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DEGREES OF LONG-CHAIN BRANCHING IN DEXTRANS*

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ABSTRACT

To estimate the content of long-chain branching in dextrans, gel-permeation chromatography (g.p.c.) and viscometric measurements were carried out. The data obtained were analyzed by an iterative, computer method, proposed by Kurata *et al.*, that can evaluate only the content of long-chain branching in a polymer. The dextrans used were those produced by *Leuconostoc mesenteroides* B-512 and other strains. The molecular weights of samples investigated ranged from 6.8×10^6 to 1.0×10^4, and the results indicated that the long-branch content increases with increasing molecular weight.

INTRODUCTION

By means of such chemical analyses as methylation analysis and periodate oxidation[1–6], it has been shown that the dextran produced by *Leuconostoc mesenteroides* NRRL B-512 is a (1→6)-linked α-D-glucan, with some 5% of branch linkages attached to the O-3 atoms of the backbone. The length of the branching chain cannot, however, be explicitly determined by these methods. Although most of the branchings were found to be composed of such short chains as D-glucosyl and isomaltosyl groups[7], which would give no appreciable contribution to the solution properties[8], it seems that the dextran has a few, very long branches, distributed randomly in a molecule, that are of great importance in determining the solution properties, judging from the results of viscosity, sedimentation, and light-scattering measurements of the dextran solutions[9–13].

We have now attempted to determine the content of the long branches in dextran fractions of various molecular weights, using gel-permeation chromatography (g.p.c.), together with viscometry, that had proved to be useful in evaluating the randomly branched, long-chain content and the molecular weight of synthetic polymers[14,15].

*Part II of the series G.p.c. Analyses of Polysaccharides.

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MATERIALS AND METHODS

Materials. — Dextrans T-2000, T-500, T-70, T-40, and T-10, supplied by Pharmacia Fine Chemicals Co , Ltd., Uppsala, were products of strains of *Leuconostoc mesenteroides* B-512. Dextrans T-2000 and T-70 were sub-fractionated into 11 and 10 fractions, respectively, by a fractional precipitation method, and some of those were used The fractionation method was as follows To an aqueous solution of dextran was added ethyl alcohol as a poor solvent, and the mixture was kept at 30° until the concentrated and the dilute phases were completely separated, 1–3 days usually being required.

Dextrans XH5550 and YH5592 were manufactured, and kindly provided, by Meito Sangyo Co., Ltd , Nagoya. They were products of *Leuconostoc mesenteroides* N-4 strain. Dextrans 7527, 3527, and 527 were produced by Fison Limited, Pharmaceutical Division, Loughborough, Leicestershire, England, and presented to us by Meito Sangyo Co., Ltd Dextrans D-195000 and D-59000 were supplied by Nakarai Chemicals Co., Ltd., Kyoto; the strains which produced them are unknown.

Pullulan (Shodex standard P-82, manufactured by Showa Denko Co., Ltd., Japan), polyethylene oxide (TSK Standard polyethylene oxide, prepared by Toyo Soda Co., Ltd., Japan), and linear amylose (synthesized by the action of phosphorylase in our laboratory[16]) were used as molecular-weight markers for the preparation of a g p.c universal calibration curve.

G.p c. measurements[17]. — G.p.c measurements were carried out with a Toyo Soda Type 802R l.c. instrument equipped with a column system which consisted of Toyo Soda G-6000PW and G-3000PW columns connected in series. The eluant used was aqueous 0.25M KCl solution, and the column was controlled at 40°. The flow rate was 0.7 cm^3/min. The detector used was a differential refractometer, and the count was marked by every 20 drops on a chart (1 count = 0 5 cm^3)

Light-scattering measurements — The light-scattering measurements were performed with a Brice–Phoenix photometer, Model 2000DM, at 436 nm, a conical cell being used An aqueous stock solution, 0.4–0.6%, was prepared by successive dilutions of the stock solution, and their turbidities were measured at scattering angles between 35 and 135° A refractive-index increment[10] of 0.151 cm^3/g was used in the calculations. By analyzing the light-scattering data by use of a Zimm plot, we determined weight-average molecular weights, *z*-average mean squares of gyration radius, and the second virial coefficients of samples.

