Thermochimica Acta, 199 (1992) 1–15
Elsevier Science Publishers B.V., Amsterdam

Specific and non-specific ion–polysaccharide interactions [1]

Attilio Cesàro [a,*], Vittorio Crescenzi [b], Franco Delben [a], Amelia Gamini [a], Gianfranco Liut [a], Sergio Paoletti [a] and Roberto Rizzo [a]

[a] *Dipartimento di Biochimica, Biofisica e Chimica delle Macromolecole, Università di Trieste, 34127 Trieste (Italy)*

[b] *Dipartimento di Chimica, Università "La Sapienza" di Roma, 00185 Rome (Italy)*

(Received in final form 12 August 1991)

Abstract

The interaction of ions with certain carbohydrate ligands in aqueous solution is examined. Simple systems are analysed to understand the role of conformational transitions and the possible specificity often advanced as an explanation for the complexity of the binding. Evidence of an extrinsic chirality of bound ionic chromophores is presented for nearly enantiomeric complexes of alginate and pectins with Pb^{2+} ions. Data on the thermodynamics of interaction between divalent cations and typical ionic polysaccharides are presented with a view to offering an interpretation based upon current theoretical models of polyelectrolyte solutions. In particular, reference is made to the linear correlation between thermodynamic state functions.

INTRODUCTION

Elucidation of the specific site binding of ions within well-defined structural pockets of enzymes and other biopolymeric chelating systems has been brought to a high degree of resolution, mainly by diffractometric investigations of solid crystalline forms. Interpretation of the thermodynamic data on the solution binding behaviour has often been derived from and complemented by other methods. The peculiarity of electrostatic interactions in biological systems has been interestingly and thoroughly summarized in the review by Tam and Williams [1], who have analysed the main concepts of ion-pair formation from small molecule/small molecule binding to three-dimensional charged surfaces. They have underlined the lack of quantitative knowledge and the increasingly empirical description of larger systems. This seems also true for cations such as Ca^{2+} that have a large radius and accept almost any irregular pattern of coordination.

Correspondence to: A. Cesàro, Dipartimento di Biochimica, Biofisica e Chimica delle Macromolecole, Università di Trieste, 34127 Trieste, Italy.

[1] Presented at the 12th National Conference on Calorimetry and Thermal Analysis, Bari, Italy, 11–13 December 1990.

The understanding of (electrostatic) interactions in solution has only advanced for schematically modelled systems, in which the chemical nature of the interacting molecules is ignored. In interactions between linear, charged polymeric species and ions in solution, the effect of changing ionic strength on the polyelectrolytic behaviour of the macromolecular component is most striking and universally accepted. The understanding of the electrostatic interactions in polymorphic macromolecular chains is not yet complete, except in some simplified circumstances. However, their range of validity has restricted application of the theoretical results to cases in which the rigidity of the chain and the absence of variations due to "specific" binding are regarded as essential.

A few exceptions in the field of polysaccharides deserve our attention. These, mostly linear polyelectrolytes present examples falling between the two extreme cases of a single-site anion/cation interaction and a complex, multi-site interaction between an ion and a conformationally defined arrangement of ligands belonging to different chains. The hydrophilicity of carbohydrate chains and the regular distribution of their charged groups make the study of ion–polysaccharide interaction particularly interesting and suitable for theoretical generalization. We propose a self-consistent framework of interpretation of the binding process based on experimental solution thermodynamics [2], extensive use of molecular mechanics for the conformational analysis of carbohydrate moieties [3], and application of polyelectrolyte theories to treat the long-range electrostatic interactions [4].

DEFINITION OF THE BINDING VARIABLES

The number of variables governing the binding of small molecules (or ions) to a polymer (or polyelectrolyte) is immense; one can only try to factorize the partition function into approximate partial forms and then describe the whole system by simple multiplication. The main problems concern the knowledge (and sometimes even the definition) of (1) the total number of sites, i.e. the stoichiometry; (2) the extent of binding, usually defined as the degree of complexation; (3) the intensity of binding, which is measured quantitatively by the binding constant (or the free energy of binding) and is not a trivial function of the degree of complexation; (4) the cooperativity, usually associated with the presence of interacting binding sites; (5) the induction of a conformational change elicited to optimize the binding; (6) the stereochemistry of the binding sites, which may induce chirality in the otherwise symmetric chromophores of the bound species.

To clarify these points with regard to the solution thermodynamics of interacting species, we will consider two cases: in one, the focus is on a small ion; in the other, it is on a polymeric chain in the random conformation.

