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Preparation, Characterization, and Pharmaceutical Application of Linear Dextrins. I. Preparation and Characterization of Amylodextrin, Metastable Amylodextrins, and Metastable Amylose

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The linear dextrin amylodextrin was prepared by enzymatic hydrolysis from waxy maize. Four metastable amylodextrins were prepared by complexation with different volatile organic compounds. All products showed partial dissolution into water at room temperature, because of dissolution of molecules with a lower DP. X-ray diffractometry revealed a helical conformation with six glucose units per turn for amylodextrin and metastable amylodextrins prepared with small molecules, and a helical conformation with seven glucose units per turn for metastable amylodextrins prepared with larger molecules. All metastable amylodextrins showed a helix with reduced distance between two turns as compared to amylodextrin. Metastable amylose, prepared from Amylose V, showed a helical conformation again with a reduced distance between two turns compared to Amylose V. FTIR analysis indicated a more flexible conformation for Amylose V and metastable amylose than for the amylodextrins.

KEY WORDS: amylose; amylodextrin; complexation; enzymatic hydrolysis; linear dextrins; structural characterization.

INTRODUCTION

Most native starches consist of two polymers of glucose, called amylose and amylopectin (1). The former is a mainly linear chain molecule composed of α-D-glucopyranose residues linked by α-1,4 glycosidic bonds, while each of the branches of the amylopectin molecule has the same structure as amylose but, in addition, contains α-1, 6 glycosidic bonds at the points where branching occurs (Fig. 1). Hydrolysis of starch, amylose, and amylopectin yields dextrins, maltose, or glucose (2). Dextrins have the same chemical structure as amylose and amylopectin, but the degree of polymerization is much lower (generally <100). Dextrins can be linear, branched, or cyclic. The latter, cyclodextrins, are of interest because of their ability to improve drug bioavailability by means of inclusion complexes (3).

Since linear dextrins are not used in pharmaceutical practice, we explored the utility of the linear dextrin amylodextrin. While the ability of amylose to form complexes with organic molecules is well documented (4–6), only one paper (7) and two patents (8,9) deal with complexation of molecules by dextrins. Because complexes of amylose tend to dissociate in the presence of water with subsequent precipitation (retrogradation) (10), this type of amylose may be characterized as metastable amylose. In analogy, possible complexes of amylodextrin are called metastable amylodextrins. From the ability of amylose to complex organic molecules, it is expected that amylodextrin will also be able to complex drug molecules. In analogy with the cyclodextrins (3), amylodextrin could thus serve as an excipient to enhance or to control drug release from solid dosage forms.

This paper reports the preparation and characterization of amylodextrin, metastable amylodextrins, and metastable amylose. Four products of metastable amylodextrins were prepared by using different complexing agents. The preparation was performed in analogy with the preparation of metastable amylose from Amylose V. The different products were characterized by their solubility and sterical conformation, analyzed by X-ray diffraction and FTIR analysis, and compared with literature values as published for amylose.

MATERIALS AND METHODS

Chemicals

Waxy maize starch and pullulanase (Promozyme) were supplied by Kon. Bijenkorf (Koog a/d Zaan, The Netherlands) and NOVO (Bagsværd, Denmark), respectively. Amylose V and β-cyclodextrin were obtained from Avebe (Veendam, The Netherlands). All other products and reagents used were of analytical grade.

Preparation of Amylodextrin, Metastable Amylodextrins, and Metastable Amylose

Amylodextrin, metastable amylodextrins, and metastable amylose were prepared according to the procedures as presented in Scheme I.

Determination of the Degree of Polymerization (DP)

The mean DP of amylodextrin was determined by measuring the reducing capacity and the total carbohydrate content. The former method was carried out according to Nelson and Somogyi (11) and the total carbohydrate was mea-

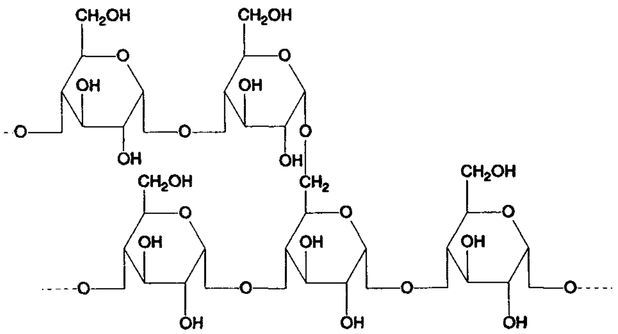

Fig. 1. The chemical structure of amylopectin.

