Carbohydrate Research, 74 (1979) 31–40

THE α-D-GLUCOPYRANOSIDIC LINKAGES OF DEXTRANS: COMPARISON OF PERCENTAGES FROM STRUCTURAL ANALYSIS BY PERIODATE OXIDATION AND BY METHYLATION*

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(Received September 15th, 1978; accepted for publication, December 27th, 1978)

ABSTRACT

The analyses of 25 dextrans by g.l.c.–m.s., methylation–fragmentation, and periodate-oxidation techniques have been compared. Although in general agreement for slightly branched dextrans, the two techniques yield divergent analyses for highly branched dextrans. Employing the methylation–fragmentation data as the standard, the periodate-oxidation data were examined to establish the extent of deviation for the types of α-D-glucopyranosidic linkages that occur in dextrans, that is, the (1→6)-like, the (1→4)-like, and the (1→3)-like. The unexpected behavior of the dextrans was correlated with whether branching occurs through either C-2 or C-4, or C-3, or both of these types. Possible causes for the effects observed are discussed.

INTRODUCTION

Analytical, periodate-oxidation analysis had provided structural characterization of a large number of diversely constituted dextrans, most of which were produced by strains of *Leuconostoc mesenteroides* and *L. dextranicum*[2,3]. This information has been especially valuable for selecting the dextrans for a variety of other investigations, and for correlating the data therefrom. The availability of methylation structural analyses on 25 of these dextrans[4–8] now makes possible, for the first time, an absolute evaluation of the accuracy of the periodate-oxidation analyses. Heretofore-unrecognized effects of specific, non-(1→6)-linkages in the dextrans on the results of periodate-oxidation analyses are disclosed, as well as previously undefined sources of inaccuracies in the structural details.

Results of the periodate-oxidation, structural analysis are expressed as the percentage of α-D-glucopyranosyl residues in three categories of linkages. These categories are: (*a*) the (1→6)-like, in which the residues are linked through C-1 only, or

*Unusual Dextrans, Part VII. For part VI, see ref. 1.

TABLE I

COMPARISON OF DATA ON LINKAGES OF DEXTRANS BY PERIODATE-OXIDATION AND METHYLATION-STRUCTURAL ANALYSES

Dextran[a]		*Methylation-structural analysis*[a]						*Periodate-oxidation analysis*						
NRRL strain[b] *No.*	*Fraction*	*Methyl ethers of* D-*glucose in molar percent*						*Initial*[a,3] *percentage*			*Difference (1→6)-like, periodate minus methylation*[c]	*Corrected*[d] *percentage*		
		2,3,4,6 plus 2,3,4	*3,4*	*2,3*	*2,3,6*	*2,4*	*2,4,6*	*(1→6)-like*	*(1→4)-like*	*(1→3)-like*		*(1→6)-like*	*(1→4)-like*	*(1→3)-like*
Group I														
B-742[4,e]	L	87		12			1	81	19	0	−6	87	13	
B-1420[f]		86	1	12		1		81	19	0	−5	86	14	
B-1422[5]		80	19			1		74	26	0	−6	80	20	
B-1526[f]	I	78		19		2		77	23	0	−1	78	22	
B-1254[6]	S[L]	77		20	3			69	31	0	−8	77	23	
B-1424[6]		76	21			3		72	28	0	−4	76	24	
B-1402[4]		75	22			3		66	34	0	−9	75	25	
B-1399[4]	P-37	73	24			3		65	35	0	−8	73	27	
B-1299[4]	L	66	27			1	6	58	36	6	−8	66	28	(6)[g]
B-1299[6]	S	65	35					50	50	0	−15	65	35	
B-1399[4]	S	65	31			3	1	55	45	0	−10	65	35	