Viscosities. — Viscosities were measured by using an Ubbelohde type of capillary viscometer for aqueous 0.25M KCl solution containing 0.02% (w/w) of NaN_3, at 40 ±0.02°. Flow times of the solvent with the viscometer were >120 s, and a correction for kinetic energy was not carried out. Intrinsic viscosity [η] was calculated by using the method of least squares

Correction of g.p c elution curve[18]. — Elution curves in g.p.c. generally show an instrumental, spreading effect which causes an erroneous evaluation of mole-

cular-weight distribution for a sample. Hence, the curve obtained was corrected by the Hamielec II method[19] through which we could obtain a corrected chromatogram that has no spreading effect.

Working model of a dextran molecule, and basic equations. — The B-512 dextran has been considered[1,6,10,12] to be a branched polymer bearing short and long side-chains amounting to a total of ~5% of branching points. Wales *et al.*[9] postulated a comb-type of structure for the B-512 dextran molecule, and interpreted the observed value of the g-factor, which is the ratio of the squares of the unperturbed radii of gyration of branched and unbranched molecules having the same chemical constitution. However, this model was criticized and discarded by Senti *et al.*[10]. They considered that there should exist, in the dextran molecule, long side-chains which would also be branched, in a manner equivalent to that for the main chain, and indistinguishable from the main chain. Bovey[12] also concluded that the B-512 dextran chain has a few very long branches, in addition to a number of very short branches. From these observations and the hydrodynamic theory[8], it is not unreasonable to consider that the effect of the short chain-branchings can be ignored in reducing the intrinsic viscosity compared with that of the long chain-branchings. This model can be regarded as equivalent to a random-branching model. Hence, the dextran can be dealt with using a randomly branched flexible polymer as concerns its solution behavior. For this kind of polymer, the g.p.c.–viscometric method was presented by Kurata *et al.*[14,15] to determine the content of branching.

In accord with their treatment, we used the following equations, which are applicable to a randomly branched polymer having a trifunctional branching point, for evaluating the branching parameter λ (expressed as the number of branches per unit molecular weight of the polymer) and the molecular weight, M, from intrinsic viscosity and the g.p.c. elution curve

$$\log Q_i = C - DV_i \tag{1}$$

$$Q_i = [\eta]_i M_i = KM_i^{a+1}\,[(\lambda M_i/7 + 1)^{0.5} + 4\lambda M_i/9\pi]^{-0.3}, \tag{2}$$

$$[\eta]_{\mathrm{cal}} = \Sigma f_i[\eta]_i \tag{3}$$

where Q represents the "universal" molecular-size parameter, which determines elution volume in g.p.c., V is the elution peak volume, and subscript i expresses correspondence with the i-th component of the sample. C and D are constants in a given system. K and a are the constants which appear in the Mark–Houwink–Sakurada equation for linear polymers, *i.e.*, $[\eta] = KM^a$; f represents the weight fraction of each molecular species.

Computer program. — In the case of a linear polymer, the g.p.c. elution curve can be directly converted into the molecular-weight distribution f_iM_i with the aid of Eqs. *1* and *2*. Then, the intrinsic viscosity can be calculated from Eq. *3*. The calculated value, $[\eta]_{\mathrm{cal}}$, should be in agreement with the observed value, $[\eta]_{\mathrm{obs}}$. For

