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ENZYMATIC SYNTHESIS OF CYCLODEXTRINS WITH α-GLUCOSYLFLUORIDE AS A SUBSTRATE FOR CYCLODEXTRIN-α(1→4)GLUCOSYLTRANSFERASE

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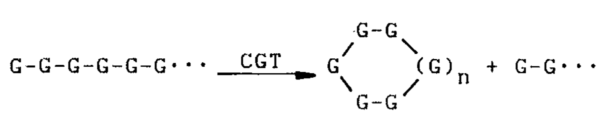
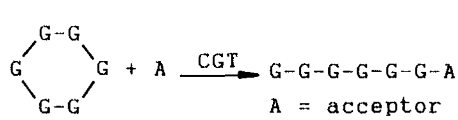
Summary:
By use of immobilized cyclodextrin-α(1→4)glucosyltransferase α-glucosylfluoride is transformed in high yield predominantly into cyclodextrins and maltooligomers as side products.

General Part

Cyclodextrins are well known products obtained by reaction of starch with cyclodextrin-α(1→4)glucosyltransferase [E.C. 2.4.1.19] from Bacillus macerans.[1,2] Recently T. Ogawa et al.[3] succeeded in the first chemical synthesis of α-cyclodextrin. Cyclodextrins form inclusion complexes with a large number of physiologically effective substances and are of great interest in pharmaceutical research.[4] So far the following reactions catalyzed by cyclodextrin-glucosyltransferase (CGT) are known:[5-7]

1. Cyclization: 

α-,β-,γ-Cyclodextrin (n=1,2,3)

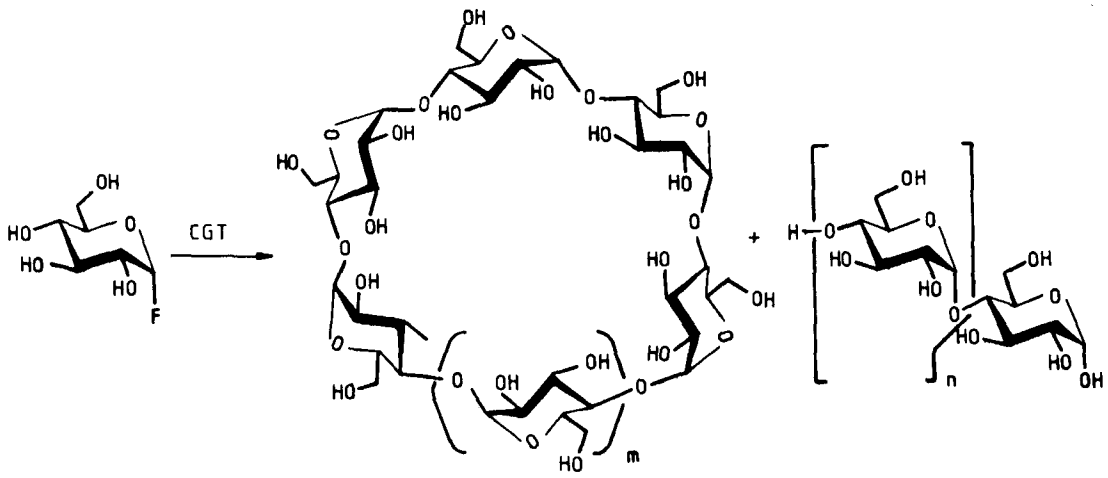
2. Coupling: 

3. Disproportionation:

$$G_m + G_m \xrightarrow{CGT} G_{m+x} + G_{n-x}$$

Recently several studies could demonstrate that certain enzymes in carbohydrate chemistry accept α-glucosylfluoride as a substrate.[8-10]

We observed that the enzyme cyclodextrin-glucosyltransferase {(1→4)-α-D-glucan: [(1→4)-α-D-glucopyranosyl]-transferase (cyclizing), E.C. 2.4.1.19, CGT} is able to transform glucosylfluoride into a mixture of α- and β-cyclodextrins as well as maltooligomers:



Immobilization of the Enzyme

Cyclodextrin-α(1→4)glucosyltransferase (500 mg = ~9000 units) is dissolved in 0.05 M sodiumacetate buffer, pH 6.0 (10 ml) and shaken with a silica gel support functionalized with glutardialdehyde (5 g) for 4 h at 20°C.[11] Subsequently the gel is washed with bidist. water (200 ml). The immobilized enzyme is now directly available for the reaction. It can be stored at 4°C for more than four weeks without any loss of activity.

