[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, COLUMBIA UNIVERSITY]

The Molecular Weight of α-Amylo-dextrin (erythro-Granulose) from Potato Starch

BY CHARLES O. BECKMANN AND QUICK LANDIS

The starch-like "grenz dextrin"[1,2] remaining from the exhaustive action of the β-amylase component of the malt amylase system is perhaps the best characterized of the enzymic dextrins and Haworth[3] recently has deduced its mean chain length to be about 12 glucopyranose units by the method of end-group assay. Accordingly the molecular weight and the heterogeneity of this material become of importance.

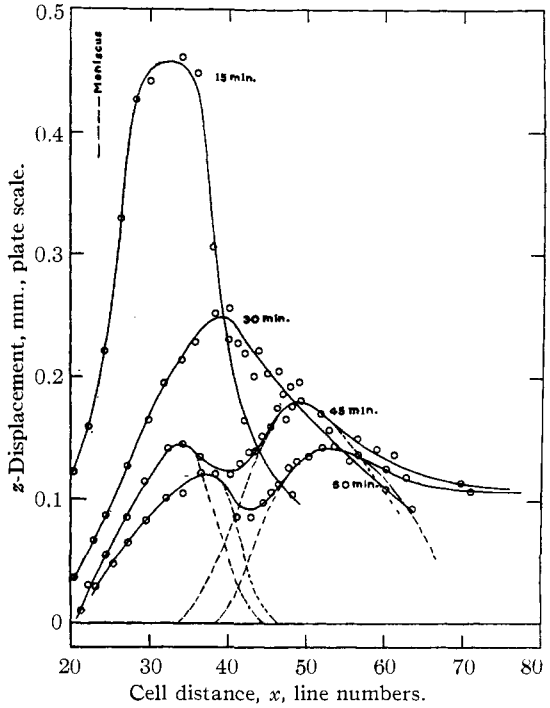

Fig. 1.—α-Amylodextrin from gelatinized potato starch: concn. 0.75%; temp. 26°; speed, 1226 r. p. s.; $\omega^2 = 5.92 \times 10^7$.

Time, sec.	Sedimentation curve data, mm., plate scale Z_m	Δx_c	x_m	$s_{26} \times 10^{13}$	$s_{20} \times 10^{13}$ I	II	Mean
900	0.458	1.67	45.78	6.86			5.80
1800	.252	3.22	46.55	6.50			5.50
2700	.146	2.12	46.00	2.89	2.44		
3600	.122	2.76	46.32	2.79	2.36		
2700	.182	5.67	47.78	7.44		6.30	
3600	.144	6.65	48.27	6.46		5.46	

Range $s_{20} = 2.36$–6.30; mean 4.64×10^{-13}. Component I; mean $s_{20} = 2.40 \pm 0.04 \times 10^{-13}$. Component II; mean $s_{20} = 5.88 \pm 0.42 \times 10^{-13}$. D_{20} extrapolated; approximately 13×10^{-7}. $D^2(d(1/D)/dt) = -42{,}000$.

(1) Wijsman, *Rec. trav. chim.*, **9**, 1 (1890).
(2) Baker, *J. Chem. Soc.*, **81**, 1177 (1902).
(3) Haworth, Hirst, Kitchen and Peat, *J. Chem. Soc.* 791 (1937).

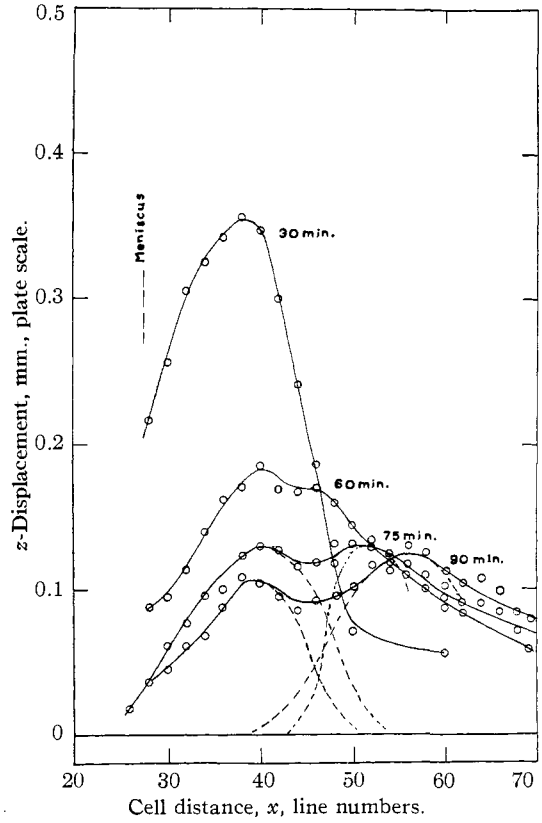

Fig. 2.—α-Amylodextrin from Lintner soluble starch: concn. 0.75%; temp. 27°; speed, 1229 r. p. s.; $\omega^2 = 5.96 \times 10^7$.