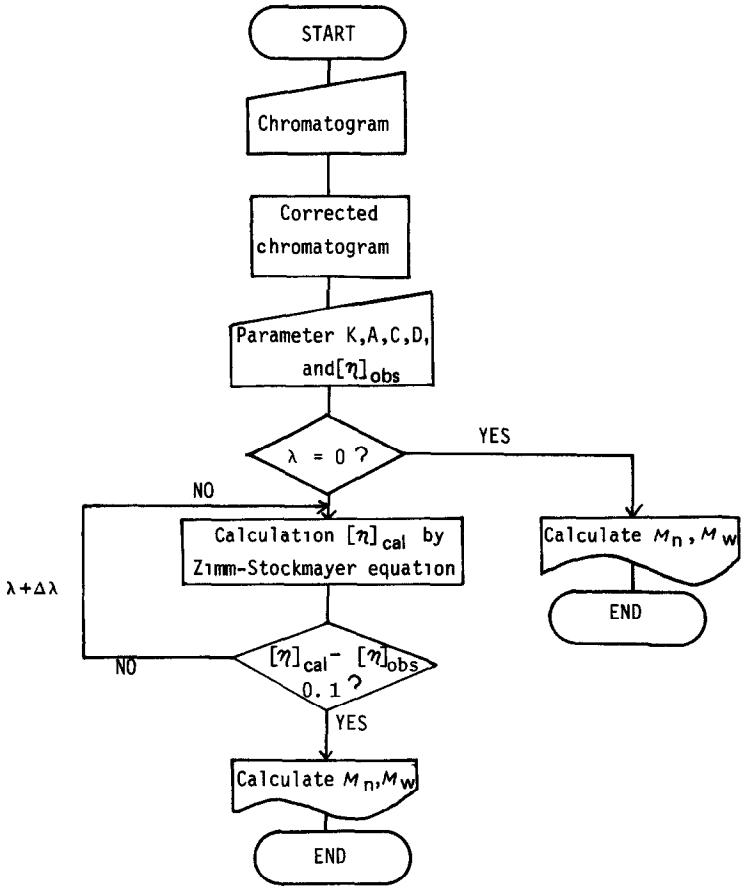

Fig 1 Flow chart of computer program for g p c –viscometric method

a branched polymer, however, $[\eta]_{cal}$ must be overestimated. In such a case, an iterative increment of λ is tested until agreement is achieved between $[\eta]_{cal}$ and $[\eta]_{obs}$. A flow chart of the computer program used is given in Fig. 1. The uncorrected g.p.c. data are fed in, and corrected by the Hamielec II method, to get a true elution profile of the g.p.c. The value of $[\eta]_{obs}$ and constants, *K, a, C,* and *D* are entered. Then, calculation of $[\eta]_{cal}$ is carried out according to Eqs. *1* to *3*, with the assumption that $\lambda = 0$. If $[\eta]_{calc} - [\eta]_{obs} < 0.1$ cm^3/g, the assumption is accepted, namely $\lambda = 0$, and the number average and weight-average molecular weights, $\overline{M}_n$ and $\overline{M}_w$, are immediately calculated. Otherwise, the program proceeds downward in Figure 1 with a finite value of λ. If $|[\eta]_{cal} - [\eta]_{obs}| > 0.1$ cm^3/g, λ is changed by a small amount, $\Delta\lambda$, and the calculation is repeated. In this way, the calculation of $[\eta]_{cal}$ can be iterated until agreement is achieved between $[\eta]_{cal}$ and $[\eta]_{obs}$ to within an allowance of 0.1 cm^3/g. Then, $\overline{M}_n$ and $\overline{M}_w$ can be calculated with the value of λ used, and printed out. The computer used was a FACOM M382 at Kyoto University

RESULTS AND DISCUSSION

We had already established[17] that the "universal" molecular-size parameter can determine the elution volume in g.p.c. of polysaccharides, irrespective of their molecular structure, *i.e.*, linear, or branched. For the g.p.c. system used here, Eq. *1* can well represent the relation between Q and V, as shown in Fig. 2, and constants C and D in Eq. *1* were determined to be 20.54 and 0.237, respectively.

The intrinsic viscosities observed, and the weight-average molecular weight, the z-average mean-squares, $\langle s^2 \rangle$ of gyration radius, and the second virial coefficients, A_2 of samples that were measured by the light-scattering method are summarized in Table I. Plotting the intrinsic viscosity against the molecular weight in log–log scale, we obtain the relationship shown in Fig. 3, in which the relation appears to be linear only for $\overline{M}_w < 10^5$, for all dextrans investigated, in agreement with the results of other authors[9–11]. It may be assumed that the dextran molecules of $\overline{M}_w < 10^5$ have no long branches that can appreciably affect their intrinsic viscosities, and they can be regarded as linear polymers. For the hypothetical, linear dextran, the relation between the intrinsic viscosity in water at 40° and the molecular weight as expressed by $[\eta] = 2.01 \times 10^{-2} \overline{M}_w^{0.637}$ cm³/g can be derived. The downward curvature seen in the range of $\overline{M}_w > 10^5$ has been pointed out by several authors[10–12].

