NOTES

Effect of Chain-End-Initiated Depolymerization in Alkaline Solution (Peeling) on the Molecular Weights of Amylose and Hydroamylose (Amylodextrin) *

1,4-Glycans such as starch and cellulose depolymerize in hot alkaline solution from the reducing chain ends with sequential release of the monosaccharide residues as soluble saccharinate degradation products. The mechanism of this peeling or unzipping process involves a Lobry de Bruyn–Alberda van Ekenstein isomerization of the terminal D-glucose to an anionic 2,3-enediol residue, which undergoes a beta-alkoxy-carbonyl elimination of the shortened polysaccharide chain from C-4. Thus the reducing chain terminus is regenerated, bearing an anionic anomeric oxygen atom that facilitates propagation of the depolymerization along the molecular chain.[2]

Measurements of the spectrophotometric properties of the iodine complex with amyloses after peeling did not detect a change in chain length.[3] We therefore examined the molecular weight of the amylosic residues remaining after the alkali-catalyzed decomposition by measuring the viscosity of dilute aqueous solutions during capillary flow.

After degrading amylose anaerobically in decimolar sodium hydroxide solutions for 3 hr at 98°C to approximately one-half of the initial amount, the residual polymer was reprecipitated from the neutralized reaction mixture with acetone. The values obtained for the limiting viscosity number (intrinsic viscosity) (Table I) indicate that the average molecular weight of the amylose is reduced to 41% of its value when treated at 0.2% initial concentration and to 22% at 0.5% concentration. This dramatic decrease may be accounted for *a priori* in several ways:

1. The introduction of oxidized functional groups in amylose through the action of atmospheric oxygen during storage would permit an alkali-catalyzed "random" scission of the polymer chains.[2] However, no iodine color was generated when the untreated polysaccharide was shaken with acidified aqueous potassium iodide solution, indicating the absence of peroxides. Furthermore, reduction with borohydride does not increase the limiting viscosity number of amylose when dissolved in normal potassium hydroxide solution.[4] We conclude that alkali-labile links had not been introduced into the chains.
2. A random alkaline hydrolysis of glucosidic linkages cannot be reconciled with the formation[5] of an alkali-stable residue (50% yield) after amylose is treated with the alkali at a concentration of 0.5% for 6 hr at 98°C.
3. The possibility of an artifact due to the reprecipitation procedure was tested by directly measuring the capillary viscosity of the cooled reaction mixture itself, without previous isolation of the polymer (Table II). The viscosity number was much smaller than that of an untreated amylose solution of the same concentration, thus confirming the validity of the data presented in Table I.
4. Having rejected alternatives 1–3, we conclude that the change in molecular weight results from endwise depolymerization.

This result leads to further conclusions regarding the molecular weight distribution and the kinetics of the degradation. Polymers with a "most probable," exponential distribution of molecular weights have the unique property that they may undergo chain-end-activated degradations of all chains present without a reduction in the average chain length.[6–8] Therefore, since the degraded amylose here exhibits a diminished degree of polymerization, the molecular weight distribution of the parent polysaccharide is not an exponential function. A narrower distribution function is suggested. The kinetic zip length is given by the ratio of the first-order rate constants for propagation (k_1) and termination (k_t). From our previous kinetic data,[5] the amylose of initial chain length 1050 has a zip length of 1050 when degraded at an initial concentration of 0.2% and a zip length of 533 at 0.5%. In the former case of a more dilute substrate concentration, the chain and zip lengths are equal, and therefore the polysaccharide depolymerizes quantitatively if allowed to react for a long enough period

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TABLE I
Properties of the Reprecipitated Reaction Products of Alkaline Degradation

Initial substrate conc.,[a] %	Reaction time, min	Yield of degraded residue, %		Viscosity, in *M* KOH	
		Iodine stain	Reppt.	$[\eta]$, ml/g	k'
Substrate amylose					
0.2	0	100	88	140	0.50
0.2	180	42	47	57	0.40
0.5	180	53	59	31	0.21
Substrate hydroamylose[b]					
0.2	0	—	85	26	0.30
0.2	10	—	74	24	0.52
0.2	10	—	71	26	0.32
0.2	15	—	57	28	0.00

[a] In hermetically sealed, 500-ml stainless-steel vessel containing aqueous sodium hydroxide solution (400 ml, 0.1*M*).
[b] 18-hr hydrolysis at 15°C.

TABLE II
Properties of the Degradation Reaction Mixtures Without Isolation of Product

Initial conc. of substrate,[a] %	Yield of degraded residue,[b] %	Capillary flow time,[c] sec		Viscosity number, ml/g	
		Final reaction mixture	Soln. of untreated substrate at same conc.	Final reaction mixture	Soln. of untreated substrate at same conc.
Substrate amylose[d]					
0.2	42	240.1	257.1	52	145
0.2	42	241.3	256.5	66	140
0.2[e]	48	244.7[f]	269.5[f]	48	151
0.5	53	259.0	323.4	53	155
0.5[g]	58	280.1	363.0	78	237
Substrate hydroamylose[h]					
0.2	79	249.1	248.1	55	49

