at this stage we tend to believe that our findings are more in line with the cell-directed processing hypothesis, further studies are needed to clarify this issue. If indeed the active form of cellulose synthase arises by the proteolytic processing of an inactive precursor, this will be analogous to the mechanism of zymogen activation of another β-linked homopolysaccharide-polymerizing enzyme, the chitin synthase from yeast, which has similarly been suggested to involve proteolytic cleavage of the structural gene product to its active form (23). Another (somewhat remote) possibility would be that the 67- and 54-kDa peptides are not derived from the *bcsB* gene product but rather arise from other structurally related gene(s).

It should be noted, however, that other reports (24, 25) have described the cellulose synthase of *A. xylinum* as comprising a single 83-kDa subunit and, at present, these differences have not been reconciled on the basis of variations in purification methodology or strain-specific features of the enzyme.

Of great potential importance is the immunochemical evidence presented herein, which may provide the long-sought link between c-di-GMP-dependent cellulose synthase of *A. xylinum* and other bacterial and plant cellulose-synthesizing systems. Immunochemical analysis employing antibodies against the *bcsB* gene product indicates that two cellulose-producing organisms, *Agrobacterium tumefaciens* and *Rhizobium leguminosarum*, as well as a variety of plant species including barley, cotton, mung bean, and pea, contain peptides immunologically related to those comprising the *A. xylinum* cellulose synthase. Although the regulatory interaction of c-di-GMP with cellulose synthase has not been demonstrated for the plant enzyme, most likely due to the inactivation of the enzyme upon cell disruption (1); recent affinity labeling studies have revealed the presence in cotton extracts of a peptide that specifically binds both c-di-GMP and UDP-Glc and has the same relative molecular mass as that reacting with the above antibody (R.M., M.B., Y. Amor, & D. P. Delmer, unpublished data). Taken together, these findings suggest a high degree of homology among the cellulose synthases of diverse organisms and, ultimately, may lead to a more unified theory for cellulose biogenesis and its regulation. This immunochemical demonstration of homology among various cellulose-producing organisms at the peptide level awaits further clarification at the genetic level by probing of the DNA from these plants and cellulose-producing bacteria with the *bcsB* gene from *A. xylinum*.

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