Synthesis of Cyclodextrins and Maltooligomers

α-Glucosylfluoride (1.0 g, 5.5 mmole) is dissolved in 0.05 M sodium acetate buffer, pH 6.0 (25 ml) and the immobilized enzyme added (1 ml gel ~1000 units). The reaction proceeds under slight shaking at 45°C with the pH-value held constant by automatic titration with 0.5 M NaOH. After 20 min the reaction is terminated and the gel can be filtered. The reaction is followed by the tlc on silica gel plates (propanol/ethanol/water = 5:3:2).[12] Yields: α-cyclodextrin: 300 mg (30%), β-cyclodextrin: 380 mg (38%), maltooligomers (glucose up to maltononaose): 320 mg (32%). R_F-values: α-GlcF: 0.78; Glc: 0.68; α-cyclodextrin: 0.57; β-cyclodextrin: 0.54; [γ-cyclodextrin: 0.51].

Analytical Methods

The reaction mixture can be separated[13] on a RP-18 HPLC-column (0.8 × 50 cm, 7 μm, Merck). Using water as the eluent and an elution velocity of 3 ml/min the linear maltooligomers from the mono- to the nonasaccharide are obtained exclusively. The cyclodextrins are retained completely due to strong interactions with the column material. Further elution with water/methanol = 9:1 gives all linear maltooligomers as one unseparated fraction. This is followed by the nicely separable α- and β-cyclodextrins the analytical data of which are fully in agreement with authentic material.[14]

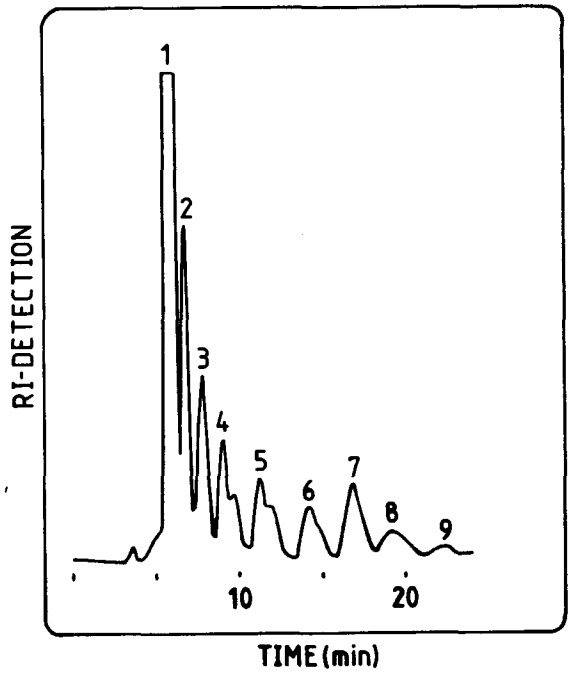


Fig. 1. Elutionprofile of Maltooligomers: Glucose (1) to Maltononaose (9); Column : RP-18 (0.8×50 cm, 7 μm, Merck); Solvent: water; Flow-rate: 3 ml/min; Refraction index detection.

Fig.2. Elutionprofile of Cyclodextrins: Maltooligomers (1), α-Cyclodextrin (2), β-Cyclodextrin (3); Solvent: water/methanol, 9:1; other conditions as in Fig. 1.