Time, sec.	Sedimentation curve data, mm., plate scale Z_m	Δx_c	x_m	$s_{27} \times 10^{13}$	$s_{20} \times 10^{13}$ I	II	Mean
1800	0.354	1.94	46.1	3.93			3.32
3600	.174	2.96	46.6	2.95			2.50
4500	.132	4.50	47.4	3.54			3.00
4500	.123	2.80	46.6	2.26	1.91		
5400	.107	2.79	46.6	1.86	1.57		
4500	.125	5.53	47.9	4.32		3.66	
5400	.122	6.18	48.3	3.98		3.37	

Range $s_{20} = 1.57$–3.66; mean 2.76×10^{-13}. Component I; mean $s_{20} = 1.74 \times 10^{-13}$. Component II; mean $s_{20} = 3.52 \times 10^{-13}$. D_{20}, extrapolated; approximately 12×10^{-7}. $D^2(d(1/D)/dt) = -27{,}000$.

Experimental

β-Amylase, prepared from commercial soft wheat flour by Hanes'[4] modification of Van Klinkenberg's[5] procedure was allowed to act on potato starch prepared in three different ways. The same starch[6] was used in all cases.

(4) Hanes, *Can. J. Research*, **13**, 185 (1935).
(5) Van Klinkenberg, *Ergeb. Enzymforsch.*, **3**, 73 (1934).
(6) Standard grade B. M. K. F. (1934 crop).

TABLE I

α-AMYLO DEXTRINS FROM B. M. K. F. POTATO STARCH

Type (source)	Conversion, %	Yield, %	$[\alpha]_D$	A. L.	Mean $s_{20}\cdot 10^{13}$	Extrapolated $D_{20}\cdot 10^{7}$	$D^2\frac{d(1/D)}{dt}$	$M\frac{f_D}{f_s}$ [a]	$\frac{f_s}{f_0}$	12 unit chains per molecule	C_6 units per molecule
Gelat.	66.5	20	182	23.0	4.64	13 ±	−42,000	23,000[b]	0.94	11.8	142
Gelat. I	..	..	...	..	5.88	...	.....	29,100[b]	.88	14.9	180
Gelat. II	..	..	...	..	2.40	...	.....	11,900[b]	1.20	6.1	74
Lint.	63.1	25	173[c]	34.5	2.76	12 ±	−27,000	13,700[b]	1.15	7.0	85
Lint. I	..	..	...	..	3.52	...	.....	17,500[b]	1.01	9.0	108
Lint. II	..	..	...	..	1.74	...	.....	8,600[b]	1.39	4.4	53
Ground	71.2	23	186	42.6	1.67	8.7	− 5,200	11,800	1.72	6.1	73
Lint. (precursor)	..	...	...	35.2	2.43	8.05	− 3,040	18,700	1.57	..	...
Ground (precursor)	..	..	...	46.3	2.30	8.15	− 2,100	12,700	1.80	..	...

[a] Taking the partial specific volume, V, to be 0.606. [b] Assuming $D = 12.5\cdot 10^{-7}$. [c] Haworth's[8] purest preparation, $[\alpha]_D$ 167.

Substrate I was prepared by heat gelatinization of the raw starch in 10% suspension, with moderate stirring. One half the amylase solution was added when the temperature had fallen to 50°, the remainder was added at 40° and the mixture allowed to digest at 30° until the conversion on two successive days remained substantially the same (seventy-two hours). Substrate II was Lintner soluble starch, prepared in the usual manner with 2 *N* hydrochloric acid acting for seven days at 18°. Substrate III was prepared by dry grinding[7] for 667 hours in a pebble mill. After dispersion of these last two in hot water, they were treated with the enzyme and digested as for substrate I. The resulting conversion mixtures were filtered and the α-amylo dextrins precipitated with acetone-free methyl alcohol. They gave a dark red or plum color with iodine. They were redispersed without drying to suitable concentration for ultracentrifugal analysis. The apparatus and methods of calculation have been described previously.[8] The results are summarized in Table I.