Furthermore, in the case of ionic polymers, it must not be forgotten that the binding process may alter the topology of distribution of the fixed charges on the polyelectrolyte. This in turn changes the overall electrostatic free energy, especially in connection with possible conformational transitions and/or chain aggregations.

Emphasis must, above all, be placed on the important driving force arising from the polyelectrolytic nature of the ionic polysaccharide chain. This fundamental physical aspect is very often overlooked: as recently as 1989, enhancement of the strength of the calcium complex formation, affected by the length of the polyuronate chain, was ascribed to steric reasons [5], without any mention of the effect of the electrostatic field on the polyelectrolyte.

CONFORMATIONAL TRANSITION INDUCED UPON BINDING

Calcium binding to sugars in solution

Our first example is calcium binding to a non-ionic monosaccharide [5]. The choice is pertinent, not only because the uncharged monomer avoids all complications due to electrostatic and long-range conformational interactions, but also, in particular, because it reveals the complexity of any multifunctional sugar ligand for which a number of conformational forms coexist in solution. It has been stated repeatedly that it is hard to establish an a priori dependence of the physico-chemical properties of a carbohydrate molecule in solution on its constitution, at least in the absence of a detailed knowledge of its conformational status. For an α-pyranoid ring, in fact, the rotation about carbon–carbon bonds gives rise to a number of conformers that can be identified by their position on the "conformational sphere" (Fig. 1). There, for instance, the angle θ is the Cremer–Pople puckering parameter [6], which specifies the conformational form (chair, twist or half-chair). However, not all the conformers are significant, so that one ends up with the approximation of selecting the most probable rotational isomeric states.

For example, the D-ribose molecule undergoes an equilibrium giving rise to the mixture composition given in Fig. 2. The percentage of each form is taken from Angyal's data [7], with integration of the sub-splitting between the $^4C_1$ and the $^1C_4$ forms [8]. The prime purpose of this analysis is to make it clear that the stability of each conformer is not determined solely by the intrinsic internal energy, which can be evaluated by means of e.g. ab-initio quantum calculations, but is strongly governed by all the solvation contributions as well as by solute–solute interactions. Therefore, a shift is induced in the conformer population [8–10] by changing temperature, solvent composition or any other external variable, as well as by adding divalent cations (see Table 1). It may be less evident that the measured