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Amylodextrin

Waxy maize starch (500 g)
| 4 L water, 100°C
↓
Gelatinized starch
| 55°C + 15,000 PUN[a] pullulanase,
| 20 hr
↓
Dissolved amylodextrin + precipitated
amylose and starch proteins
| Heating to 100°C
| Centrifugation (9000*g*, 15 min)
↓
Dissolved amylodextrin
| 20°C, 24 hr
↓
Precipitated amylodextrin
| Centrifugation (9000*g*, 15 min)
↓
Solid amylodextrin
| Wash 3 times with ethanol 96%
| Wash 1 time with abs. ethanol
| Dry at 50°C
↓
Amylodextrin (yield, 80%)

Metastable amylodextrins

Amylodextrin (32 g)
| 800 mL water, 80°C
↓
Dissolved amylodextrin
| 80°C + 1-octanol (0.4%, v/v)
| or 2-methyl-1-butanol (3.1%, v/v)
| or cyclohexanol (5.7%, v/v)
| or 3-methylcyclohex. (5.7%, v/v)
↓
Precipitated complex of amylodextrin
| Cooling to 20°C during 20 hr
| Spray-drying (Büchi 190, Flawil, Switzerland)
| (flow, 400 normliter/hr, T_{in} 100°C, T_{out} 60°C)
↓
Metastable amylodextrins (yield, 70%)

Metastable amylose

Amylose V (60 g)
| 1 L 1 *N* NaOH, 20°C
↓
Dissolved amylose
| 50°C glacial acetic acid,
| adding glacial acetic acid to neutral pH
| + 2-Methyl-1-butanol
| (2.2%, v/v)
↓
Precipitated amylose complex
| Cooling to 20°C (20 hr)
| Centrifugation (9000*g*, 15 min)
| Wash 4 times with ethanol 96%
| Wash 1 time with abs. ethanol
| Dry at 20°C
↓
Metastable amylose (yield, 80%)

Scheme I. The preparation of amylodextrin, metastable amylodextrins, and metastable amylose. [a]1 PUN is defined as the amount of enzyme that, under normal conditions, hydrolyzes pullulan, liberating reducing carbohydrate with a reducing power equivalent to 1 µmol of glucose/min.

sured after reaction with anthrone according to Corrigan and Stanley (12). The amylodextrin could be separated into ranges with a different mean DP value by means of HPLC according to Kobayashi *et al.* (13). The column used was a TSK 20-XL (300 * 7.5 mm), while the eluent was milli-Q-water. The temperature was 44°C and the flow was 1 mL/min. The reducing capacity and the total carbohydrate content of the fractions isolated were measured.

Determination of Aqueous Solubility

The aqueous solubility of Amylose V, metastable amylose, amylodextrin, and the four metastable amylodextrins was determined by adding 10 mL water to varying quantities of the substance. The samples were shaken during 5 days at 20°C and filtered through a 0.45-µm filter (Schleicher & Schuell, Dassel, Germany), and the concentrations of dissolved substance in the resulting filtrates were measured spectrophotometrically after reaction with anthrone (12).

X-Ray Diffraction and Fourier Transform Infrared Spectroscopy

A Guinier Hagg camera (XDC-700, Jungner Instrument, Stockholm, Sweden) generated X-rays with a wavelength of 1.5406 Å, which were used for powder X-ray diffractometry.

FTIR spectroscopy was carried out as transmission spectroscopy on a Bruker 113v FTIR, operating at 2-cm^{-1} resolution using a TGS-detector. The number of scans was 128. The apodization function was Happ Genzel and the zero filling factor was 2. KBr was used as matrix diluter in all measurements.