Discussion

The theoretical conversion of 60–70% required to reduce a chain of 30 units to one approximating 12 is achieved in all cases, yet, excepting that from the gelatinized starch, the molecular weights of the dextrins differ but little from those of the original materials. The gelatinized starch, still exhibiting remnants of granule organization, had a sedimentation constant too high to observe with certainty, and the molecular weight of the dextrin from this material is correspondingly high.

Somewhat suprising is the heterogeneity of the dextrins from substrates I and II, the curves (Figs. 1 and 2) showing definite resolution into two components. Thus the comparative homogeneity of the dextrin from the ground material (Fig. 3) is of considerable significance. Remembering the heterogeneity exhibited by the β-amylose from corn starch ground for 168 hours[8] we are justified in assuming even greater heterogeneity for the gelatinized potato starch. The Lintner soluble starch, although more homogeneous than the ground corn starch, shows definite evidence of an appreciable amount of a

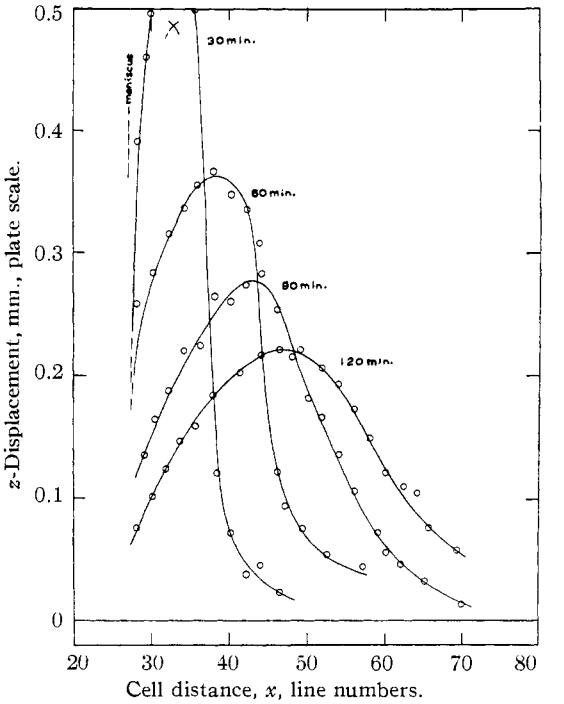

Fig. 3.—α-Amylodextrin from ground (667 hours) potato starch: concn. 0.75%; temp. 27°; speed, 1230 r. p. s.; $\omega^2 = 5.96 \times 10^7$.

Time, sec.	Sedimentation curve data, mm., plate scale Z_m	Δx_c	x_m	$s_{27} \times 10^{13}$
1800	0.550	0.95	45.5	1.95
3600	.362	1.95	46.0	1.98
5400	.274	2.95	46.5	1.97
7200	.222	4.07	47.1	2.03

Mean $s_{20} = 1.98 \pm 0.02 \times 10^{-13}$. $s_{20} = 1.67 \pm 0.02 \times 10^{-13}$. D_{20} extrapolated; 8.74×10^{-7}. $D^2(d(1/D)/dt) = -5200$.

(7) Taylor and Beckmann, THIS JOURNAL, **51**, 294 (1929).

(8) Beckmann and Landis, *ibid.*, **61**, 1495 (1939).

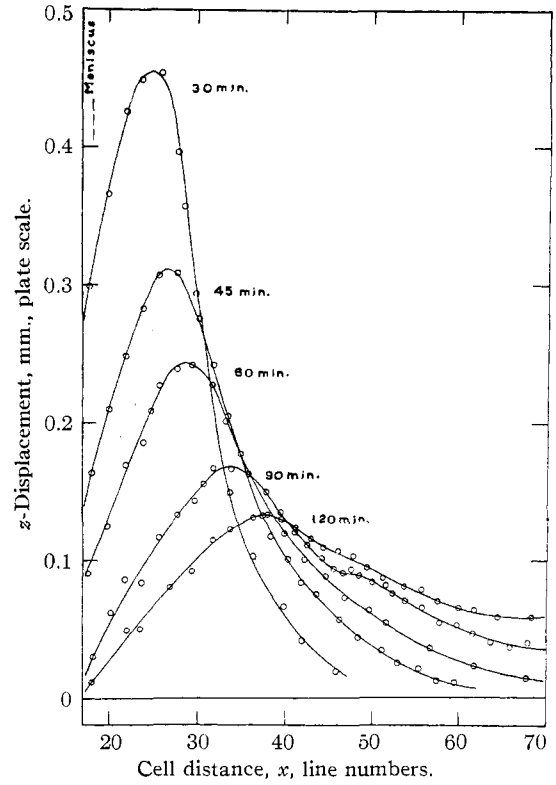

Fig. 4.—Lintner soluble starch: concn. 0.75%; temp. 27°; speed, 1046 r. p. s.; $\omega^2 = 4.32 \times 10^7$.

