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Enzymic synthesis of cyclothiomaltins

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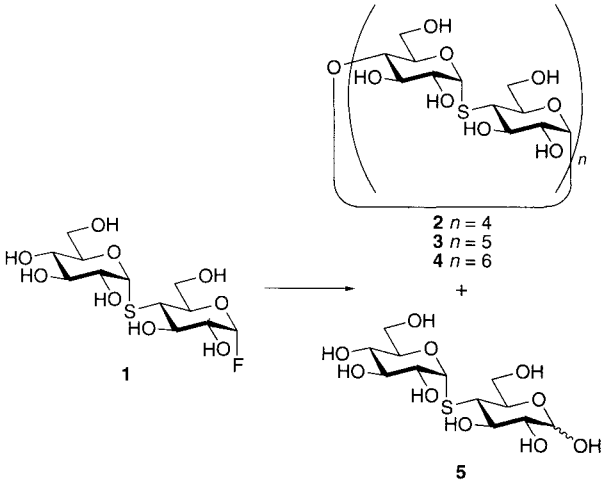
The effective conversion of 4-thio-α-maltosyl fluoride 1 into cyclothiomaltins 2, 3 and 4, using cyclodextrin glycosyltransferase enzymes from *Bacillus circulans*, is described.

Cyclodextrin glycosyltransferases (CGTase, EC 2.4.1.19) are bacterial enzymes that catalyse the transformation of starch and related maltooligosaccharides 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).[1]§ However, cyclo-α(1 → 4)-gluconononaoside (δ-CD) and cyclo-α(1 → 4)-glucododecaoside (η-CD) have also recently been isolated in minute amounts and characterised.[2] In 1983, enzymic conversion of α-maltosyl fluoride into α-, β- and γ-CDs was demonstrated,[3] 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.[4,5] 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,[4] 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 8[6] and CGTase 2 from *B. circulans* strain 251.[7] 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,[8] we might expect that they present some difference in their specificity.



Scheme 1

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 NH_2 column. The TLC patterns and HPLC profiles were identical and showed that oligomerisation occurred. After treatment with β-amylase, an *exo*-glucanase which hydrolyses the penultimate bond of the non-reducing end of linear maltooligosaccharides and which is unable to attack cyclodextrins,[9] 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 NH_2 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 D-glucose and maltose. It is interesting that, in the first experiment, cyclo-α(1 → 4)-4-thiomaltotrioside was not obtained and only the linear hemithiomaltohexaose 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.[10,11] The complexation 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 Immel for the natural compounds,[1b] 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 mm^3) was added to a solution of compound **1** (55 mg, 0.15 mmol) in phosphate buffer (0.2 mol dm^{-3}, pH 6.5, 5 cm^3). The mixture was incubated at 40 °C for 10 h. The reaction was stopped by boiling for 10 min and then the proteins eliminated by spinning. The supernatant was freeze-dried, diluted in the same phosphate buffer (1.25 cm^3) and then treated with β-amylase (20 U cm^{-3}, 1 mm^3) at 40 °C for 24 h. After boiling, the reaction mixture was treated with TMD-8 mixed bed resin (Sigma, St Louis, MO USA), freeze-dried and purified on HPLC (μ-Bondapak NH_2 column, Interchim, Montluçon, France) with a 60:40 MeCN–water mixture as eluent. All new compounds gave satisfactory high resolution mass spectra.

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