RESULTS AND DISCUSSION

Preparation of Amylodextrin, Metastable Amylodextrins, and Metastable Amylose

Acid hydrolysis of starch generally results in dextrins with a highly branched structure. Amylodextrin was therefore prepared by selectively hydrolyzing the α-1, 6 glycosidic bonds of amylopectin with the enzyme pullulanase (13). Previous isolation of amylopectin from waxy maize starch, which accounts for almost 100% of amylopectin (1), is not necessary. Waxy maize was therefore used in this study as raw material.

Amylodextrin was prepared as described in two patents (8,9) of the Hayashibara Company (Okayama-shi, Japan). The first patent (8) reports the preparation of linear dextrin from potato starch by enzymatic hydrolysis of the α-1,6 glycosidic bonds of the amylopectin fraction. Potato starch contains 20% amylose which is removed after hydrolysis by fractional precipitation. The patent reports different methods of fractionation including the precipitation of amylose at a temperature between 40 and 90°C. In this temperature range the linear dextrin remains in solution. The linear dextrin is subsequently precipitated by cooling down to room temperature. In this study this method of fractionation was applied after previous coagulation of the enzyme at 100°C to remove the enzyme, the amylose (waxy maize contains about 1% amylose), and the starch proteins present.

The patent reports alternative ways of fractionation in-

Table I. Critical Concentrations of Some Complexing Agents and Their Corresponding Saturation Concentrations in Water

Complexing agent	Critical concentration (%, v/v)	Solubility (%, v/v)
1-Octanol	0.04	0.13
2-Methyl-1-butanol	1.8	3.1
Cyclohexanol	0.5	5.7
3-Methylcyclohexanol	0.5	5.7

cluding complexation with 1-butanol. In the present study this method was applied for the preparation of metastable amylodextrins from dissolved amylodextrin. Contrary to the patent, four different chemicals were used instead of 1-butanol.

Muetgeert (6) published a study on the fractionation of starch into amylose and amylopectin by complexation. He demonstrated that most complexing agents possess a rather narrow region of concentration in which optimum fractionation is obtained. This optimum concentration of a complexing agent is designated the "critical concentration." Table I illustrates the critical concentrations of four complexing agents and their corresponding saturation concentrations in water. At concentrations higher than the critical concentration, increasing amounts of amylopectin precipitate. The lower affinity of branched amylopectin for the complexing agent can be explained by steric hindrance. This enables only the outer branches to complex the agent. Reducing the degree of polymerization (DP) of amylose resulted in a reduced affinity for complexing agents (7). Because the DP of the outer branches of amylopectin is very low compared with the DP of amylose, the latter has a much higher affinity for complexing agents. Accordingly, dextrins exhibit a lower affinity for complexing agents than amylose (7). Indeed, for each complexing agent concentrations higher than the critical concentration were needed in the present study to complex and precipitate amylodextrin. For 2-methyl-1-butanol, cyclohexanol, and 3-methyl-cyclohexanol amounts equal to the saturation concentration were found to be sufficient to precipitate the metastable amylodextrin. However, for 1-octanol higher amounts were needed. The suspensions of metastable amylodextrin were finally spray-dried. According to the second patent (9), the inlet temperature was not higher than 100°C.

The procedure as applied for the preparation of metastable amylodextrins could not be applied to the preparation of metastable amylose. In contrast to amylodextrin, which is soluble in water at 85°C, Amylose V [V = *verstuivingsgedroogd*[4] (Dutch) = spray-dried] needs a water temperature of 160°C, because of the difference in DP of the two compounds. Because Amylose V dissolves completely in alkaline solutions at room temperature, a solution of 1 *N* NaOH was used to dissolve this compound. The solution was neutralized before the addition of 2-methyl-1-butanol as complexing agent. In contrast to the preparation of metastable amylodextrin, the critical concentration of 2-methyl-1-butanol should be sufficient to precipitate metastable amylose (6). Because of the absence of amylopectin in Amylose V, a concentration higher than the critical concentration was used to ensure complete precipitation of metastable amylose and stabilization of the complex during workup. The suspension of metastable amylose was subsequently filtered and washed with ethanol, because spray-drying was troublesome.

A minimum concentration of complexing agent to ensure the formation of complexes of amylose or amylodextrin implies that the complexes dissociate in the absence of complexing agent. The complexes are therefore thermodynamically unstable in water and thus called metastable.