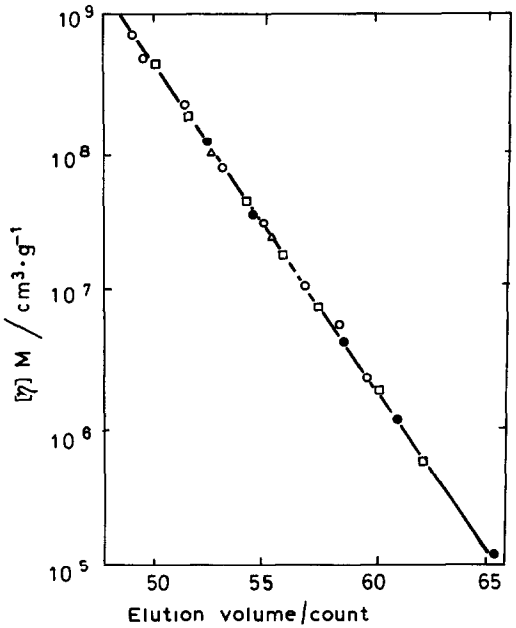

Fig 2. Universal calibration curve for polysaccharides in g.p.c [Column system used consisted of Toyo Soda G-6000PW and G-3000PW and was eluted with aq. 0.25M KCl solution at 40° Key: dextran (○), pullulan (●), amylose (△), and polyethylene oxide (□).]

TABLE I

LIGHT-SCATTERING DATA AND INTRINSIC VISCOSITIES

Sample	$\overline{M}$ *(10^4)*	$[\eta]$ *(cm^3/g)*	$\langle s^2 \rangle$ *(10^{-12} cm^2)*	A_2 *(10^5 g^{-2} cm^2 mol)*
T-500	46 6[a]	49 9	—	—
T-70	7 16[a]	25 1	—	—
T-40	4 0[a]	17 2	—	—
T-10	1 0[a]	8 5	—	—
D-195000	19 5[a]	38 8	—	—
D-59000	5 90[a]	19 9	—	—
XH5550	18 0[a]	36 3	—	—
YH5592	42 0[a]	46 4	—	—
7527	4 1	16 4	5 48	61 8
3527	8 2	25 1	—	—
527	11 2	29 2	—	—
Dextran T-2000 fraction				
Fr Ia	681	91 2	61 5	3 52
Fr Ib	563	83 7	61 0	6 99
Fr Ic	281	76 2	28 5	7 93
Fr Id	116	63 9	13 2	14 1
Fr Ie	55 1	54 5	8 36	19 3
Fr If	25 9	40 9	5 87	31 3
Fr IV	15 0	33 2	5 05	35 8
Fr V	8 3	27 3	—	33 8
Dextran T-70 fraction				
Fr 5	7 0	24 1	—	110

[a]Provided by manufacturers

A typical example of a corrected g.p.c. elution curve is compared with an uncorrected one in Fig. 4. It may be seen that the corrected elution curve is narrower than the uncorrected one, but the elution peak volume remains unchanged Results of the calculated using these elution curves are shown in Table II, in which it may be seen that, by use of the correction, the calculated $\overline{M}_{w\,cal}$ approaches the observed $\overline{M}_{w\,obs}$.

Table III summarizes the results obtained by analyzing the corrected elution curves and the viscometric data for the fractions of T-2000 and other dextrans. Eq. 2 had, on the basis of the Zimm–Stockmayer theory[20,21], been semi-empirically proposed by Kurata *et al.*[14,15] for randomly branched polymers with trifunctional branching points and flexible, long branches. Hence, λ obtained may correspond to the content of long, flexible branches which can enable dextran molecules to alter their hydrodynamic properties from those of a linear chain. Thus, the long-branch contents (%) was derived from λ.

