

THE INFLUENCE OF SIDE-CHAIN MODIFICATIONS ON THE SOLUTION BEHAVIOR OF THE CAPSULAR POLYSACCHARIDE FROM *Rhizobium trifolii* STRAIN TA-1

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(Received March 28th, 1988; accepted for publication in revised form, September 15th, 1988)

ABSTRACT

The effect of chemical modification of the side chain on the solution properties of the capsular polysaccharide from *Rhizobium trifolii* strain TA-1 has been investigated. The gel stability of the unmodified polymer has been measured by the sigmoidal change of optical activity as a function of temperature. The transition temperature decreases with increasing side-chain modification by periodate oxidation and further borohydride reduction. A series of carboxylic derivatives has been obtained, in addition to the non-ionic ones, by chlorite oxidation after the periodate splitting. Polyelectrolytic (*i.e.*, apparent pK) and optical dissymmetric properties (*i.e.* circular dichroism, c.d.) have been studied on the ionic derivatives as a function of pH. The change of c.d. bands as a function of the degree of oxidation is related to the fractions of the oxidized sites by assuming that periodate reaction occurs according to the magnitude of the rate constants found for simple sugars.

INTRODUCTION

Capsular bacterial polysaccharides have been identified for a long time, but only a few of them have been studied from the physicochemical point of view. One of these biopolymers, extracted from *Rhizobium trifolii*, strain TA-1 (TA-1-CPS), has received considerable attention, as it has recently been extracted and its structure described for the first time by Zevenhuizen and van Neerven¹. In fact, this polysaccharide has been studied by different research groups who have elucidated a number of its structural features and solution properties^{2–5}.

The chemical structure of TA-1-CPS, disclosed by a combination of chemical and spectroscopic methods, exhibits a quite unusual double-branched repeating-unit (see Fig. 1). Such a feature is shared by some capsular polysaccharides from *Klebsiella* (*i.e.*, strains 27, 30, 33, 38, and 64), although all of these contain uronic acids in the repeating unit⁶.

Gidley *et al.*³. In particular, both the hysteresis and the temperature dependence of the rigidity (storage) modulus in water and in aqueous urea solution support the presence of an intermediate step for the formation of the aggregate structure³.

The presence of side chains in TA-1-CPS has stimulated investigations aimed at an understanding of their role in gel formation. The presence of side chains on a polysaccharidic backbone is normally considered to be a perturbing factor with respect to gelation. Examples can be taken from the literature, and include the welan and rhamnan family, as well as the curdlan-schizophyllan case^{8,9}. The case of TA-1-CPS points to just the opposite: in fact, either cleavage or modification of the side chains has been reported to destroy the ability of this polysaccharide to form a gel^{1,3,4}. In one case, cleavage of both side-arms of TA-1-CPS (see Fig. 1) has been obtained by Smith degradation of the polymer, previously fully oxidized with periodate. As immediately deduced from the chemical structure (see Fig. 1), such a reaction produces a neutral, linear polymer with a trisaccharidic backbone repeating-unit¹ without any gelling property³. In the second case, partial oxidation with periodate and further chlorite oxidation has been used to prepare an ionic carboxylate derivative which, once again, does not form a gel either on addition of calcium ions or by lowering the pH⁴.

These observations have stimulated a more-systematic study on a series of ionic and nonionic derivatives of TA-1-CPS, the results of which are now reported. Chemical modification of side chains has been carried out to various extents by periodate oxidation followed either by chlorite oxidation, to produce ionic derivatives, or by borohydride reduction to produce nonionic derivatives. The aim of the work has been, besides obtaining new functionalized polysaccharides, a better understanding of the correlation between the structure and the peculiar gel-forming propensity of TA-1-CPS.

EXPERIMENTAL

Sample of TA-1-CPS. — Samples of the capsular polysaccharide TA-1-CPS used in this study were kindly provided by Dr. L. P. T. M. Zevenhuizen, Department of Microbiology, University of Wageningen, The Netherlands. TA-1-CPS extracted following the procedure previously reported¹, was received as a freeze-dried solid. An average content of 13.5% by weight of water has been determined by the Karl Fischer method, using a Mettler DL 18 automatic titrator. Samples of TA-1-CPS contained traces of non-saccharidic impurities.

Aqueous solutions of TA-1-CPS were prepared by heating the polymer suspended in water in a microwave oven for a few minutes, until complete dissolution occurred.

Determination of contaminants. — Aqueous solutions of native TA-1-CPS may show substantial u.v. absorption at 260 nm. After several dialyses against water, both TA-1-CPS and the external dialysis solutions showed an absorption at the same wavelength. No appreciable variations in the gelling behavior of TA-1-

CPS were noted after the removal of these impurities by dialysis. Some of the contaminants were reactive to periodate, consuming part of the oxidizing reagent. The periodate consumption has been ascribed to contaminant D-mannitol or ethylene glycol, used in large quantities during the microbial culture. Chemical analysis of TA-1-CPS was carried out, to determine the presence of non-saccharidic impurities, and it revealed a contamination of $\sim 2\%$ of proteins and $\sim 0.5\%$ of D-mannitol in all of the samples considered.