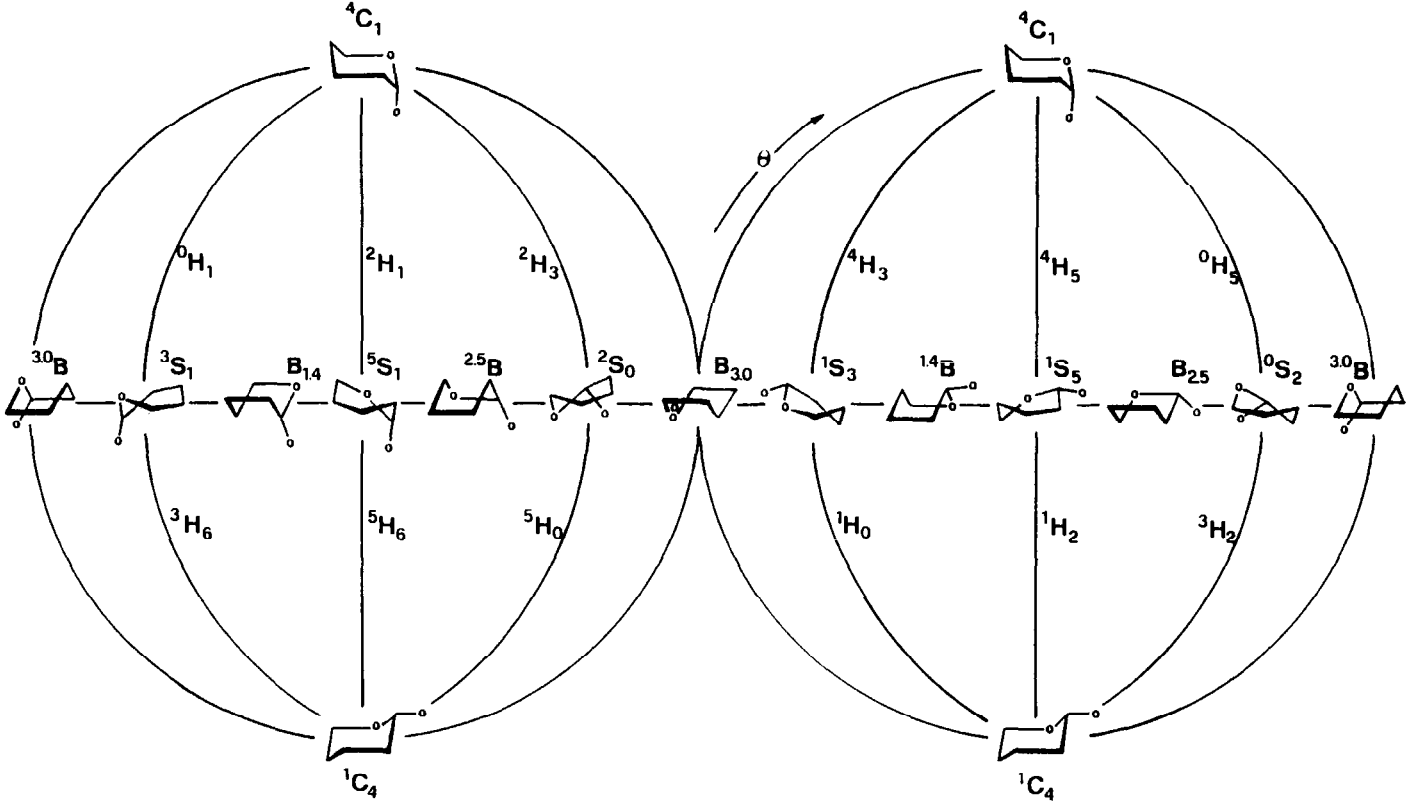

Fig. 1. Map of the α-pyranoid ring interconversion and the position of the conformers (chairs, C, boats, B, skews, S, and half-chairs, H) on the conformational sphere defined by Cremer and Pople [6].

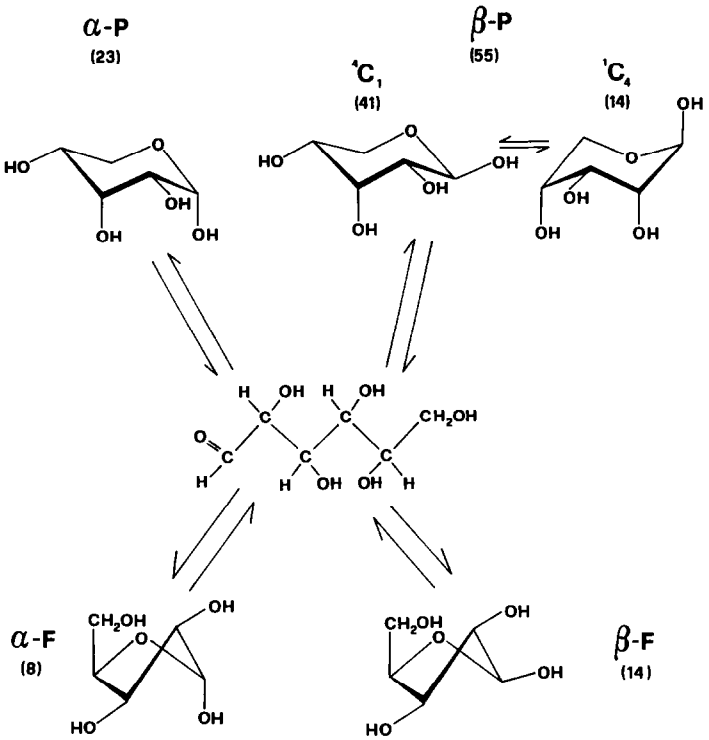

Fig. 2. Selected isomers of D-ribose (pyranose, P, and furanose, F) and percentage composition (in brackets) of the equilibrium mixture in water (the conformations drawn do not necessarily correspond to a minimum of conformational energy).

TABLE 1

Percentage composition of furanose and pyranose forms of D-ribose and its methyl derivatives in various solvent systems [a]

Solvent	α-F	β-F	α-P	($^4C_1$) β-P	($^1C_4$) β-P
D-Ribose					
H_2O (31°C)	13.5	6.5	21.5	44.0	14.5
D_2O (30°C)	8.0	14.0	23.0	41.0	14.0
DMSO (30°C)	6.0	22.0	16.2	31.0	24.8
Ca^{2+} 1.27 M	13.0	5.0	40.0	14.0	28.0
1-*o*-Methyl-β-D-ribose (P)					
H_2O				57	43
Ca^{2+} 2.08 M				12	88
5-*o*-methyl-D-ribose (F)					
H_2O	30	67			
Ca^{2+} 2.08 M	70	30			

[a] Data obtained from refs. 7 and 8.

thermodynamic properties accompanying a complexation are thus apparent quantities averaged over a population of states which is not constant before and after the reaction. The Gibbs energy terms (or the equilibrium constants) can be easily calculated by taking into account the actual concentration of the conformer involved in the complexation [11]. No such simple procedure is straightforwardly applicable for the enthalpy change, because it is measured in a calorimeter and therefore includes all the energetic contributions due to rearrangements of the species in solution.