It has been said that some 5% of residues in the dextran have branching linkages, and that the branch content does not depend on the molecular weight[9].

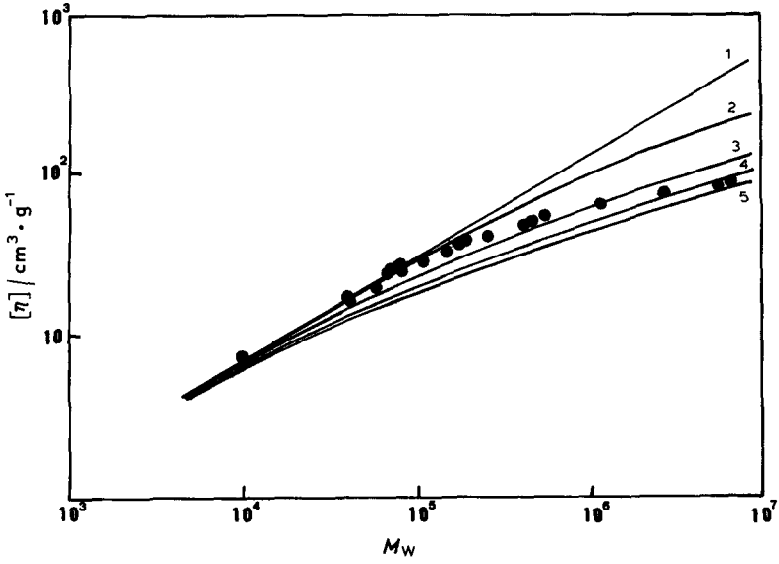

Fig. 3 Log–log plot of $[\eta]$ against $\overline{M}_w$ for dextran samples in water at 40° [Key (●) represent values observed in this experiment. Lines are those calculated theoretically by using Eq 2, with various values of λ (1, 0, 2, 0 8 × 10^{-5}, 3, 5 90 × 10^{-5}, 4, 14 0 × 10^{-5}, and 5, 24 0 × 10^{-5}) and given values of K (0 0201) and a (0.627).]

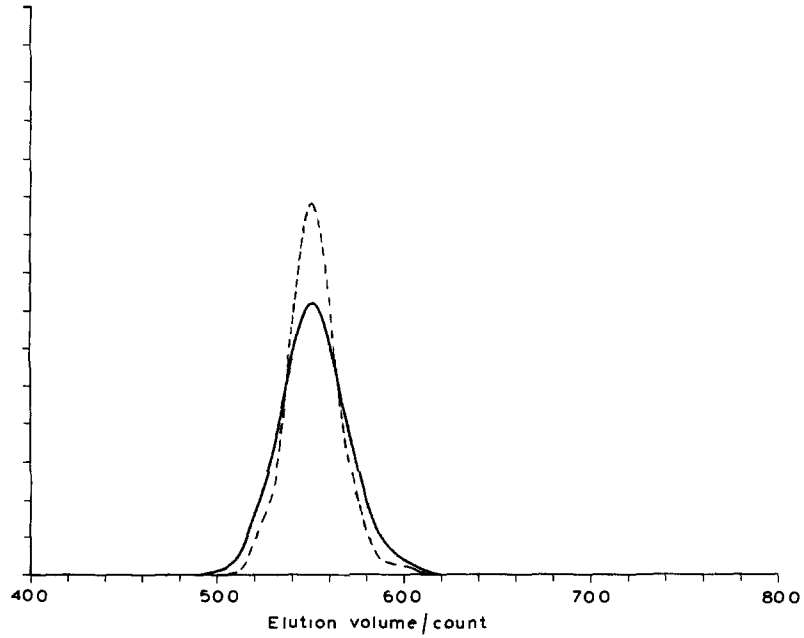

Fig 4 Uncorrected (——) and corrected (------) elution profile for Dextran T-2000, Fraction I_c