Some samples of TA-1-CPS were not purified by dialysis before the periodate reaction. No changes in the properties of the derivatives between the purified and the unpurified samples were observed. Most of the contaminants, in fact, were rapidly destroyed by periodate, and then eliminated during the procedures of purification of the derivatives.

Preparation of modified TA-1-CPS. — Samples of modified TA-1-CPS were prepared by using the following pathway. Various amounts of periodate were added to separate aqueous solutions of native TA-1-CPS ($C_p = 1$ g/L) to obtain different molar ratios of periodate to polysaccharide repeating-units and, therefore, different final degrees of oxidation. The reactions were allowed to proceed for 20 h at 25° . Thereafter, the pH of the reaction mixtures was measured, in order to check for the possible formation of formic acid, and the amount of formic acid was determined by potentiometric titration with 0.1M NaOH.

Each reacted sample was split into two parts. One part was further oxidized with sodium chlorite, in order to convert the aldehyde groups into carboxylate groups, giving, as a final product, a polyelectrolyte¹⁰. The reaction mixture was first acidified with 0.5M acetic acid, and then the sodium chlorite was added to the mixture in a molar ratio of chlorite to aldehyde which was always 4:1. After reaction for 12 h, the mixture was dialyzed against water many times, and then freeze-dried. The other part of the periodate-oxidated sample was reduced with an excess of sodium borohydride¹. The reaction was allowed to proceed for 12 h, and then the sample was dialyzed and freeze-dried.

Clear aqueous solutions of the nonionic modified TA-1-CPS samples were prepared as for the native polymer. The free acid form of the polyelectrolytic TA-1-CPS derivatives was obtained by repeated dialysis against large volumes of mM perchloric acid, and then against H_2O . All samples were stored in the freeze-dried state.

Methods. — U.v. absorption experiments were performed by using a Varian Cary 219 spectrophotometer. Periodate consumption was monitored by measuring the decrease in absorption of the periodate solution at 255 nm.

Polarimetric experiments were performed at different wavelengths as defined in previous work^{4,7} with a Model 141M Perkin–Elmer spectropolarimeter.

A Jasco J500-A dichrograph equipped with a DP 500 data processor was employed for circular dichroism measurements. Smoothing of the spectra was obtained by accumulating at least four runs for each experiment.

Potentiometric titrations were carried out by using a Radiometer PHM 52

pH-meter. The degree of ionization, α , the pK, and the equivalent weight were calculated from plots of the potentiometric pH vs. the degree of neutralization, $\bar{\alpha}$, with standard procedures already used in previous work^{11,12}.

RESULTS

Modification reactions

1. Oxidation by periodate. — The particular (1→3) linkage structure of the glucomannogalactosidic backbone^{1,3} makes the oxidation by periodate possible only on the galactosidic side-chains of TA-1-CPS, leaving the principal chain unaltered. Thus, appearance of new properties, or modification of the existing ones, can be ascribed exclusively to the progressive perturbation of the lateral galactopyranosides.

The behavior of the polysaccharidic molecules under periodate oxidation conditions has previously been described^{13–16}.

The oxidation of the vicinal diol of pyranosidic molecule has been studied in detail by Aalmo and Painter¹⁷, and recently also investigated in our laboratory¹⁸. Periodate oxidation of a triol group produces formic acid in amount proportional to complete oxidation of the site, with a total consumption of two mol of periodate per mol of triol site. Therefore, determination of formic acid in the reaction solutions provides the extent of the oxidation of galactosidic site on TA-1-CPS.

2. Ionic derivatives obtained by oxidation of aldehyde groups with sodium chlorite. — Further oxidation of the polysaccharidic aldehyde sites to carboxyl groups, using sodium chlorite, has been carried out in order to (i) determine the “reaction degree” and (ii) obtain polyelectrolytes with an increasing charge density as a function of the reaction degree.

The degree of modification of the polymeric chain has been determined by potentiometric titration of the carboxyl groups, which provides the equivalent weight (e.w.) inversely proportional to the number of carboxyl groups per repeating unit.

The actual degree of reaction, $N(\text{ox})$, has been evaluated from the experimentally determined equivalent weight by the equation:

$$\text{e.w.} = \frac{\text{m.w.} + 15 \times N(\text{ox}) - 30 \times N(\text{HCO}_2\text{H})}{N(\text{ox})}, \quad (1)$$

where m.w. is the molecular weight of the unaltered repeating unit (972.9), $N(\text{ox})$ is the oxidation degree, $N(\text{HCO}_2\text{H})$ is the number of equivalents of formic acid released per g of polymer, 15 is the molecular weight increase per mole for every carboxyl group introduced along the chain, and 30 is the molecular weight decrease per mole for every formic acid unit released.

