Carbohydrate Research, 91 (1980) 93–96
Elsevier Scientific Publishing Company, Amsterdam – Printed in The Netherlands

Note

Measurement of diad frequencies in lucerne- and clover-seed galactomannans by periodate oxidation

JOSÉ J. GONZÁLEZ
Agder Ingeniør og Distriktshøgskole, N-4890 Grimstad (Norway)
AND TERENCE J. PAINTER
Institutt for Marin Biokjemi, N-7034 Trondheim-NTH (Norway)

(Received September 4th, 1980; accepted for publication, October 6th, 1980)

Reactions of polymers are usually co-operative*, in the sense that the reactivity of any monomeric unit depends upon the reacted-or-unreacted state of other units in the same chain[1,2]. The theory is well-developed for binary, linear copolymers in which the reactivity depends upon the state of nearest-neighbour units only[3,4]. It allows inferences to be drawn about the sequence of monomer units (herein denoted by **1** and **2**, respectively) in the chains, from studies of the kinetics of the reaction. In principle, any kind of sequence can be studied, but one is normally restricted by experimental error to measurements of diad frequencies (F_{11}, F_{12}, *etc.*) and triad frequencies (F_{111}, F_{112}, *etc.*). A sequence that is fully defined by its diad frequencies alone is described as a "first-order Markov chain"; one that is fully described by its diad and triad frequencies alone is a "second-order Markov chain"; and so on.

In the periodate oxidation of (1→4)-linked glycans, there is a strong, nearest-neighbour, auto-inhibitory effect, arising from the spontaneous formation of six-membered hemiacetal rings between the aldehyde groups of oxidised units and the secondary hydroxyl groups of unoxidised, adjacent units[5-9]. The legume-seed galactomannans[10] are particularly suitable for study, because their high molecular weight eliminates end-group effects, and over-oxidation[11] is blocked by the 6-*O*-substitution of D-mannosyl residues. They fit the available mathematical model[3,4], because they can be formally regarded as a linear sequence of 6-*O*-substituted, and unsubstituted, (1→4)-linked β-D-mannopyranosyl residues (monomer units **1** and **2**, respectively). A further simplification is that the hemiacetals formed by oxidised **1** units are so stable that there is virtually complete protection of adjacent, unoxidised units from subsequent oxidation, whereas those formed by oxidised **2** units are much

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*The term embraces positively co-operative, or "auto-acceleratory" reactions, and negatively co-operative, or "auto-inhibitory" reactions.

less stable[12]. This situation gives rise to fairly well-defined, and easily measured, oxidation limits that are dependent upon both composition and sequence[12]. These limits are denoted here by y_∞, the fraction of oxidisable D-mannosyl residues in the mannan chains. The ratio, d_∞, of unoxidised **1** to unoxidised **2** units in the limit-oxidised products is also easily measured, by methylation analysis after reduction with borohydride; this gives d_∞ as the ratio of 2,3-di-*O*-methyl- to 2,3,6-tri-*O*-methyl-D-mannose in the final hydrolysate[13].

Fig. 1 shows theoretical curves which illustrate the dependence of y_∞ and d_∞ upon sequence for first-order Markovian galactomannans of the usual structural type (represented by guaran[10]). In the calculations, account has been taken of the different, initial rates of oxidation of **1** and **2** units ($k_2/k_1 = 1.4$)[13,14], and the partial back-protection afforded by oxidised **2**. These results have not been published before, and should be generally applicable to all galactomannans of this type. The quantity p_1 is the absolute probability of finding a unit **1** anywhere in the chain, and is numerically identical with the Gal:Man ratio for the galactomannan. The quantity p_{11} is the conditional probability that a unit **1** will be followed by another unit **1** when the chain is scanned in any given direction. Hence, the diad frequency F_{11} is $p_1 \times p_{11}$. For the special case of a random distribution, $p_{11} = p_1$. The four diad frequencies for a first-order Markov chain are fully defined by p_1 and p_{11}, because $p_2 = 1 - p_1$, $p_{12} = 1 - p_{11}$, $F_{12} = F_{21} = p_1p_{12} = p_2p_{21}$, and $F_{22} = 1 - F_{11} - F_{12} - F_{21}$.

If the sequence in a given galactomannan conformed to a first-order Markov chain, the measured values of y_∞ and d_∞ should intersect in Fig. 1 at a point corresponding to the polymer's composition, p_1, and, from the corresponding value of p_{11}, it would be possible to calculate all four diad-frequencies with the relationships