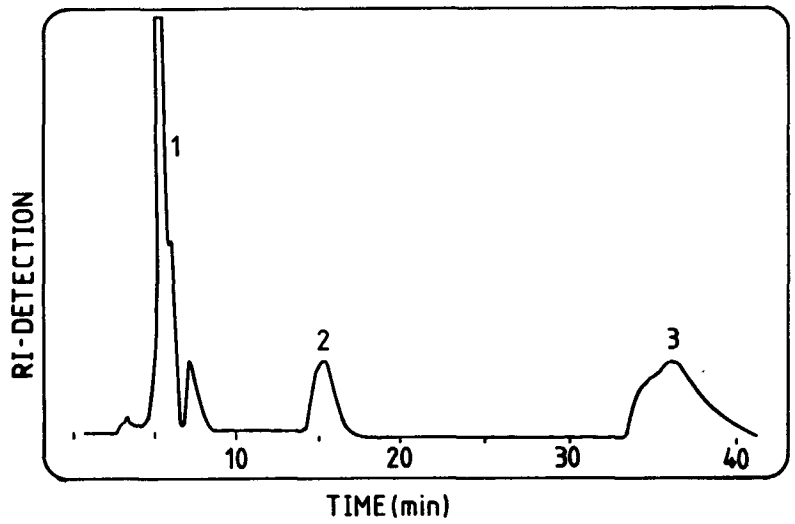


Fig. 2

Results

The enzyme cyclodextrin-α(1→4)glucosyltransferase [E.C. 2.4.1.19] is immobilized for the first time using a silica gel support functionalized with glutardialdehyde as favourably applied for other purposes recently.[15] The immobilized enzyme shows no loss of activity after four weeks when stored at 4°C.

α-Glucosylfluoride is accepted as a substrate by the enzyme which results in high yields of cyclodextrins (approx. 70%) and in addition some maltooligomers (approx. 30%). In order to prevent the released hydrogen fluoride from acidolysis of unreacted educt as well as maltooligomers the pH-value must be maintained constant by regular addition of sodium hydroxide.

Acknowledgement

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References

1. D. French, *Adv. Carbohydr. Chem. Biochem.* **12**, 189 (1957).
2. W. Saenger, *Angew. Chem., Int. Ed. Engl.* **19**, 344 (1980).
3. T. Ogawa and Y. Takahashi, *Carbohydr. Res.* **138**, C5 (1985).
4. M.L. Bender and M. Komiyama: *"Cyclodextrin Chemistry"* Springer Verlag, Berlin 1978.
5. H. Bender, *Carbohydr. Res.* **78**, 133 (1980).
6. K. Wallenfels, P. Földi, H. Niermann, H. Bender and D. Linder, *Carbohydr. Res.* **61**, 359 (1978).
7. H. Bender, *Carbohydr. Res.* **78**, 147 (1980).
8. P.J. Card and W.D. Hitz, *J. Am. Chem. Soc.* **106**, 5348 (1984).
9. D.G. Drueckhammer and C.-H. Wong, *J. Org. Chem.* **50**, 5912 (1985).
10. A.M. Gold and M.P. Osber, *Biochem. Biophys. Res. Commun.* **42**, 469 (1971).
11. H.H. Weetall, *Meth. Enzymol.* **44**, 134 (1976).
12. K. Koizumi, T. Utamura and Y. Okada, *J. Chromatogr.* **321**, 145 (1985).
13. G.D. McGinnis, S. Prince and J. Lowrimore, *J. Carbohydr. Chem.* **5**, 83 (1986).
14. ^{1}H-NMR (300 MHz, D_2O): α-cyclodextrin: δ = 4.89 (d, $J_{1,2}$ = 3.7 Hz, 1-H); β-cyclodextrin: 4.97 (d, $J_{1,2}$ = 3.7 Hz, 1-H); [γ-cyclodextrin: 5.05 (d, $J_{1,2}$ = 4.0 Hz, 1-H)].
15. J. Thiem and W. Treder, *Angew. Chem.*, submitted.

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