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Note

Pyruvate-free xanthan

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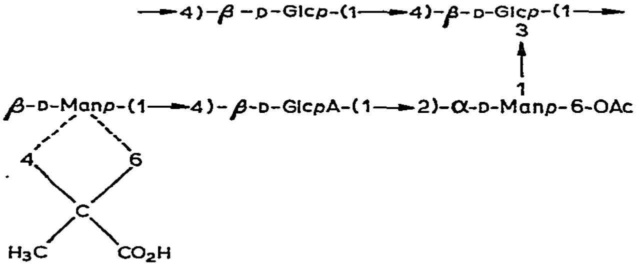
Xanthan, the extracellular polysaccharide of the bacterium *Xanthomonas campestris*, was shown by Lindberg and coworkers[1] to have a comb-like primary structure with the pentasaccharide repeating-unit **1**. However, the presence of acetal-linked pyruvate at the end of the side chain is not stoichiometric. Only 0.14–0.65 of the terminal mannosyl groups bear a pyruvate group[1-4], the proportion depending on the strain and growth conditions[5,6]. It is known already that the average pyruvate-content of the polysaccharide may vary during the course of fermentation[6] and that the final fermentation-product is composed of a mixture of species having various contents of pyruvate[4]. However, the distribution of the pyruvate groups on individual molecules is unknown, although these groups play an important role in determining the properties of xanthan[3] and other bacterial polysaccharides[7].



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In order to elucidate the structural role of different parts of the molecule **1**, we have sought methods for selectively removing the pyruvate or acetyl groups. It is possible by one method to remove 90% of the pyruvate groups from xanthan without removing acetyl groups or causing substantial breakdown of glycosidic bonds; a second method removes 100% of the acetyl groups while leaving the pyruvate groups intact.

Fig. 1 shows the optical rotation at various temperatures ("melting curves") for unmodified xanthan and also for such "pyruvate-free" xanthan at three ionic strengths. Unmodified xanthan exhibits a broad transition whose midpoint tempera-

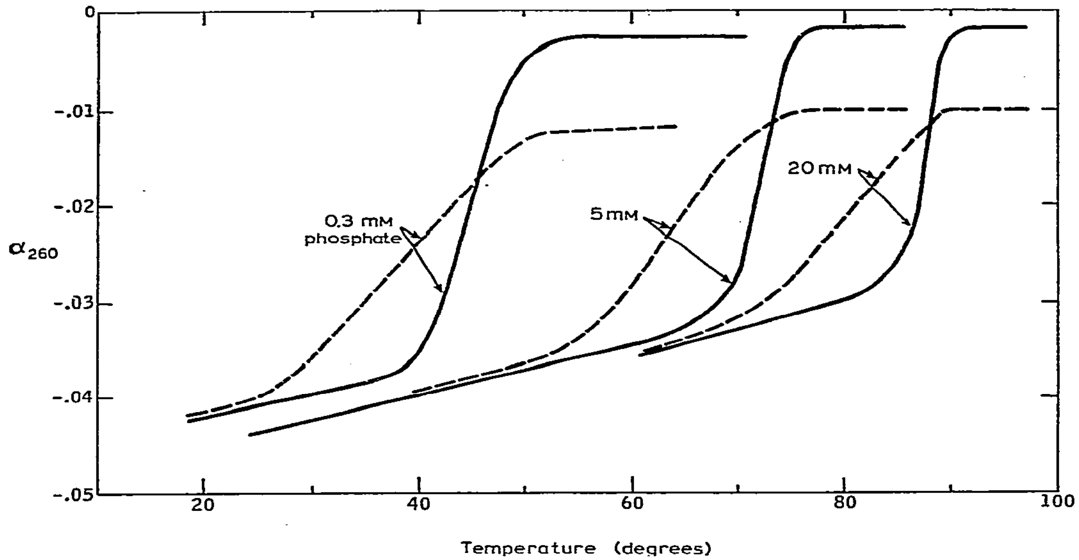

Fig. 1. Optical rotation (α, degrees) at 260 nm for native (– –) and pyruvate-free (——) xanthan (0.6 mg/mL) in a 10-cm cell, between 20 and 100°. The concentration of phosphate buffer (pH 7) is indicated.

ture (T_m) increases with ionic strength, as reported previously[8,9]. The removal of pyruvate groups sharpens the transition markedly, so that the slope at the midpoint is steeper by a factor of 3–5. In fact, the sharpness becomes comparable to that of DNA[10]. Moreover removal of pyruvate leaves unchanged the temperature at which melting is complete. Finally, it increases T_m by 5–10°.