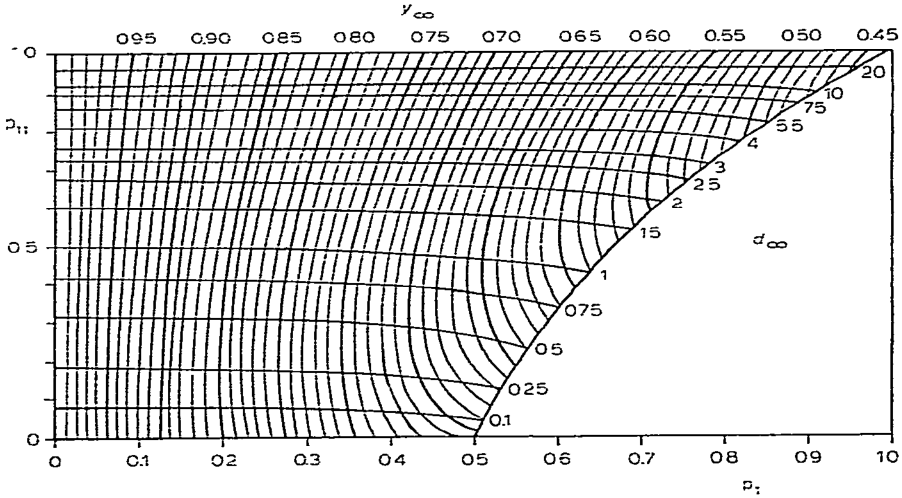

Fig. 1. Theoretical curves showing dependence of the conditional probability (p_{11}) upon the oxidation limit (y_∞) and methylation ratio (d_∞) for galactomannans having different Gal:Man ratios (p_1).

TABLE I

GAL:MAN RATIOS (p_1), OXIDATION LIMITS (y_∞), AND METHYLATION RATIOS (d_∞) FOR LEGUME-SEED GALACTOMANNANS

Galactomannan	y_∞	d_∞	*Gal:Man ratio (p_1)*		*Value of p_{11} indicated*[a]
			Expected[a]	*Found*[b]	
Carob	0.86[c]	0.35 ±0.02	0.18	0.24	No fit
Guar	0.59	1.75 ±0.20	0.63	0.56	No fit
Clover	0.55	4.20 ±0.60	0.72	0.72	0.79
Lucerne	0.52	5.00 ±0.70	0.78	0.79	0.83

[a] For a first-order Markov chain (from Fig. 1). [b] By periodate oxidation, n.m.r. spectroscopy[18], and sugar analysis[12,13,16]. [c] Value obtained by extrapolation[15]. The terminal stage of oxidation of this galactomannan is extremely slow, and the value of 0.80 reported earlier[13] was measured after an insufficient period of reaction.

TABLE II

DIAD FREQUENCIES IN LUCERNE- AND CLOVER-SEED GALACTOMANNANS, DETERMINED BY PERIODATE OXIDATION (METHOD A) AND ^{13}C-N.M.R. SPECTROSCOPY (METHOD B[18])

Galactomannan	F_{11}		$F_{12} = F_{21}$		F_{22}	
	A	*B*	*A*	*B*	*A*	*B*
Lucerne	0.65	0.59	0.14	0.18	0.07	0.05
Clover	0.57	0.56	0.15	0.16	0.13	0.12

just mentioned. In earlier studies[3,14,15] of guaran and locust-bean (carob) gum, however, this condition was not fulfilled, as shown in Table I. These galactomannans therefore needed at least a second-order Markov chain to describe them, and this prompted a more detailed, theoretical analysis, which is reported elsewhere[14,15].

We now report that application of the same methods to the galactomannans from lucerne[16,17] and clover[16] seeds gave results that fitted the first-order Markovian statistics within the limits of experimental error (Table I). From the indicated value for p_{11} (Fig. 1), the two complete sets of diad frequencies were calculated, and, in Table II, these are compared with values determined on the same samples by ^{13}C-n.m.r. spectroscopy[18]. Within the limits of experimental error of both methods[14,15,18], the agreement is good. In conclusion, we have found by this method that p_{11} is slightly larger than p_1, which implies that the groups of contiguous, substituted D-mannosyl residues in the chains are a little longer than expected for a random distribution, and that the same is true for the groups of contiguous, unsubstituted D-mannosyl residues.

Insofar as the distribution may be presumed to be of the first-order Markovian type, the measured values of p_1 and p_{11} are sufficient for calculation of everything concerning sequence in the molecules, including multad frequencies of higher order and sequence-length distributions[19]. The consistency of the data with this model does not, however, prove that a more-complex type of structure could not account for them, and the distribution cannot therefore be said to have been determined unequivocally.

EXPERIMENTAL

The materials[18] and methods[12-14] have been described. In the oxidations. 1-propanol (2% v/v) was included in the reaction mixtures. to inhibit free-radical depolymerisation[14]. Oxidation was continued for 11 days, after which the consumption of periodate and the liberation of formic acid had stopped. The yield of formic acid was used to calculate the Gal : Man ratio (p_1), which was also measured by ^{13}C-n.m.r. spectroscopy[18]. Methylation analysis[13] was performed on the borohydride-reduced, limit-oxidised products. to give d_∞; and also on the starting-materials, to correct d_∞ for any selective losses incurred during work-up, and to calibrate the detection system of the gas chromatograph. To improve the solubility of the un-oxidised galactomannans in the dimethyl sulphoxide used for methylation, they were slightly depolymerised with acid, as described elsewhere[18]. For g.l.c.. an SP-1000 glass column at 220° was used.

ACKNOWLEDGMENTS

We thank Dr. J. Hoffman for performing the methylation analyses, Professor B. Lindberg for helpful discussion, and Norges almenvitenskapelige forskningsråd and Nansenfondet for financial support (to J.J.G.).

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