Group II													
B-1308[6]		96		4		95	5	0	−1	96	4 →	0[h]
B-512(F)[7]		95		5		95	5	0	0	95	5 →	0
B-640[5]		95		5		95	5	0	0	95	5 →	0
B-1355[4]	L	95		4	1	88	9	3	−7	95	2 →	3
B-1254[4]	L[$]	94		4	2	93	7	0	−1	94	6 →	0
B-1351[6]	S	89		11		88	6	6	−1	89	5 →	6
B-1191[f]		81		17		77	9	14	−4	81	5 →	14
B-1501[8]	S	67		9	24	65	15	20	−2	67	13 →	20
B-1142[f]		67		28	3	63	8	29	−4	67	4 →	29
B-1498[8]	S	61		10	29	62	11	27	+1	(62)[g]	11 →	27
B-1355[6]	S	54		11	35	57	8	35	+3	(57)	8 →	35
B-742[4]	S	50		50		57	17	26	+7	(57)	17 →	26
Group III												
B-1396[5]		88	9	3		81	19	0	−7	88	12[i]	(0)[g]
B-1399[6]	L	87	7	6		81	19	0	−6	87	13	(0)

[a]Methylation-structural analysis by g.l.c.–m.s. of the per-*O*-acetylated aldononitriles[9] of the dextrans. See also footnote *e*. [b]All strains are *Leuconostoc mesenteroides*, except *L. dextranicum* B-640 and B-1420, *Streptobacterium dextranicum* B-1254, *Streptococcus viridans* B-1351, and *Streptococcus sp.* B-1526. [c]This is the difference between percentages of (1 →)- and (1 → 6)-linked residues, as determined by methylation and periodate-oxidation analyses. A "—" indicates that the periodate value is the lower, and "+" indicates that it is the greater. [d]In Groups I and III, and where applicable in Group II, periodate-oxidation values were corrected by deducting the "difference" from the percentage of (1 → 2)- or (1 → 4)-linked residues [(1 → 4)-like], or both, and adding it to the percentage of the (1 →)- and (1 → 6)-linked residues [(1 → 6)-like]. Further correction was made for Group II, but not Group III, dextrans by adding the value for the (1 → 4)-like linkages, after correction as just stated in this footnote, to that for the (1 → 3)-like linkages. This transfer is indicated by a horizontal arrow, "→". Thus, throughout Group II, the final, corrected value for (1 → 4)-like linkages is "0". See, also, footnotes *g*, *h*, and *i*. [e]Published data on the methylation-structural analysis is cited by the reference given at the strain number. [f]F. R. Seymour and E. C. M. Chen, unpublished data. [g]Parentheses indicate that the initial value has not been changed. [h]See footnote *d* for the explanation of "→". [i]In Group III, the (1 → 4)-like linkages are corrected in the same way as for Group I (see footnote *d*), but further correction as for Group II cannot be made.

through both C-1 and C-6; one mole of each of these types of residue would be expected to reduce two moles of periodate and liberate one mole of formic acid; (*b*) the (1→4)-like, in which the residues are linked individually through (1→4)- or (1→2)-bonds or both; these residues also carry (1→6)-bonds; the expected action of these residues is reduction of one mole of periodate per mole; and (*c*) the (1→3)-like, which include 3-mono- and 3,6-di-*O*-substituted residues, respectively involved in linear chain-extension and branching; these residues are inert to periodate. Alternative techniques, and numerous variables in the periodate oxidative and analytical procedures were evaluated[3], in order to establish the reliability of the analyses. It was concluded that the accuracy of measurements obtained, under the standard conditions established for the control dextran [which had 95% of (1→6)-like linkages], was 99% for the formic acid liberated and 95% for the periodate reduced.

RESULTS AND DISCUSSION

The results of analysis of the dextrans by methylation–fragmentation[9] and periodate-oxidation techniques are compared in Table I. This comparison shows that, in their behavior in periodate-oxidation analysis, the dextrans are distributed among three, well-defined groups on the basis of whether branching occurs through either C-2 or C-4, or C-3, or both of these types. The relationship between the identity and proportion of linkages that characterize the three groups and the results of periodate-oxidation analysis are discussed. The major influences resulting in deviation of periodate-oxidation values from those from methylation relate to branching.