Degree of Polymerization of Amylodextrin

Figure 2 represents the distribution of the DP of amylodextrin determined by HPLC. The chromatogram shows a small fraction and a large bimodally distributed fraction. The former fraction (DP ≈100) is related to β-limitdextrin (14). The bimodally distributed fraction accounted for about 75% of the molecules with a median DP of 20 and for 25% of the molecules with a DP of 50. A bimodal distribution agrees with the structure of amylopectin; a molecule of this compound accounts for 75% of so-called A-chains and for 25% of B-chains and it contains one C-chain (15). The A-chains (mean DP = 20) are joined to the remainder of the molecule with a single 1,6-glycosidic bond. B-chains (mean DP = 50) are joined through a 1,6 bond but carry one or more A- and/or B-chains on primary hydroxyl groups; the single C-chain carries the sole reducing group.

The enzyme pullulanase hydrolyses the outer branches of amylopectin. This process would theoretically result in bimodally distributed dextrin. However, due to the inhibitory effect of the product on pullulanase (14), amylopectin is hydrolyzed incompletely, leaving molecules with a highly branched structure, called β-limitdextrin.

Aqueous Solubility of the Products

The solubility of Amylose V and metastable amylose was too low for analysis as a result of their high DP (>100). For both amylodextrin and the metastable amylodextrins, increasing concentrations of dissolved amylodextrin at room

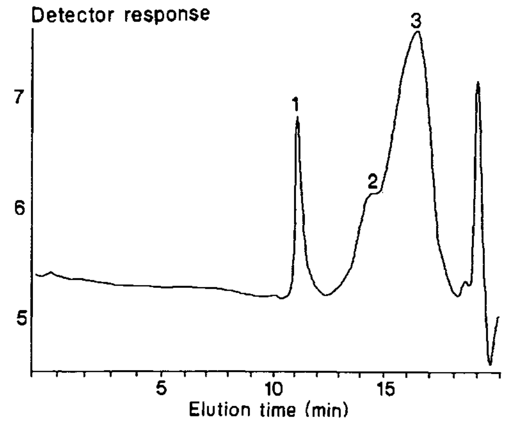

Fig. 2. Chromatogram representing the distribution of the degree of polymerization of amylodextrin. (1) β-Limitdextrin; (2) median DP 50; (3) median DP 20.

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[4] By Avebe (Veendam, The Netherlands).

temperature were found with increasing amounts of substance added to water. This relationship appeared to be linear for all five compounds (Fig. 3). This new phenomenon points to a constant percentage of each compound soluble in water. The fraction dissolved in water at room temperature could merely represent the molecules with a lower DP. This assumption is supported by determination of the DP: amylodextrin showed a mean DP of 35, while for the dissolved fraction a DP of 24 was measured. Linear regression analysis showed that between 16 and 20% of amylodextrin and the metastable amylodextrins prepared with 1-octanol or 2-methyl-1-butanol dissolved in water at room temperature (Table II). However, the metastable amylodextrins prepared with cyclohexanol or 3-methylcyclohexanol dissolved about 35% into water (Fig. 3 and Table II). The mean DP of dissolved metastable amylodextrin prepared with cyclohexanol was found to be 28, indicating that also from metastable amylodextrins merely molecules with a low DP dissolved in water at room temperature. The difference observed between the metastable amylodextrins prepared with 1-octanol or 2-methyl-1-butanol and the metastable amylodextrins prepared with cyclohexanol or 3-methylcyclohexanol might be explained by differences in conformation between the two types of metastable amylodextrins.

The conformation of amylose in the solid state has been reported to be a double helix, each strand containing six glucose units per turn (16). The conformation of amylose in solution has been shown to be a random coil (17). Because X-ray diffraction showed that starch-related compounds have the same conformation as amylose (18), it is generally accepted that the conformation of dextrins is also a double helix. Moreover, the conformation of dextrins with a DP >10 was reported to be a double helix (19). Consequently, during dissolution amylodextrin will go from the helical conformation to a random coil. Because the energy barrier between these conformations is lower for shorter molecules, only these molecules might be able to dissolve at room temperature.