The calculated e.w. and $N(\text{ox})$ parameters of native TA-1-CPS oxidized with different molar ratios of periodate to repeating unit are collected in Table I. From

TABLE I

CALCULATED EQUIVALENT WEIGHT AND N(OX) PARAMETERS OF SAMPLES OF OXIDIZED NATIVE TA-1-CPS

Sample	E.w. ^a	$N(\text{HCO}_2\text{H}) \times 10^{-4}$ (eq/g) ^b	N(ox) ^c
A	1167		0.84
B	1149		0.86
C	818		1.21
D	660	1.06	1.50
E	443	1.39	2.28
F	442		2.28
G	342	2.19	2.98
H	329	3.34	3.10
I'	276	2.20	3.73
I	252	5.10	4.10
L	206	7.89	5.09
M	205	8.47	5.12
N	166	9.39	6.26

^aEquivalent weight, e.w. experimentally determined from potentiometric titrations of the poly acids.^bEquivalents of formic acid experimentally determined in the periodate reaction mixtures. ^cDegree of oxidation calculated through Eq. 1.

these results, it is clear that the following may be concluded. (a) E.w. values at the low oxidation ratio, $N(\text{ox}) < 2$, are quite scattered with respect to the stoichiometric amount of periodate used. This could be explained, in some cases, with the ascertained presence of impurities. (b) Increasing amounts of formic acid are generated at higher oxidation ratios (*i.e.*, lower e.w.); further oxidation of an already modified site is, in fact, kinetically less favored than the first attack on an unaltered diol¹⁷. (c) The calculated $N(\text{ox})$ is subject to the uncertainty of the experimentally determined values of both e.w. and $N(\text{HCO}_2\text{H})$.

3. *Nonionic derivatives obtained by reduction of polyaldehyde by sodium borohydride.* — Reduction of the aldehyde groups on the polysaccharide provided a series of nonionic polymers having different degrees of modification corresponding to those of the carboxylate derivatives.

The nonionic derivatives were prepared in order to investigate whether increasing perturbation of the galactosidic arms could, *per se*, modify the solution properties and the gelling behavior of TA-1-CPS, even in the absence of charged groups. In fact, the alcoholic groups, restored by borohydride reduction, do not present chemical heterogeneity with respect to the unmodified TA-1-CPS, although they have a greater conformational freedom which may affect the strength of topological constraints (*e.g.*, hydrogen bonds).

Solution properties

1. *Optical activity.* — Optical activity measurements were carried out as a function of temperature on aqueous solutions of native samples of TA-1-CPS and

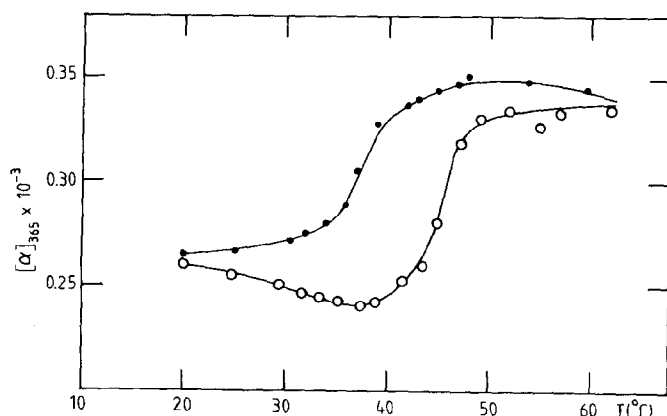


Fig. 2. Temperature-dependence of specific optical rotation for unmodified TA-1-CPS polysaccharide in aqueous solution ($C_p = 1$ g/L; $\lambda = 365$ nm; 10 cm pathlength). [Empty symbols: heating; full symbols: cooling.]

of the modified ones (see Figs. 2-5). The optical rotation of native TA-1-CPS shows a sigmoidal change with temperature, with the characteristic hysteresis between the heating and the cooling curves. Optical rotation was measured at 365 and 313 nm at different concentrations of polysaccharide, but no appreciable dependence of $[\alpha]$ on the concentration was observed. In addition to the disappearance of the dip (which is characteristic of the native sample, as may be seen in Fig. 2 and in the data of refs. 3 and 4), modification of the polysaccharide highly affects melting temperature, hysteresis, and the absolute value of $[\alpha]$ (see Figs. 3 and 4). It has been assumed that sigmoidal change of optical rotation due to some conformational transition is associated with the gel formation. A regular decrease of the transition temperatures (T_m) occurs with an increase of the degree of chemical modification of the side chains, so that the nonionic derivatives having the highest values of

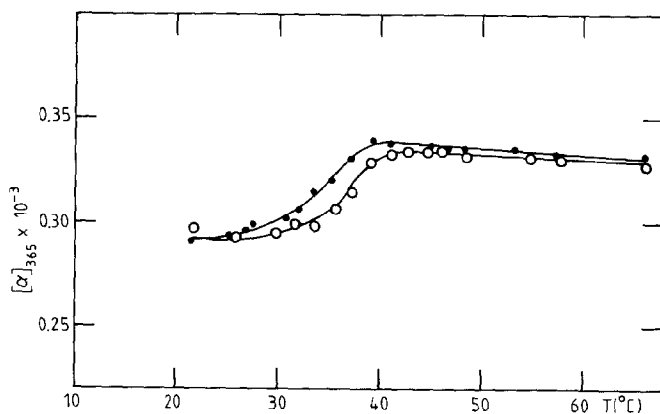


Fig. 3. Temperature-dependence of specific optical rotation for the modified-reduced polysaccharide sample A red ($C_p = 1.25$ g/L; for other conditions, see Fig. 2).