Time, sec.	Curve data, mm., plate scale Z_m	Δx_c	x_m	$s_{27} \times 10^{13}$	$s_{20} \times 10^{13}$
1800	0.455	0.87	43.44	2.57	2.17
2700	.312	1.46	43.73	2.87	2.43
3600	.244	2.00	44.00	2.92	2.47
5400	.168	3.14	44.57	3.01	2.55
7200	.133	4.17	45.09	2.97	2.51

Mean $s_{20} = 2.43 \pm 0.10 \times 10^{-13}$. D_{20}, extrapolated 8.05×10^{-7}.

heavy component which was not completely resolved (Fig. 4). On the other hand, the ground potato starch precursor (Fig. 5) is the most homogeneous carbohydrate sample we have yet encountered. The inference that the original means utilized for dispersion of the starch largely determines the character of the resulting dextrin is obvious.

It is noteworthy that with few exceptions the dissymmetry or form factors (f_s/f_0) do not differ greatly from 1 and in no case are they as high as 2. Thus it appears that the dextrins have relatively little of the long thread-like components which were observed in the case of ground corn starch.[8] These are either converted entirely by the enzyme, or the "frayed ends," so to speak, are hydrolyzed back to a more spherical nucleus. It is tempting to attempt to identify the relatively spherical dextrins with a spherical component similar to that recognized in ground corn starch, *i. e.*, to substantiate the theory that α-amylo dextrin preëxists as an independent component in the granule. However, this is quite inconsistent with the definite changes exhibited in homogeneity when the samples are dispersed in different ways. The concept of a number of chains bound together into a unit through the multiple hydroxyl associative forces so plentifully scattered along the amylose chain, the associative links being successively broken as chemical or mechanical treatment is continued, is less inharmonious with the observations. Exposed chains and thread-like molecules may be readily attacked, by the β-amylase, 2 units at a time until the steric hindrance produced by an adjacent associated chain prevents it, and an unattackable kernel is then exposed whose char-

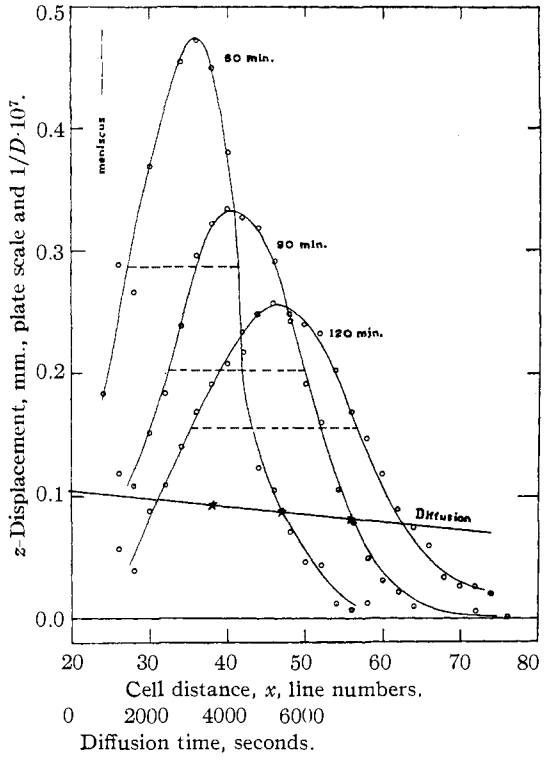

Fig. 5.—β-Amylose from potato starch ground for 667 hours: concn. 0.75%; temp. 27°; speed, 1152 r. p. s.; $\omega^2 = 5.24 \times 10^7$.

Time, sec.	Curve data, mm., plate scale Z_m	Δx_c	x_m	$s_{27} \times 10^{13}$
3600	0.474	2.30	45.28	2.69
5400	.334	3.58	45.92	2.75
7200	.256	4.79	46.59	2.73

Mean $s_{27} = 2.72 \pm 0.03 \times 10^{-13}$. $s_{20} = 2.30 \times 10^{-13}$. D_{20} extrapolated, 8.15×10^{-7}. $D^2(d(1/D)/dt) = -2100$.

acter depends on the original disruptive method. This would mean that the 12 unit length deduced by Haworth[1] for the dextrin chain is an average value, and may vary with the disruptive method used.