Group I. — The presence of (1→4)-like linkages at branch points appears to be responsible for the unusual behavior, with periodate, of the dextrans of this group. For these dextrans, the (1→6)-like linkages found by the periodate-oxidation method are low, usually by 5–10%, and the (1→4)-like linkages are correspondingly high. The difference between the percentage of (1→6)-like linkages by the two methods is shown in column 12 of Table I. The sign "–" indicates that the periodate-oxidation value is the lower. If this difference is deducted from the periodate value for (1→4)-like linkages, and added to the value for the (1→6)-like, the "corrected" percentages of (1→4)-like linkages are in good agreement with those from methylation-structural analysis. For a number of the dextrans, however, the corrected value for (1→4)-like linkages is 1–3% higher than that from methylation analysis. This deviation is especially notable for dextrans B-1420, B-1422, B-1526 fraction I, B-1424, B-1402, B-1399 (P-37), B-1299 fraction L, and B-1399 fraction S. For each of these dextrans, the methylation products (see Table I) include small molar percentages of 2,4-di-*O*-methyl D-glucose which, if completely methylated[4], would appear in the analyses as either 2,3,4,6-tetra- or 2,3,4-tri-*O*-methyl-D-glucose. If these percentages of the dimethyl ether are added to those of the tetra- and tri-methyl ethers, the corrected values for the (1→4)-like linkages by periodate-oxidation analysis agree almost exactly with the corresponding values from methylation analysis. Several other methyl ethers, in proportions of ~1%, which are included in the methylation analyses for dextrans

B-742 fraction L, B-1420, and B-1399 fraction S, also diminish the agreement of the corrected, (1→4)-like values with the methylation analyses.

The difference value (see column 12, Table I) appears to be a measure of the α-D-glucopyranosyl residues linked (1→6)-like that have reduced only one mole of periodate per mole instead of two, and have failed to liberate one mole of formic acid. These residues, therefore, enter the calculation of periodate-oxidation results as if they were linked (1→4)-like.

Dialdehydes formed by periodate oxidation of carbohydrates are well known to form cyclic, inter-residue[10-13] and intra-residue[11,13,14] hemiacetals. In polysaccharides, these hemiacetals may inhibit the first glycol-bond cleavage of otherwise susceptible residues [11-13] or prevent the second glycol-bond cleavage that is necessary to liberate a mole of formic acid per mole[11,14]. Heretofore, study of cyclic hemiacetals and related forms[10,13] in periodate-oxidized polysaccharides has been almost exclusively limited to those in unbranched polysaccharides[11,14]. Two branched polysaccharides have been studied: (*a*) guaran[15], for which the restricted oxidation observed was proportional to the degree of branching, but was interpreted as resulting from hemiacetal formation in the β-D-(1→4)-linked D-mannosyl backbone, and (*b*) a β-linked diheteroglycan[16] for which it was concluded that the restricted oxidation of D-glucosyl residues resulted from both hemiacetal formation and steric hindrance caused by the equatorial orientation of the side-chain attachment. Kinetic study[17] of the periodate oxidation of a dextran having 93% of (1→6)-like linkages detailed the transient influences of intra-residue, hemiacetal formation by singly oxidized residues, and inter-residue, hemiacetal formation between doubly oxidized residues and intact D-glucosyl residues adjacent to them in the chains. The expected reaction of all oxidizable residues was, however, finally achieved.