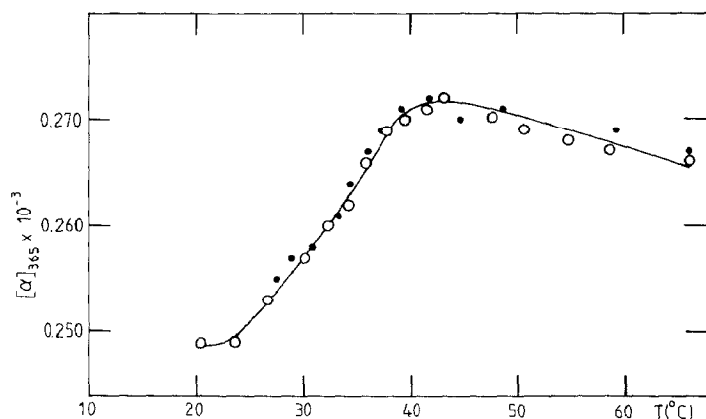


Fig. 4. Temperature-dependence of specific optical rotation for the modified-reduced polysaccharide sample *B red* ($C_p = 1.5$ g/L; for other conditions, see Fig. 2).

$N(ox)$ do not form gel at all, and $[\alpha]$ remains constant in the whole range of temperature explored. Hysteresis, which is evident in native samples, is no longer recognizable for samples having $N(ox)$ higher than 1.2, which yield, during the heating-cooling cycle, the same curve profile (see Fig. 4). Absolute values of $[\alpha]$ (max and min) decrease when the polymer modification increases.

It is also noteworthy that there is a dependence of optical rotation on temperature for the carboxylate sample having the lowest oxidation value (0.84) at two different pH values (1 and 4.75; see Fig. 5). Such ionic samples may be able to give rise to some conformational change at low temperature and degree of ionization $\alpha = 0$. When dissociation of carboxylic groups occurs, the charge density of the chain increases, and this capability is lost.

Melting temperatures as a function of $N(ox)$ are reported in Fig. 6 for the

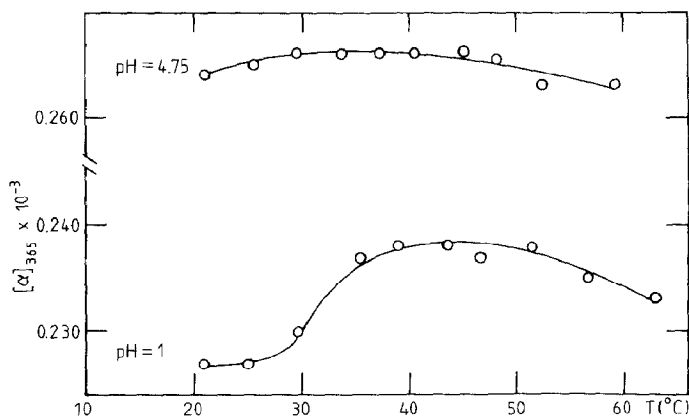


Fig. 5. Temperature-dependence of specific optical rotation for the modified-oxidized polysaccharide sample *A ox*, at two different pH values ($C_p = 1$ g/L; for other conditions, see Fig. 2).

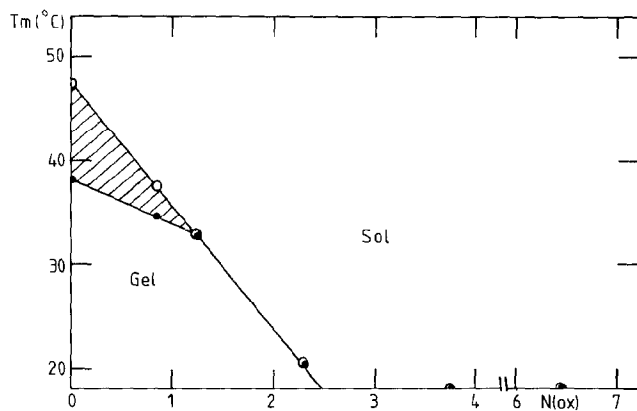


Fig. 6. "Phase diagram" of TA-1-CPS nonionic derivatives: the temperature of transition is plotted vs. the oxidation degree, $N(\text{ox})$. [Empty symbols: heating; full symbols: cooling. The shaded area refers to hysteresis phenomena.]

reduced forms of modified TA-1-CPS. This phase diagram is referred to as the sol-gel state diagram of the modified samples, and it identifies the $N(\text{ox})$ limit for gel formation.

2. *Potentiometric titration.* — Potentiometric titration curves for different modified TA-1-CPS samples were prepared, in order to calculate the dependence of pK_a on the degree of ionization, α .