[a] In sealed 20-ml glass ampuls containing aqueous sodium hydroxide solution (11 ml, 0.1*M*). Multiple entries are repeat reactions to test reproducibility.
[b] Determined by iodine stain (Ref. 14).
[c] Solvent flow time, 228.7 sec, unless otherwise stated.
[d] Peeling reaction time, 3 hr.
[e] Initial titer, 94-m*M* NaOH; final, 87-m*M* NaOH.
[f] Solvent flow time, 233.7 sec.
[g] Initial titer, 118-m*M* NaOH; final, 103-m*M* NaOH.
[h] 18 hr hydrolysis at 30°C; peeling reaction time, 5 min.

of time (18 hr).[5] At the elevated amylose concentration, however, the zip is half of the chain length, so that half of the polysaccharide is degraded, leaving an alkali-stable residue of reduced molecular weight.

Kainuma and French[9] obtained a "Nageli amylodextrin" in 83% yield by heterogeneous treatment of Superlose potato amylose with 16% sulfuric acid (ca. 1.7*M*) at an ambient temperature for 3 months. Hydrolysis of Avebe potato amylose for 2 hr yields a stable, solid residue of 34% recovery of poly-

saccharide ([η] = 45 ml/g) in 0.5*M* sulfuric acid[10] at 98°C and of 40% recovered solids ([η] = 18 ml/g) after 2 hr in 5*M* hydrochloric acid[3] at an ambient temperature. The limiting viscosity number, the iodine-stain characteristics, and the yield of these "hydroamyloses" are unaltered on prolonged acid treatment, suggesting[3] that a "leveling-off degree of polymerization" may have been attained, as occurs[11] on acid hydrolysis of cellulose.

On homogeneous alkaline peeling for 15 min, 18-hr hydroamylose was degraded to 57% of the initial amount. Such a fast degradation rate, that is an order of magnitude greater than was found for amylose, is expected for a substrate reacting at a 10-fold higher (molar) concentration in a first-order process. In contrast to the behavior of the parent amylose, viscosity measurements revealed no decrease in the average molecular size of the hydroamylose during the alkaline treatment (Tables I and II), thus confirming the findings[3] based on iodine-stain data. According to the kinetic data,[3] the zip length is 120, the same value as the average degree of polymerization of the hydroamylose.

A constant average molecular weight may be accounted for in principle by an unaltered distribution of molecular weights for the hydroamylose during peeling. Scission of glucosidic bonds by peeling off in alkaline solution is two orders of magnitude faster in the hydroamylose chain than in the homologous disaccharide maltose.[3] This difference is in keeping with the hypothesis[5,12] that the first glucosidic linkage at the reducing end of hydroamylose molecules is broken at a slower rate than the other glucosidic linkages, which are subsequently severed as unzipping progresses along the polymer chain. Consequently, entire molecular chains would unzip, so that the distribution of chain lengths in the remaining polymer would not change during the depolymerization. Such a mechanism would be valid in principle whatever the initial shape of the molecular weight distribution of hydroamylose.

However, if the chain length distributions of both the substrate and the product are exponential functions, one cannot exclude the possibility that chain end degradation without any reduction in average chain length is attained in a mechanism whereby all molecular chains have been shortened to the same extent.[6-8] In that case, the chain length distribution would be displaced to shorter chain length after peeling.[6-8] It is therefore proposed to test this alternative by using the method of GPC to determine whether the distribution is in fact unaltered after peeling.

It is of interest to note that amylose is also subject to enzymatic unzipping by the action of beta-amylase, which releases a zip of four maltose moieties from each nonreducing chain terminus.[13]

EXPERIMENTAL

Avebe potato amylose ([η] = 142 ml/g, $DP \sim 1000$, Blue Value[14] = 1.35, 10 g) was shaken in hydrochloric acid (5*M*, 500 ml) at an ambient temperature for 18 hr. The solid residue was collected by centrifugation and washed with water until neutral and then 5 times with methanol. After being ground to a powder, the hydroamylose (amylose amylodextrin) product was dried over phosphorus pentaoxide *in vacuo*. Recovery at summer ambient temperature (ca. 30°C) was 40% after 2- or 18-hr hydrolysis ([η] = 15 ml/g), while at winter ambient temperature (ca. 15°C) the yield was 47% after 18 hr ([η] = 26 ml/g).

Depolymerizations were performed with solutions in aqueous sodium hydroxide (0.1*M*) under nitrogen in sealed vessels that were immersed in boiling water. The residual polymer was assayed spectrophotometrically with iodine reagent.[3,14] For reprecipitation[15] of the residue, the reaction mixture (ca. 400 ml) was neutralized with hydrochloric acid (1*M*, ca. 40 ml), and acetone (300 ml) was added. After 18 hr the precipitate was recovered by centrifugation and washed once with acetone–water (1:3), 5 times with acetone, and once each with methanol and ether. After drying in air, the material was desiccated *in vacuo* to constant weight over phosphorus pentaoxide.

Flow times were measured in a No. 50 Cannon-Fenske viscometer at 30 ± 0.1°C. The limiting viscosity numbers [η] and Huggins slope constants k' were determined in aqueous molar potassium hydroxide solution.

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