Studies on helix–coil transitions in DNA and in polypeptides[11,12] provide a quantitative molecular framework for comprehending thermally induced, conformational transitions in polysaccharides. Indeed, the dependence of T_m of xanthan on ionic strength, counter-ion species, and pH has been shown previously to fit into this framework[8]. The one-dimensional Ising model provides a particularly attractive way to describe this transition; for a homogeneous polymer near T_m it predicts[13] that the sharpness is given by

$$\partial f/\partial(1/T) = -\Delta H/4\sigma^{1/2} \qquad (1)$$

where f is the fraction of monomeric groups in the helical form, and ΔH and σ characterize the transition enthalpy and cooperativity. For such chemically heterogeneous samples as DNA, the transition may be broadened, because T_m is different for different portions of the molecule[14].

The data in Fig. 1 may be incorporated into this framework if it is recognized that unmodified xanthan is chemically heterogeneous, with some molecules or sections of molecules being rich in pyruvate and others depleted in pyruvate. The increased charge-density along the chain in pyruvate-rich regions would be expected to lower T_m in the same way that decreasing ionic strength lowers T_m or decreasing pH increases[8] T_m. The lowering of T_m originates in electrostatic repulsion among the carboxylate groups of pyruvate and D-glucuronic acid; this repulsion is greater in native xanthan than in denatured xanthan, because the charged groups are more closely spaced in the native conformation.

In order to make this argument more quantitative, we have examined the effect of pH on T_m. Changing the degree of ionization from 1.0 to 0.75 increases T_m by 6°; at a degree of ionization of 0.6, T_m is increased by 20°. Hence, it is reasonable that pyruvate-rich sections should have T_m values lower by 10–15° than pyruvate-free polymer.

By contrast, pyruvate-poor sections of unmodified xanthan would be expected to have T_m values near that of pyruvate-free polymer. The net result of a mixture of pyruvate-rich and pyruvate-poor regions would be a broadening of the overall, apparent melting-curve.

The fact that a similar temperature is needed to complete the transition in unmodified and in pyruvate-free xanthan supports the suggestion that unmodified xanthan contains regions essentially free from pyruvate groups. Although it is possible that all of these observations could arise from changes in some unsuspected group, rather than pyruvate, this possibility seems unlikely. Indeed, samples from which only 75% of the pyruvate was removed show a transition breadth intermediate between that of the native and the pyruvate-free samples.

What would be the effect on the melting curve if the pyruvate groups were distributed uniformly along the chain? One would expect T_m to increase upon removal of pyruvate, but the sharpness of the melting curve should be largely unaltered, in contrast to the experimental results.

The sharpness of the transitions depicted in Fig. 1 would permit a calculation of the cooperativity parameter σ in Eq. *1* if ΔH were known. The largest value of $\partial f/\partial T$ at T_m is $0.25^{\circ -1}$, if the pretransition (gradual change in α before the sharp transition) is neglected. Attempts to measure ΔH show it to be less than 500 cal/mol (pentasaccharide). This result means that $\sigma < 10^{-5}$, which is indicative of a highly cooperative transition.

It is interesting to contrast the effect of removal of pyruvate with that of removal of the acetyl group from O-6 of the innermost mannosyl residue of each side chain. Acetyl-free xanthan exhibits melting transitions as broad as those of unmodified polymer, but with the T_m value decreased by about 15°. This result is consistent with the fact that every side chain bears one acetyl group and suggests that the acetyl groups, in contrast to the pyruvate groups, stabilize the native conformation.

EXPERIMENTAL

Materials and procedures. — Xanthan ("Keltrol", Kelco Co.) was purified by centrifugation and ethanol precipitation as previously described[8]. This sample had a pyruvate content[15] of 3.1%, which means that 33% of the terminal mannosyl groups bear a pyruvate moiety.

The pyruvate groups were removed by heating a solution of xanthan (3–5 mg/mL in 1mM oxalic acid, 0.1M sodium chloride, pH 3) for 2 h at 95°. Under these conditions, about 90% of the pyruvate groups were removed, as measured by the lactate-dehydrogenase method[15]. The molecular weight of the pyruvate-free polymer, measured by band-sedimentation as previously described[16], was 7×10^6, which means that the molecular backbone remained essentially intact. The acetyl content[17] was unaltered.

Mere heating of xanthan, for example in 0.1M sodium chloride, pH 7, to 95° for several h did not remove the pyruvate groups and did not produce a xanthan displaying a sharpened melting-curve.

Acetyl-free xanthan was prepared by dissolving the unmodified polymer (3–5 mg/mL) in water and then altering the solvent to 0.025M potassium hydroxide, 0.1M potassium chloride for 2–3 h at 20° under nitrogen[2]. Complete removal of acetyl groups was achieved without removal of pyruvate and with less than 30% decrease in viscosity.

Optical rotation–temperature curves were obtained with a Jasco spectropolarimeter modified as described previously[8] to provide direct display of rotation and sample temperature on an *x–y* recorder.

Solutions were prepared to the desired ionic strength by dialysis against appropriate buffers.

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