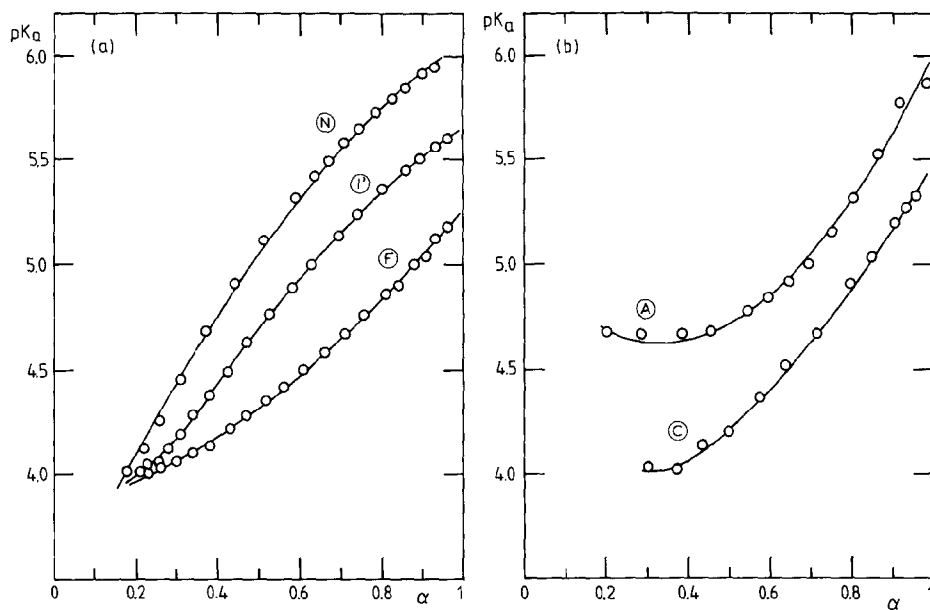


Fig. 7. Dependence of the pK_a on α (the degree of ionization) for different samples of oxidized TA-1-CPS, of higher modification degree (samples $F \text{ ox}$, $I' \text{ ox}$, $N \text{ ox}$, Fig. 7a) and of lower modification degree (samples $A \text{ ox}$, $C \text{ ox}$, Fig. 7b).

Curves show an increase of pK_a with α , in accordance with the polyelectrolytic nature of weak poly acids, at least for samples having a high $N(ox)$ value (see Fig. 7a). The dependence on $N(ox)$ of such curves is in analogy with the trend theoretically predicted for the dissociation of weak poly acids. Indeed, the slope increases, as expected, with the number of carboxyl groups per repeating unit in the polymeric chain. On the other hand, the dependence of pK_a for TA-1-CPS samples with a low $N(ox)$ value shows a plateau at $\alpha < 0.45$ and an increase of pK_a at higher values of α (see Fig. 7b). This behavior at low degree of ionization is related to the formation of aggregates, which are disrupted only above critical values of charge density. Similar phenomena had already been described for other polysaccharides¹².

Comparison of different samples having increasing $N(ox)$ shows that two cumulative effects (increasing charge density and increasing solubility, *i.e.*, disaggregation) result in the drop of pK_a ($\alpha = 0.5$) to a minimum value for sample C, and in the further increase of pK_a ($\alpha = 0.5$) due to polyelectrolytic effects.

3. *Circular dichroism.* — C.d. spectra of the protonated carboxyl groups of modified TA-1-CPS (pH 2, $\alpha = 0$) are collected in Fig. 8. At low $N(ox)$, the principal absorption band has a maximum at λ 205 nm; upon increasing the oxidation degree, this absorption intensity decreases, while a new negative band appears at 225 nm. At the highest values of $N(ox)$, this negative absorption band becomes predominant, and its intensity further increases as a function of the oxidation degree, with a shift toward lower wavelengths.

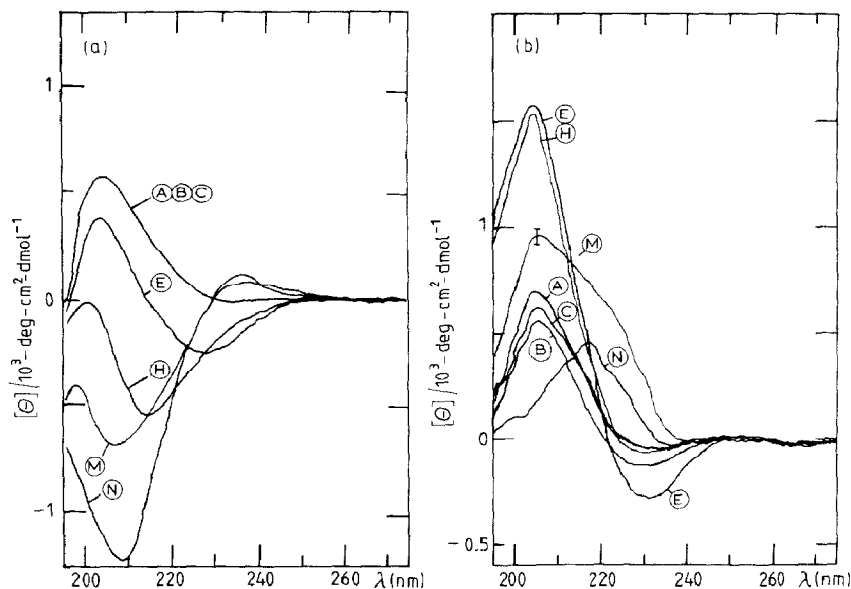


Fig. 8. Circular dichroic spectra of oxidized TA-1-CPS samples, at different pH conditions (Fig. 8a: pH 2; Fig. 8b: pH 8).