Complexation of amylose with large molecules such as tertiary butanol resulted in a single helix with seven glucose units per turn (20,21), instead of the single helix with six glucose units per turn obtained on complexation with small molecules. The energy level of the former conformation is 10.3 kJ/mole higher compared to that of the helix with six glucose units per turn (21). The molecules of metastable amylodextrins prepared with cyclohexanol or 3-methylcyclohexanol, which might also have a helical conformation with seven glucose units per turn, might therefore be in the solid state in a higher energy level than the molecules of the other amylodextrins. Hence for the former there is a lower energy barrier to go from the helical conformation to the random coil, which might permit more molecules of these amylodextrins to dissolve. To confirm the suggested conformations of the different products, X-ray diffraction analysis was performed.

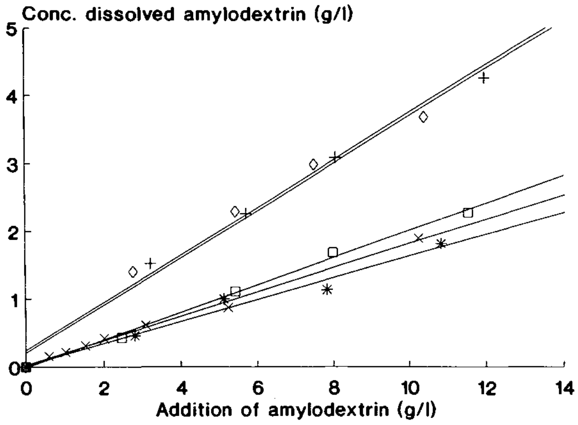

Fig. 3. Relationship between the concentration of dissolved amylodextrin at room temperature and the addition of amylodextrin to water. (x) Amylodextrin; metastable amylodextrin prepared with (□) 1-octanol, (∗) 2-methyl-1-butanol, (+) cyclohexanol, and (◇) 3-methylcyclohexanol.

Table II. Percentages of Amylodextrin Dissolved in Water After 5 Days at Room Temperature Calculated by Linear Regression Analysis

Amylodextrin	Percentage dissolved
Amylodextrin	18
Metastable amylodextrin prepared with	
1-Octanol	20
2-Methyl-1-butanol	16
Cyclohexanol	35
3-Methylcyclohexanol	35

X-Ray Diffractometry

This technique was applied because, for each conformation possible for amylose, its own characteristic diffraction pattern has been reported in the literature. The patterns of amylose and of its complexes were defined as the B- and V-pattern [V = *Verkleisterung* (German)], respectively (10). Two V-patterns have been reported (20): one for the complex of amylose with large molecules such as t-butanol and one for the complex with small molecules.

Table III presents the positions of the diffraction bands of the patterns as reported (22) for B-amylose and both types of V-amylose and the positions as found for Amylose V and for the products prepared in this paper. All compounds analyzed showed a lack of crystallinity, which is consistent with the literature (23). The diffraction pattern of Amylose V and of amylodextrin appeared to be similar to that of B-amylose. This confirms that both Amylose V and amylodextrin have the same conformation as B-amylose, being a double helix, where each strand contains six glucose units per turn repeating in 21.0 Å. In contrast, both types of V-amylose are known to have a single helical conformation with six or seven glucose units per turn repeating in 8.0 Å, the so-called collapsed helix (20). Metastable amylose and the metastable amylodextrins prepared with 1-octanol or 2-methyl-1-butanol indeed showed the pattern of V-amylose prepared with ethanol. The conformation of all three products is thus the collapsed helix with six glucose units per turn. The metastable amylodextrins prepared with cyclohexanol or 3-methyl-cyclohexanol showed a pattern similar to that of V-amylose prepared with tertiary butanol. Therefore, it is con-

Table III. Diffraction Bands of B-Amylose and V-Amyloses, as Reported in the Literature and Experimental Data for Amylodextrin, Metastable Amylodextrins, Amylose V, and Metastable Amylose

Compound	Position of strongest diffraction bands (Å)		
Values obtained from literature[a]			
B-Amylose			
(6 glucose/turn repeating in 21.0 Å)	6.4	5.2	4.0
V-Amylose (from ethanol)			
(6 glucose/turn, repeating in 8.0 Å)	7.0		4.5
V-Amylose (from t-butanol)			
(7 glucose/turn)		5.0	
Amylodextrin	6.5	5.3	4.0
Metastable amylodextrin			
1-Octanol or 2-methyl-1-butanol	7.0	5.2	4.6
(3-Methyl)cyclohexanol		5.0	
Amylose V	6.3	5.2	3.9
Metastable amylose	7.1	5.2	4.5

[a] Taken from Ref. 22.

cluded that these types of amylodextrin have a collapsed helical conformation with seven glucose units per turn.