TABLE II

RESULTS OF MOLECULAR-WEIGHT CALCULATIONS WITH UNCORRECTED AND CORRECTED G P C ELUTION CURVES

Sample	*Molecular weight observed*	*Molecular weight calculated with*					
		Uncorrected curve			*Corrected curve*		
	$\overline{M}_w$ (10^4)	$\overline{M}_n$ (10^4)	$\overline{M}_w$ (10^4)	$\overline{M}_w/\overline{M}_n$	$\overline{M}_n$ (10^4)	$\overline{M}_w$ (10^4)	$\overline{M}_w/\overline{M}_n$
Dextran T-2000							
Fr Ia	681	261	741	2 84	286	660	2 30
Fr Id	116	86 4	164	1 90	97 3	148	1 52
Fr V	8 3	5 9	8 8	1 50	6 5	8 2	1 26

TABLE III

RESULTS OBTAINED FOR DEXTRAN FRACTIONS

Sample	$\overline{M}_{w\,obs}$ (10^4)	$\overline{M}_{n\,calc}$ (10^4)	$\overline{M}_{w\,calc}$ (10^4)	$\overline{M}_{w\,obs}/\overline{M}_{w\,calc}$	$\overline{M}_{w\,calc}/\overline{M}_{n\,calc}$	λ (10^{-5})	*Long branch* content (%)
Dextran T-2000							
Fr Ia	681	286	660	1 03	2 30	16	2 5
Fr Ib	563	347	782	0 72	2 25	24	3.9
Fr Ic	281	179	392	0.72	2 19	14	2.3
Fr Id	116	97 3	148	0 78	1 52	8 8	1.4
Fr Ie	55 1	43 0	58 8	0 94	1 37	4 3	0 70
Fr If	25 9	19 3	28 1	0 92	1 46	3 5	0 57
Fr IV	15 0	10 4	13 9	1 08	1 34	1 4	0 23
Fr V	8 3	6 5	8 2	1 01	1 26	0 0	0 0
T-500	46 6	33 4	47.4	0.98	1 42	4 1	0 66
T-70	7 2	4 6	6 1	1 18	1 33	0 0	0 0
D-195000	19 5	13 3	21 4	0 91	1 61	2.1	0 34
D-59000	5 9	4 0	6 7	0 88	1 68	0 0	0 0
XH5550	18 0	11 0	18 8	0 96	1 71	2 0	0 32
527	11 1	7 1	10 7	1 04	1 51	0 8	0 13
3527	8 2	5 2	8 5	0 96	1 63	0.0	0 0
7527	4 1	2 4	3 6	1 14	1 50	0 0	0 0

The long-branch content, however, alters with the molecular weight, as may be seen in Table III

Eq. *2* shows that the sensitivity of detecting the degree of branching, using $[\eta]$, decreases with decreasing molecular weight. However, it is not necessary to alter our conclusion describing the relation between the content of long-chain branching and molecular weight for the following reasons.

At a given, finite value of λ, log $[\eta]$ should increase, with downward curvature, with increasing log M obeying Eq. *2*, At first glance, this would appear to

express well the experimental results obtained. Then, the theoretical curves of Eq. 2 for various λ values with given values of K and a were calculated (and are shown in Fig. 3). No curves, however, can represent the experimental data. Even the best-fitting curve, 5.90×10^5 of λ, crosses the experimental points. From this fact, it is certain that the long-branch content should increase as the molecular weight increases.

Such physicochemical methods as are used in this study usually lack accuracy, compared with chemical methods. However, the fact that the ratio of $\overline{M}_{w\,cal}$ to $\overline{M}_{w\,obs}$ is distributed around 1.0 shows that this method has no systematic error, and is suitable for determining the long-chain content only, not counting such short branches as a D-glucosyl or maltosyl group.

Both the second virial coefficient and the radius of gyration of the dextran molecule changed with molecular weight and showed a linear relationship when plotted on a log–log scale. these parameters appeared not to be appreciably affected by the long branchings. This seems due to the fact that the contraction of radius of gyration owing to the branchings would be obscured, because the radius of gyration determined by light-scattering is of z-average and may be over-estimated, as the sample used is more or less polydisperse. Hence, it can be said that the radius of gyration is insensitive for detection of the effect of branching for a polydisperse branched polymer, as was pointed out by several authors[22,23].

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