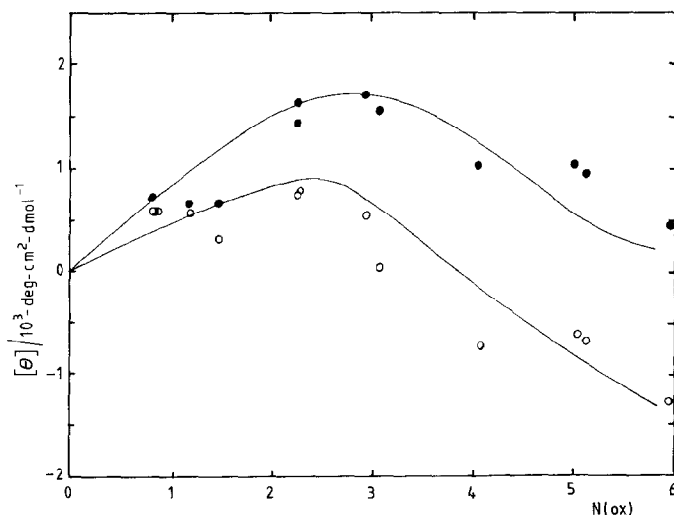


Fig. 9. Variation of the dichroic absorption (λ 205 nm) for the oxidized samples of TA-1-CPS as a function of the oxidation degree, at two different pH values. [Empty symbols: pH 2; full symbols: pH 8. The full lines are calculated according to the model described in the text.]

Polycarboxylates in the sodium salt form (pH 8) show a different behavior (see Fig. 8b). The principal band (λ 205 nm) is always positive, initially, and increases in intensity with the oxidation degree; subsequently, the absorption band shifts to higher wavelength values (up to ~ 220 nm) and the intensity decreases.

The results of c.d. measurements are cumulatively shown in Fig. 9, which reports the molar ellipticity $[\theta]$ at λ 205 nm as a function of $N(\text{ox})$ or e.w. The reported values are normalized for nanomoles of polymer, calculated on the basis of the average molecular weight of the modified repeating-unit.

An interpretation of solution properties of TA-1-CPS derivatives

Inspection of the chemical structure of TA-1-CPS and scrutiny of the results presented herein allow some conclusions on the role of the side arms in gel formation. One is obviously that side-chain integrity is a necessary condition for gel formation. A detailed, quantitative description of the properties of the derivatives can be drawn only from knowledge of the sites of oxidation. On the basis of kinetic arguments, an attempt may be made to show that *any* chemical modification of the chains seriously perturbs the gel stability of TA-1-CPS.

Therefore, for the periodate oxidation, a second-order kinetic equation dependent on the particular site i ($i = 1, \dots, 5$) on the concentration of each galactose unit $[G(i)]$, and on periodate concentration $[P]$ can be written.

$$d[\text{ox}(i)]/dt = k(i) \cdot [G(i)] \cdot [P] \quad (2)$$

Let us recall the stereochemical difference of the five oxidizable sites,

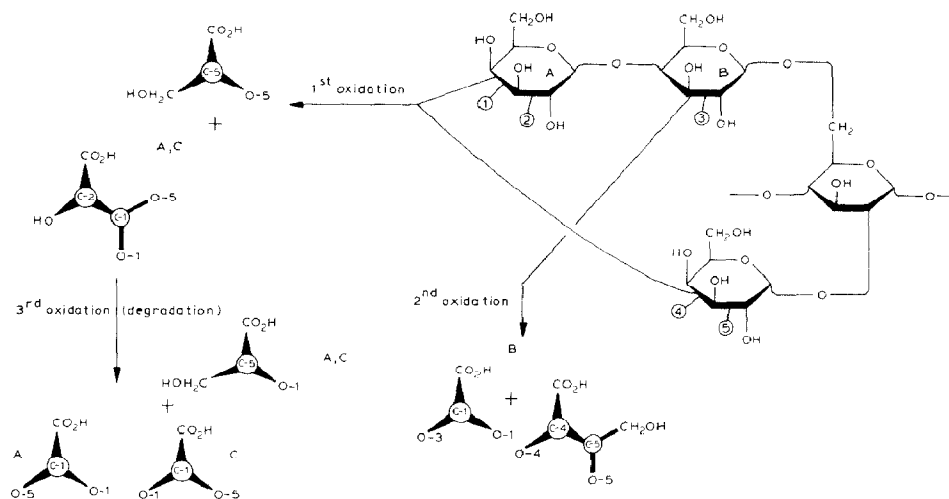


Fig. 10. Periodate-oxidizable sites on the galactosidic residues of TA-1-CPS, and configuration of the carbon atoms neighboring carboxyl groups.

identified with numbers 1 to 5 in Fig. 10. All diols C-2-C-3, *i.e.* sites 2, 3, and 5, are indeed characterized by a *trans* orientation of the hydroxyl groups. Only diols C-3-C-4 of the terminal galactosidic residues (sites 1 and 4) contain the hydroxyl groups in *cis* orientation. It is now well known that periodate oxidation of a *cis* diol is kinetically more favored than that of a *trans* diol^{17,19}.