For our dextrans, close agreement between results from periodate-oxidation and methylation-structural analyses was achieved only for those having the lowest degree of branching; that is, the first three listed in Group II of Table I. For all of the other dextrans, partial inhibition of normal oxidation, or other unexpected effects observed with Group II dextrans, persisted to the final measurements[3]. For Group I dextrans, the reduction of an excess of periodate without corresponding release of formic acid correlates with intraresidue formation of hemiacetal in singly oxidized residues, and seems to preclude a role by hemiacetal formation between oxidized residues and intact residues. An intra-residue, cyclic structure adjacent to a branch-point residue might derive unusual stability from steric hindrance. An additional stabilizing effect on an intra-residue hemiacetal situated adjacent to a residue branched through C-2 or C-4 might result from blockage of hemiacetal formation through these positions which, in essentially unbranched dextrans, are sterically favored for formation of cyclic compounds[17]. Another possible factor in the restricted oxidation of dextrans of Group I is constituted by the branches themselves, which frequently consist of a single residue[4-6,8]. Kinetic observations have not been made on the role of such residues in the periodate oxidation of α-D-glucans. Oxidation by periodate would be extremely rapid; and the oxidized residue would have unusual freedom from

steric restraints, especially if attached to a branch residue in which the pyranose ring has also been cleaved by periodate.

Group II. — The presence of (1→3)-like linkages is the distinguishing, structural feature of the dextrans in this Group; the percentage of these linkages is in the range of 4–50%. Values for the content of (1→6)-like linkages by methylation and periodate-oxidation analyses agree much more closely for this Group than for Group I: most of the differences are in the range of 0 to −4, although there is one at −7, and several "+" (positive) differences (+1, +3, and +7) occur (see Table I). Periodate-oxidation analysis shows (1→4)-like linkages (in the range of 5–17%) in all of these dextrans, although methylation analysis shows none. The percentage of (1→3)-like linkages by periodate-oxidation analysis is low throughout this Group.

Further discussion is simplified by excluding from this paragraph, and the three that follow, the first three dextrans listed in Group II. They appear to constitute a special sub-group, and will be considered later in this discussion. If the type of correction that was used for Group I is applied to Group II dextrans for which the difference value is negative (see column 12, Table I), the percentage of (1→4)-like linkages is still high by some 2–13%, and the (1→3)-like values are correspondingly low. [Interrelationship between the percentage of (1→3)-like and (1→4)-like linkages from periodate-oxidation measurements is inherent, as the (1→3)-like consist of the calculated difference between 100% and the sum of the other two types of linkage, which are determined by titrimetric measurements[3].] The periodate-oxidation values can, however, be brought into agreement with the methylation analyses by adding the corrected percentage of (1→4)-like linkages to that of the (1→3)-like. By this adjustment, the (1→4)-like values become zero, and the (1→3)-like become equal to those obtained from methylation analysis. It is concluded that, in Group II, the dextrans that have negative difference-values appear to show two concurrent side-effects with periodate: (*a*) the same as that shown by Group I dextrans, that is, inhibition, by cyclic bond-formation, of the second, glycol bond-cleavage necessary for liberation of formic acid, and (*b*) reduction of periodate by a reaction that does not involve normal, glycol-bond cleavage or liberation of formic acid. The percentage of linkages calculated from periodate-oxidation measurements is, therefore, erroneously high for (1→4)-like linkages, and low for (1→3)-like.

The last three dextrans in Group II contain (1→3)-like linkages in the range of 39–50%, and show positive difference-values (see Table I). The periodate-oxidation values obtained from them provide no logical basis for applying the type of correction for (1→6)-like and (1→4)-like linkages that serves satisfactorily for the other dextrans in Groups I and II. Possibly, these three dextrans, with their high content of non-(1→6)-linkages, do not undergo the effect characteristic of Group I dextrans, and their abnormal reduction of periodate is accompanied by liberation of formic acid in molar percentages of 1, 3, and 7, respectively, as is shown in Table I. Another possibility is that the percentage of (1→6)-like linkages (as found by periodate oxidation) for these three dextrans is an average of the Group I effect that would result in a negative

difference-value, and in the novel, unexplained effect that liberates an excess of formic acid.

