Enzymic synthesis of cyclothiomaltins

The effective conversion of 4-thio-α-maltosyl fluoride 1 into cyclothiomaltins 2, 3 and 4, using cyclodextrin glycosyltransferase enzymes from Bacillus circulans, is described.

Cyclodextrin glycosyltransferases (CGTase, EC 2.4.1.19) are bacterial enzymes that catalyse the transformation of starch and related maltoligosaccharides to cyclodextrins (CDs), mainly cyclo-α(1→4)-glucohexaoside (α-CD), cyclo-α(1→4)-glucoheptaoside (β-CD) and cyclo-α(1→4)-glucooctaoside (γ-CD). However, cyclo-α(1→4)-gluconacontaose (δ-CD) and cyclo-α(1→4)-glucooctadecaose (η-CD) have also recently been isolated in minute amounts and characterised. In 1983, enzymic conversion of α-maltosyl fluoride into α-, β- and γ-CDs was demonstrated, and since then, this approach has been used for the synthesis of regioselectively substituted CDs starting from α-maltosyl or maltotriosyl fluorides modified at their non-reducing C-6 position. We describe here an efficient synthesis of new cyclo-α(1→4)-4-thiomaltotetraoside 2, thiomaltopentaoside 3 and thiomaltohexaoside 4 from 4-thiomaltosyl fluoride 1 using CGTase enzymes.

Attempts to obtain cyclothiomaltins using this approach have already been published, but only linear 4-thiomaltosyl dimer and trimer were formed in low amounts when an impure commercial preparation of CGTase enzyme was used. In order to expand our knowledge of carbohydrate-CGTase protein interactions and to have access to new cyclodextrins for supramolecular studies, we re-investigated the reaction of 4-thio-α-maltosyl fluoride 1 and pure CGTase 1 from Bacillus circulans strain 89 and CGTase 2 from B. circulans strain 251. Although comparison of the three-dimensional structures of the two B. circulans enzymes reveals that nearly all of the 25% differences are on the surface of the molecules, we might expect that they present some difference in their specificity.

In the first set of experiments, 4-thio-α-maltosyl fluoride 1 was incubated with CGTase 1 or CGTase 2 and the enzymatic mixtures were analysed by thin layer chromatography (TLC) on silica plates and high performance liquid chromatography (HPLC) using a μ-Bondapak NH2 column. The TLC patterns and HPLC profiles were identical and showed that oligomerisation occurred. After treatment with β-amylase, an exoglucanase which hydrolyses the penultimate bond of the non-reducing end of linear maltoligosaccharides and which is unable to attack cyclodextrins, linear hemithiomaltodextrins were converted into 4-thiomaltose 5. Under the conditions used, the time-course of the reaction shows that the optimum time for recovery of cyclothiomaltins is around 10 h, and that no interconversion occurred between these cyclic compounds. The enzymatic mixture was treated as described and purified by preparative HPLC using μ-Bondapak NH2 column. Cyclothiomaltins 2, 3 and 4 were isolated in 16, 14 and 7% yield, respectively. Importantly, it should be noted that with the same quantity of CGTase 2 enzyme, α-maltosyl fluoride afforded a mixture of α-, β- and γ-CDs after only 1 h with β-CD as the predominant product, while maltotriose was hydrolysed into glucose and maltose. It is interesting that, in the first experiment, cyclo-α(1→4)-4-thiomaltotrioside was not obtained and only the linear hemithiomaltohexaoside was observed when the β-amylase treatment was omitted. The shift in the reaction products towards larger CD ring sizes may be explained by the high flexibility of conformation of the 4-thiomaltosyl residues. The complication properties of these new molecules and their biochemical properties will be described in due course.

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Footnotes
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§ We decided to use the nomenclature proposed by Lichtenthaler and Innell for the natural compounds, and we propose the generic name of cyclothiomaltins for these new compounds consisting of 4-thiomaltosyl repeating units.
¶ CGTase 2 (19.5 U cm⁻³, 60 mm³) was added to a solution of compound I (55 mg, 0.15 mmol) in phosphate buffer (0.2 mol dm⁻³, pH 6.5, 5 cm³).

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References


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