From kinetic data from our laboratory¹⁸, which agree with other literature data^{17,19}, we have taken the following values for the second-order rate constants.

$$k(1) = k(4) = 0.2 \quad \text{L.mol}^{-1}.\text{s}^{-1} \quad (3a)$$

$$k(3) = 0.02 \quad \text{L.mol}^{-1}.\text{s}^{-1} \quad (3b)$$

$$k(2) = k(5) = 0.02/w \quad \text{L.mol}^{-1}.\text{s}^{-1} \quad (3c)$$

where the factor w has been introduced to take into account the possible formation of internal hemiacetals as a consequence of the first oxidation step¹⁷. Admittedly, this is an oversimplification, because the rate constants refer to simple isolated sugar units and do not take into account the mutual steric interactions occurring in the chain.

A simple algorithm can then be elaborated to calculate the fraction of each site oxidized as a function of the overall degree of oxidation²⁰ $N(\text{ox})$. The five exponential equations (obtained by integration of Eq. 2) were simultaneously solved by assuming the concentration of periodate to be constant during each infinitesimal interval of time and allowing the five sites to react independently with the periodate. Once these fractions are known, any experimental property $\langle Y \rangle$ may be described, in first approximation, as a sum of the contributions due to each oxidized state, present in fraction $f(i)$.

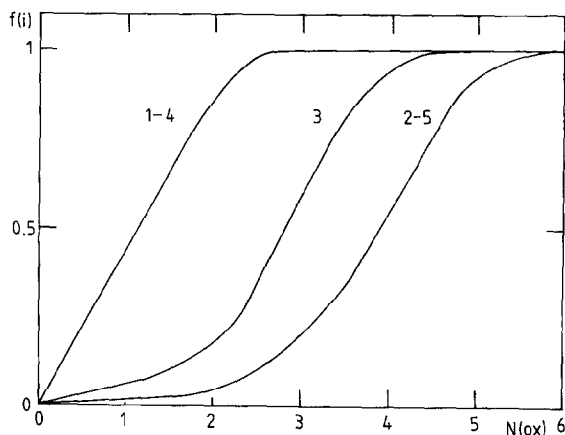


Fig. 11. Increase in the fractions of oxidized sites; $f(i)$, as a function of the oxidation degree $N(\text{ox})$, according to the model described in the text (see also Fig. 10).

$$\langle Y \rangle = f(1)Y(1) + f(2)Y(2) + f(3)Y(3) + f(4)Y(4) + f(5)Y(5) \quad (4)$$

The simplest case is the calculation of the equivalent weight, which obviously takes into account the formic acid release as a consequence of the oxidation of sites 2 and 5. The distribution function of oxidized sites shows that, up to $N(\text{ox}) < 2$, almost exclusively either one or the other of the *cis* diols of the two terminal galactose units is oxidized (see Fig. 11). Therefore, polyelectrolytic and dichroic properties of samples having $N(\text{ox}) < 2$ must be associated with the distribution and the stereochemistry of the carboxyl groups originated by C-3 and C-4 of residue A or C (see Fig. 10). The first neighboring asymmetric carbon atoms of these carboxyl groups are C-5 and C-2, which do not have the same configuration (see Fig. 10). It is reasonable to assume that they may contribute with opposite sign to the circular dichroic values (but their contributions cannot be resolved, since they must occur simultaneously). When site 3 is oxidized, it produces one carboxyl group linked on C-4 and one carboxyl group linked on the β -anomeric (C-1) atom.

The last oxidative process, which occurs with elimination of two molecules of formic acid, destroys the dissymmetry on C-2 of the terminal units, and creates two carboxyl groups attached to C-1 atoms originally belonging to a β -galactose (B) and to an α -galactose (A) residue, respectively (see Fig. 10). Although complicated by the presence of too many (seven) stereochemically different chromophores, it must be considered that these are simultaneously created in three steps only. An exemplification of the arguments is provided by the plot in Fig. 9 of the molar ellipticity $[\theta]$ at λ 205 nm as a function of the degree of oxidation, and by the fitting of the data with the assumption (taken from the experimental data) that the overall sign of $[\theta]$ is positive for the first and the second oxidative steps, while it is negative for the last one.

Once the dichroic behavior of the ionic derivatives has been coherently related with the assumed kinetic model, the distribution plot of Fig. 11 can be used to interpret the gel stability of the nonionic derivatives. The straightforward conclusion is that the gel stability decreases linearly with the first oxidative step. Therefore, side-chain modification must destroy the functionality of the arms in the intermolecular cross-linking process. Although no definitive molecular conformation of TA-1-CPS has yet been provided, preliminary information obtained from X-ray diffraction studies of oriented fibers⁵ shows that the polysaccharide could form either 2-fold helices of pitch 1.96 nm or 4-fold, half-staggered, parallel double-helices of pitch 3.92 nm. In both cases, the chain trajectory describes a zigzag staircase with side arms turned at $\sim 90^\circ$ with respect to each other. The authors also suggested⁵ two different orientations of the longer arm, since the (1 \rightarrow 6)-linked galactoside unit can quite easily rotate about the three torsional angles. Confining attention to the rotation about the ω angle, the transition from the *g* + to the *trans* conformation moves the disaccharide side-arm from the fully extended orientation (perpendicular to the main-chain axis) to a folded-down orientation (parallel to the chain axis). If any of the two proposed molecular models occurs in solution, there must be an involvement of the hydroxyl groups of residue A (see Fig. 10) in the conformational stability of the chain. The extended conformation of the side arm seems suitable to intermolecular interaction, while the folded one seems to stabilize better the ordered chain conformation through intramolecular bonding. The results so far obtained suggest that both sources of interaction would be necessary for chain ordering and gel formation, respectively. No details about the intrinsic conformational freedom of the chain backbone are available to support either one of the two proposals.

