Maltodextrin-dependent Crystallization of Cyclomaltodextrin Glucanotransferase from *Bacillus circulans*

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Crystals of cyclomaltodextrin glucanotransferase from *Bacillus circulans* (EC 2.4.1.19) suitable for high-resolution X-ray analysis were obtained by vapor diffusion against 60% (v/v) 2-methyl 2,4-pentanediol buffered with 100 mM-sodium Hepes, pH 7.55. The crystals have \(P2_12_12_1\) space group symmetry, with \(a=120.4\) \(\text{Å}\), \(b=110.9\) \(\text{Å}\) and \(c=66.4\) \(\text{Å}\), and contain one molecule of 68,000 in the asymmetric unit. Growth of single enzyme crystals was found to require the presence of either \(\alpha\)-cyclodextrin, \(\beta\)-cyclodextrin, \(\gamma\)-cyclodextrin, or maltose in high molar excess, a requirement that could not be fulfilled by glucose, the basic building block of these compounds. Although the exact role of cyclic and linear maltodextrins in enzyme crystallization is not yet known, we have preliminary evidence that these compounds are degraded by the enzyme in the crystallization droplet.

The cyclomaltodextrin glucanotransferases (CGTases\(^\ddagger\); EC 2.4.1.19) catalyze the formation of cyclodextrins from starch and related \(\alpha(1\rightarrow4)\)-linked glucose polymers (French, 1957). CGTases are functionally related to the \(\alpha\)-amylases, which hydrolyze starch into linear products; limited stretches of primary sequence homology corresponding to conserved catalytic binding sites in the \(\alpha\)-amylase \(\beta\)-barrel domain (Matsuura et al., 1984; Buisson et al., 1987) suggest that the two enzyme classes are also structurally related (Kimura et al., 1987; Svensson, 1988). In contrast with \(\alpha\)-amylases, CGTases preferentially add a "non reducing end" glycosidic C-4 ROH across the scissile \(\alpha(1\rightarrow4)\) glycosidic bond, resulting in a glycosidic exchange. Cyclodextrins containing six or more glycosidic units are produced by an intramolecular transglycosylation reaction utilizing a single linear substrate molecule, e.g. amylase, or an \(\alpha(1\rightarrow4)\) branch of amylopectin with a non-reducing terminus. The relative proportions of the \(\alpha\), \(\beta\) and \(\gamma\)-cyclodextrins produced, corresponding to six, seven and eight circularly linked glycosidic units, vary with the bacterial source of the enzyme. Related bimolecular glycosidic exchange reactions are also catalyzed by CGTases, including reversal of the cyclodextrin-forming reaction, termed coupling (French et al., 1954), and glycosidic exchange between linear substrates (Norberg & French, 1950). In these reactions, the ROH acceptor added across the scissile glycosidic bond can be a sugar other than glucose (French et al., 1954; Kitahata & Okada, 1976). Hydrolytic activity observed in pure CGTase preparations (Kobayashi et al., 1978; Shiraishi et al., 1989) might be viewed as a type of glycosidic exchange where the acceptor is HOH.

Diverse applications for cyclodextrins have been found in research and industry, due to the ability of these compounds to form inclusion complexes with small molecules (Saenger, 1980; Szejtli, 1982). In the Netherlands, AVEBE has developed a process for...
production of cyclodextrins with the Bacillus circulans CGTase†, an enzyme that produces predominantly β-cyclodextrin (Bergsma & Spjut, 1986). The industrial process could be improved by isolation of mutant CGTases with improved product specificity, decreased product inhibition, and improved stability at extremes of temperature and pH. Rational design of such mutants will require detailed knowledge of the three-dimensional structure of the enzyme, as well as elucidation of the transglycosylation reaction pathway.

We report here our success in the formation of crystals of B. circulans CGTase that are suitable for high-resolution X-ray analysis. Two other crystallographic groups (Kubota et al., 1988; Hoffman et al., 1989) are also pursuing the structure determination of CGTases from B. stearothermophilus and a separately isolated strain of B. circulans, respectively. The crystals obtained by these groups differ significantly from ours in crystal cell parameters and growth conditions. Although we predict that the overall structure of CGTase will be very similar in each case, we may be able to obtain unique information about the enzymatic activity from our crystals because they were obtained under conditions that require the presence of substrate.

The strain B. circulans 251 was isolated from flax retting water by NIKO-TNO (Nederlands Instituut voor Koolhydraatonderzoek-Toegepast Natuurwetenschappelijk Onderzoek, Groningen, The Netherlands) and is deposited as RIV no. 11115 (Rijksinstituut voor Volksgezondheid, Bilthoven, The Netherlands). CGTase was purified from culture broth by α-cyclodextrin–Sepharose affinity chromatography essentially as described by László et al. (1981). The purified enzyme migrated in a single band with M, 68,000 on SDS/polyacrylamide gels stained with silver. Isoelectric focusing revealed the complete absence of α-cyclodextrin during the course of vapor diffusion. This hypothesis is supported by the finding that completely isomorphous crystals of the enzyme can be grown if an equivalent mass of α-cyclodextrin or maltose, but not n-glucose, is substituted for α-cyclodextrin in the crystallization protocol. The complete absence of α-cyclodextrin crystals under normal CGTase crystallization conditions suggests that α-cyclodextrin must be converted into one or more soluble products by the enzyme during the course of vapor diffusion. This hypothesis is supported by the finding that completely isomorphous crystals of the enzyme can be grown if an equivalent mass of β-cyclodextrin, γ-cyclodextrin or maltose, but not n-glucose, is substituted for α-cyclodextrin in the crystallization protocol. The α-cyclodextrin requirement must therefore translate into either a general requirement for compounds containing α(1→4) glycosidic bonds, or a specific requirement for a compound that can be enzymatically obtained from both maltose and α-cyclodextrin. Thin-layer chromatographic analysis (data not shown) indicates that the cyclodextrins are degraded into short...
linear maltodextrins plus glucose, and that maltose is degraded into glucose. In each case, an additional degradation product is observed, which we suggest may be an MPD-glucoside, on the basis of chromatographic mobility and staining properties. We have not been able to purify this compound in sufficient quantity for identification by analytical methods.

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References