A structural feature common to all of the dextrans in Group II is the presence of 3,6-di-*O*-substituted α-D-glucosyl residues at branch points. Three of the Group II dextrans (B-1355 fraction S, B-1498 fraction S, and B-1501 fraction S) also contain 3-mono-*O*-substituted [that is, linearly (1→3)-linked] residues in high proportions. The rest of the Group II dextrans contain no, or only small proportions of linearly (1→3)-linked residues. The unusual behavior may, therefore, relate to 3,6-di-*O*-substituted residues alone, and not necessarily to 3-mono-*O*-substituted residues also. This possibility is supported by the reasoning that, in the dextrans (B-1501 fraction S, B-1498 fraction S, and B-1355 fraction S) that contain both of these types of residue in considerable proportions, the corrected percentage of apparent, (1→4)-like residues agrees better with the percentage of 3,6-di-*O*-substituted residues than with that of the 3-mono-*O*-substituted residues. For these three dextrans, there appears to be a roughly quantitative correlation between (*a*) the residues that branch through C-3 and (*b*) the reduction of periodate unaccompanied by liberation of formic acid. However, among the dextrans that have only 3,6-di-*O*-substituted residues (such as dextrans B-1351 fraction S, B-1191, and B-1142) there is no consistency between the proportion of these linkages and the corrected content of apparent (1→4)-linkages.

Further indication that (1→3)-linked residues in linear-chain positions in dextrans of Group II are probably not involved in the reduction of periodate unassociated with glycol-bond cleavage is that no unexpected effects are reported for the action of periodate on the linearly (1→3)-linked glucans nigeran and lichenan[10,11].

The first three dextrans included in Group II (from strains B-1308, B-512(F), and B-640) show, in common with the other Group II dextrans, high values for (1→4)-like linkages by periodate oxidation and the necessity of transferring these values to the (1→3)-like category in order to achieve agreement with the results of methylation structural analyses. It is to be noted that a number of other Group II dextrans show corrected, (1→4)-like values in the range of 2–6% (see Table I). Periodate-oxidation analysis indicated that numerous other NRRL dextrans (see ref. 2, Table I; Class A dextrans) contain 5–6% of (1→4)-like linkages, and it might now be suspected that at least some of these linkages are also, in reality, (1→3)-like; ~5% of (1→4)-like linkages is near the limit of accuracy of the analytical methods employed[3]. The major source of this limiting accuracy may be the reaction of the peroxidized polysaccharide with periodate, or with the iodine reagent itself, during the analysis for residual periodate in the analysis solution[3]. The role of this uncertainty in the results given in Table I that are under discussion here can be resolved only by repetition of the oxidations, with analysis for residual periodate by a reagent now available[18] that precludes these potential sources of error.

The corrected values for (1→4)-like linkages in a number of Group II dextrans are well beyond the uncertainties arising from the limiting accuracy of the periodate-oxidation titrimetric analysis. Likewise, this source of uncertainty is eliminated from consideration for Group I dextrans by the excellent agreement therefore between

periodate-oxidation and methylation-structural analyses. There is justification, therefore, for considering other possible explanations for the high values for (1→4)-like linkages in Group II dextrans, and for the reduction of periodate unassociated with normal, glycol-bond cleavage and release of formic acid.

Erosion from the reducing end, commonly called[19] "over-oxidation", is, for several reasons, not a plausible explanation for the unusual behavior of Group II dextrans with periodate. (*a*) Over-oxidation would require the liberation of much more formic acid than is observed, as, in over-oxidation, the molar ratio of formic acid liberated to periodate reduced is[19] 3:4. The fortuitous introduction of formic acid in such proportions into the calculation of linkages is incompatible with the percentage of (1→6)-like linkages found in Group II dextrans by periodate oxidation, a value that is consistently in close agreement with the results of methylation analysis (see Table I). (*b*) The magnitude of the abnormal effect would require extensive erosion; this would be dependent upon sizable sequences of (1→3)-linked residues starting at the reducing end of the dextran and continuing without interruption by residues having substituents at C-6, which would stop the progress of erosion[19]. Thus, 3,6-di-*O*-substituted residues could not participate in degradative erosion. The absence of contiguously situated, (1→3)-linked residues in dextran B-1355 fraction S has been established by acetolysis[20,21] and by enzymic degradation[22].