FTIR Spectroscopy

Recent studies reported the use of Fourier transform infrared spectroscopy in the field of carbohydrate chemistry (24). A more ordered conformation of the amylose helix results in sharpening of the absorption bands in the fingerprint region (between 1400 and 800 cm^{-1}) of the spectrum. Most bands in this region arise from C–O and C–C stretching modes. The line broadening of these bands for more flexible conformations is related to a higher variation in orientation of the functional groups within the molecule. The order in conformation of the products prepared in this study was therefore checked by FTIR spectroscopy and compared with that of β-cyclodextrin. The latter is expected to have a highly ordered conformation and hence sharp peaks in the fingerprint region because it is rigid.

The spectrum of β-cyclodextrin (Fig. 4a) indeed, shows three very sharp bands at 1155, 1080, and 1025 cm^{-1}. Amylodextrin and the four metastable amylodextrins surprisingly showed a similar spectrum. This points to a highly ordered helical conformation. In contrast, the spectrum of Amylose V shows three broad bands (Fig. 4b) in the fingerprint region, which points to a more flexible conformation. Moreover, the band observed for β-cyclodextrin and the amylodextrins at 1025 cm^{-1} is shifted to 980 cm^{-1} in the spectrum of Amylose V. This shift is caused by changes in the molecular environment of the hydroxyl group at carbon 6. The primary hydroxyl groups in Amylose V are intramolecularly hydrogen bonded, resulting in a folded helical conformation. A similar spectrum with three broad bands was observed for metastable amylose. The band at 1025 cm^{-1}, however, was not shifted, indicating that metastable amylose does not have a folded conformation.

In conclusion, amylodextrin and Amylose V have a double-helical conformation with six glucose units per turn. The helix of Amylose V is folded. Metastable amylose and metastable amylodextrins have a collapsed single helical conformation. The number of glucose units (six or seven) depends upon the size of the complexing agent.

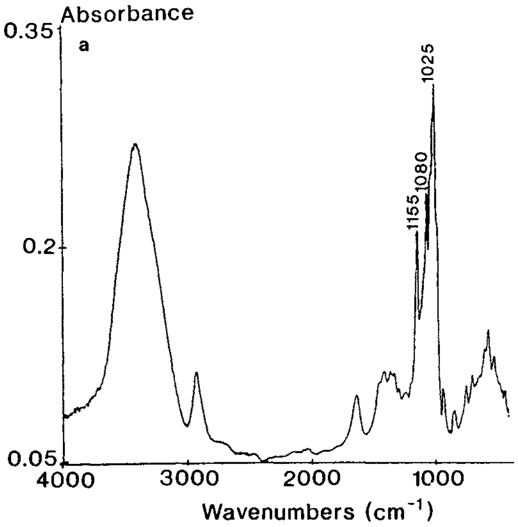

Fig. 4a. FTIR spectrum of β-cyclodextrin.

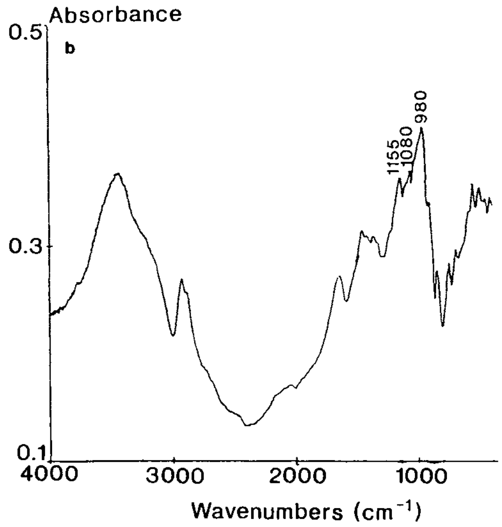

Fig. 4b. FTIR spectrum of Amylose V.

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