CONCLUSIONS

The main conclusion of this work is that gel stability of nonionic derivatives of TA-1-CPS decreases linearly with the degree of modification of the two (kinetically unresolvable) sites containing *cis*-diols in the side chains. Further modification alters other properties of a polymer which does not retain molecular order in solution.

In addition to the role of the side chains, interpreted by means of the chemical modification introduced by oxidation with periodate, the derivatives prepared herein present a number of solution properties which are summarized next.

The solution properties of the polyelectrolytes obtained are typical of carboxylated polysaccharides, the apparent *pK_a* of the proton dissociation showing a low- α behavior which is ascribable to polymer aggregation (for samples A–C). Increasing structural charge-density on the polymers is reflected by the absence of aggregation in the low α region (for samples D and N) and by the slope of the *pK* vs. α curves. It is of interest to point out that the maximum value of the average

distance between neighboring fixed charges for polymer sample N is 1.63 Å/charge, which is much smaller than that of other known ionic polysaccharides.

Finally, circular dichroic spectra of fully protonated and fully ionized carboxylic derivatives have been interpreted on the basis of the configuration of neighboring carbon atoms. In particular, the spectra of sample F,...N at low pH disclose a second negative band, which superimposes on, and eventually predominates over, the positive band exhibited by samples A, B, and C.

All these new derivatives are potential candidates both for further physicochemical characterization and for possible practical applications.

ACKNOWLEDGMENTS

The authors thank Prof. L. P. T. M. Zevenhuizen for his kind gift of the sample of native CPS-TA-1 polysaccharide. This work was carried out with financial support from the University of Trieste and from Italian CNR, "Progetto Finalizzato Chimica Fine e Secondaria", Rome.

REFERENCES

- 1 L. P. T. M. ZEVENHUIZEN AND A. R. W. VAN NEERVEN, *Carbohydr. Res.*, 124 (1983) 166–171.
- 2 L. P. T. M. ZEVENHUIZEN, *Appl. Microbiol. Biotechnol.*, 20 (1984) 393–399.
- 3 M. J. GIDLEY, I. C. M. DEA, G. EGGLESTON, AND E. R. MORRIS, *Carbohydr. Res.*, 160 (1987) 381–396.
- 4 A. CESÀRO, S. PAOLETTI, F. DELBEN, S. CAVALLO, V. CRESCENZI, AND L. P. T. M. ZEVENHUIZEN, in S. S. STIVALA, V. CRESCENZI, AND I. C. M. DEA (Eds.), *Industrial Polysaccharides*, Gordon and Breach, New York, 1987, pp. 99–109.
- 5 R. CHANDRASEKARAN, R. P. MILLANE, J. K. WALKER, S. ARNOTT, AND I. C. M. DEA, in S. S. STIVALA, V. CRESCENZI, AND I. C. M. DEA (Eds.), *Industrial Polysaccharides*, Gordon and Breach, New York, 1987, pp. 111–118.
- 6 G. G. S. DUTTON, personal communication.
- 7 S. CAVALLO, Tesi di Laurea, University of Trieste, Italy, 1985.
- 8 V. CRESCENZI, M. DENTINI, AND I. C. M. DEA, *Carbohydr. Res.*, 160 (1987) 283–302.
- 9 V. CRESCENZI, M. DENTINI, T. COVIELLO, S. PAOLETTI, A. CESÀRO, AND F. DELBEN, *Gazz. Chim. Ital.*, 117 (1987) 611–616.
- 10 B. T. HOFREITER, I. A. WOLFF, AND C. L. MEHLTRETTER, *J. Am. Chem. Soc.*, 79 (1957) 6457–6460.
- 11 A. CESÀRO, A. CIANA, F. DELBEN, G. MANZINI, AND S. PAOLETTI, *Biopolymers*, 21 (1982) 431–449.
- 12 A. CESÀRO, F. DELBEN, A. FLAIBANI, AND S. PAOLETTI, *Carbohydr. Res.*, 160 (1987) 355–368.
- 13 T. J. PAINTER AND B. LARSEN, *Acta Chem. Scand.*, 24 (1970) 813–833.
- 14 T. J. PAINTER AND B. LARSEN, *Acta Chem. Scand.*, 24 (1970) 2366–2378.
- 15 T. J. PAINTER AND B. LARSEN, *Acta Chem. Scand.*, 24 (1970) 2724–2736.
- 16 M. ISHAK AND T. J. PAINTER, *Acta Chem. Scand.*, 25 (1971) 3875–3877.
- 17 K. M. AALMO AND T. J. PAINTER, *Carbohydr. Res.*, 89 (1981) 73–82.
- 18 M. T. FERUGLIO, Tesi di Laurea, University of Trieste, Italy, 1984.
- 19 K. M. AALMO, M. F. ISHAK, AND T. J. PAINTER, *Carbohydr. Res.*, 63 (1978) c3–c7.
- 20 P. ESPOSITO, Tesi di Laurea, University of Trieste, Italy, 1987.