Periodate is known to oxidize "active" hydrogen atoms[19], but the structural residues from periodate-oxidized dextrans that contain 3,6-di- or 3-mono-*O*-substituted residues (as normally envisaged[10]) give no indication of the presence of such hydrogen atoms, either actual or potential.

Structures not normally susceptible to periodate oxidation are, however, known to be attacked when held in cyclic form, so that complexation with periodate is favored[23]. We postulate that, if structures of such a unique type occur in periodate-oxidized, Group II dextrans and account for the abnormal reduction of periodate, they must involve the oxidized branch-residues attached at C-3 of the intact, branch-point residue. Intra-residue cyclization would be favored by the presence of the primary hydroxyl group of a nonreducing end-group[13], and novel complexation with periodate might be facilitated by relatively low steric hindrance at C-3 of the branch-point residue. This postulated site of abnormal reduction of periodate appears to be the only site available in dextran B-742 fraction S. Methylation-structural analysis[4] shows that this dextran consists of only two types of residue in equal proportions, that is, linear backbone residues linked by (1→6)-bonds, each one of which carries a one-unit long branch attached at C-3. Periodate-oxidation analysis showed that each side-chain residue reduced two molecules of periodate and liberated one molecule of formic acid, a value which is expressed as 50 (±7)% of (1→6)-like residues. The analysis also showed that 17 molar percent of periodate was reduced in excess of that required to produce the 57 molar percent of formic acid (see ref. 3, and Table I). The 3,6-di-*O*-substituted, backbone residues are inert to periodate; therefore, the abnormal reduction of 17 molar percent of periodate apparently relates to novel behavior of the oxidized, branch residue.

The 3,6-di-*O*-substituted α-D-glucopyranosyl residues at branch points in dextrans are unaffected by periodate oxidation, and have been recovered quantitatively as D-glucose from acid hydrolyzates of the completely periodate-oxidized polysaccharide. From three of the dextrans discussed herein, the molar percentage of D-glucose isolated in this way[24] is: dextran B-512(F), 5%; dextran B-742 fraction S, 28%; and dextran B-1355 fraction S, 47%. Except for the B-742 fraction S, these results agree precisely with those given by methylation analysis. For dextran B-742 fraction S, the results accord with the percentage of units having (1→3)-like linkages that is indicated by periodate-oxidation analysis.

Group III. — The dextrans in this group contain both (1→4)-like and (1→3)-like linkages in significant proportions. The results of periodate-oxidation analysis show the characteristic effect of (*a*) Group I, in the low values for (1→6)-like linkages, and (*b*) Group II, in the high values for (1→4)-like linkages and low values for the (1→3)-like. The periodate-oxidation results are not brought into accord with the methylation analyses by the corrections applicable to Group I or Group II dextrans. The percentage of (1→4)-like linkages measured by periodate-oxidation analysis cannot be resolved by simple readjustment of values, because it arises from three sources: (*a*) normal oxidation of a single glycol bond, (*b*) oxidation of one member of a pair of glycol bonds (Group I effect), and (*c*) oxidation different from (*a*) or (*b*) (Group II effect).

The two dextrans in this group are probably representative of numerous other NRRL dextrans (see ref. 2, Table I) for which periodate-oxidation analysis indicates both (1→4)-like and (1→3)-like linkages. The evaluations made here also suggest that C-4 branching in dextrans may be less prevalent than has been thought heretofore[2], or lower in proportion.

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