Form factors less than unity are very seldom encountered and in the present work may be due to the uncertainties involved in the extrapolation of the diffusion constant to zero time. On the other hand, if further work should prove the extrapolation to be valid, a form factor less than unity may indicate a molecule with the shape of a prolate or oblate spheroid with rudders to stabilize an otherwise unstable orientation in the liquid.[9]

(9) C. W. Oseen, "Hydrodynamik," Akademische Verlagsgesellschaft m. b. H., Leipzig, Germany, 1927, p. 188.

Summary

1. The molecular weights of α-amylo dextrins prepared from substrates dispersed by three different methods from the same sample of potato starch varied from 8600 to 29,100.

2. The heterogeneity of the dextrins closely paralleled that of the amylose precursors.

3. In general they exhibited less departure from sphericity than the original amyloses. The evidence is interpreted to indicate the presence of an unattackable nucleus in the molecules of the amylose dispersion, the character of which varies widely depending upon the original methods utilized for disruption of the granule.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, COLUMBIA UNIVERSITY]

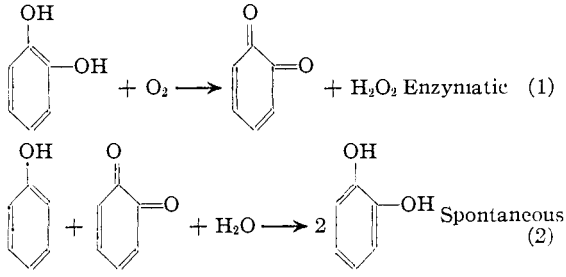
On the Oxidation of *p*-Cresol by Means of Tyrosinase

BY CHARLES A. BORDNER AND J. M. NELSON

The oxidase, tyrosinase, generally is regarded as capable of catalyzing two essentially different aerobic oxidations: first, the insertion of a hydroxyl group in a monohydric phenol, ortho to the one already present, and second, the oxidation of certain *o*-dihydric phenols to their corresponding *o*-quinones.

The rate of this enzymatic oxidation of an *o*-dihydric phenol, such as catechol, has a maximum value at the beginning of the reaction, while the initial rate in the case of a monohydric phenol is usually autocatalytic. This initial lag in the rate of oxygen uptake in the oxidation of monohydric phenols can be overcome by the addition of a trace of an *o*-dihydric phenol, such as catechol. This influence of catechol in accelerating the initial rate of oxidation of a monohydric phenol, such as *p*-cresol, has given rise to several theories concerning the mechanism of the reaction or reactions involved.

Among these theories that of Onslow and Robinson[1] seems to have been most widely accepted.[2] According to this view the monohydric phenol is oxidized spontaneously to the *o*-dihydric condition by *o*-quinone formed by the enzymatic oxidation of catechol.



Califano and Kertesz[3] even claim to have confirmed reaction (2) experimentally.

On the other hand, Pugh[4] claims that *o*-quinone, formed by oxidizing catechol in the presence of *p*-cresol by peroxidase and hydrogen peroxide, is unable to oxidize the cresol. Unfortunately her claim is weakened due to *o*-quinone and hydrogen peroxide being incompatible under these conditions as shown by Dawson and Ludwig.[5]

To overcome this objection, Miss Pugh's experiment has been repeated by oxidizing catechol to *o*-benzoquinone in the presence of phenol using an oxidase from sweet potatoes, *Ipomoea batatas*, instead of peroxidase and hydrogen peroxide. This oxidase has practically no action on phenol. By using the Warburg respirometer, it was found that the same amount of oxygen, 2.09 atoms per mole of catechol, was consumed when 1 mg. of

(1) M. W. Onslow and M. E. Robinson, *Biochem. J.*, **22**, 1327 (1928).

(2) (a) D. Keilin and T. Mann, *Proc. Roy. Soc.* (London), **125B**, 187 (1938); (b) F. Kubowitz, *Biochem. Z.*, **299**, 32 (1938).

(3) L. Califano and D. Kertesz, *Nature*, **142**, 1036 (1938).

(4) C. E. M. Pugh, *Biochem. J.*, **23**, 456 (1929).

(5) C. R. Dawson and B. Ludwig, THIS JOURNAL, **60**, 1617 (1938).