Gibbs energy, enthalpy and entropy data for the interaction of the D-ribose (mixture) with various cations (Ca^{2+}, Sr^{2+}, Ba^{2+}, La^{3+}, Gd^{3+}) have been reported by Alvarez et al. [12], with a linear Barclay–Butler correlation of $-T\Delta S$ versus $-\Delta H$, while the ΔG values range from -1.7 to -3.6 kJ mol^{-1} with no relation to the charge or to the size of the cation. The linearity may simply derive from a common mechanism of reorganization of hydration spheres (dehydration) and rearrangement of different (hydrated) forms in the presence of the cations: then the extrapolation to zero enthalpy ($\Delta G = -T\Delta S = -6$ kJ mol^{-1}) signifies the free-energy change associated with one mole of rearranged species in the absence of enthalpy contribution. One may wonder whether this value should be related to the shift of the conformational equilibria of the sugar ring in water.

Iodine–triiodide binding to amylose

The second example is the dark blue complex of amylose with iodine in the presence of iodide, which is the oldest (1815) and best known example of inclusion compound formation. In the dissolved complex, iodine and triiodide are situated within the annular cavity of a more-or-less regular helical amylose chain, as described in earlier works [13]. Because the hydrodynamic volume of the polymer decreases upon complexation with iodine, the helical character of the complex does not imply a rigid, rod-like conformation for the complexed polymer (Fig. 3).

The complex presents strong absorption and circular dichroic bands near 600 nm. These change as a function of many variables, such as the average degree of polymerization of the polymer, the degree of saturation of the complex, and the iodide ion concentration. Changes in the optical properties, e.g. the wavelength of the maximum absorption λ_{max} of the complex, have been ascribed to changes in the chain length of the polyiodine arrays [13].

The composition, i.e. the relative amount of triiodide and iodine, is also a function of these variables. In particular, the ratio $R = I_3^-/(I_2 + I_3^-)$ seems to vary in the range 0.3–0.5 for many experimental conditions [14]. At the macroscopic level, one may find that the complex exists in a variety of different compositions which, however, with the limit of $R = 1$, i.e. I_3^-

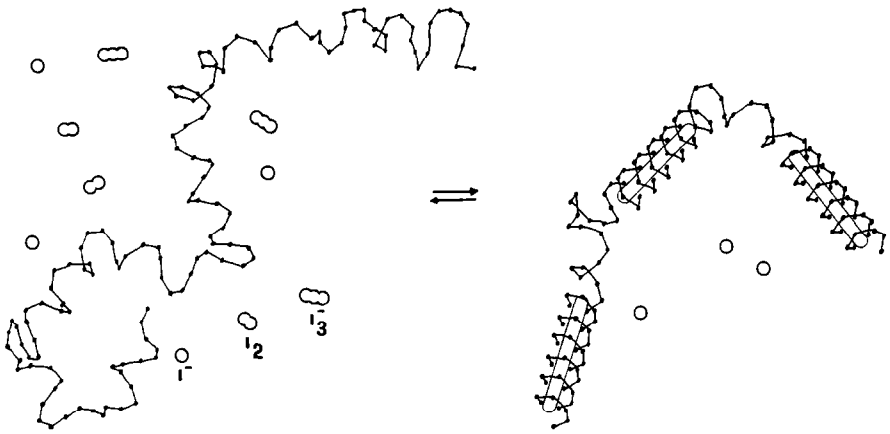

Fig. 3. Conformational change of the amylosic chain upon complexation with iodine–triiodide. The scheme of the reaction also shows the presence of the free species, I^-, I_2 and I_3^-, in addition to the linear polyiodine chains inserted in the helical amylosic cavity, which confers the blue colour to the complex.

alone, do not produce the other known optical properties typical of the blue complex.

Evaluation of the binding energetics has been the subject of many investigations, most of them directed to determination of the fraction of binding as a function of the external variables, including temperature. The equilibrium in solution between iodine and triiodide ions in the presence of iodide ions must be borne in mind. At 293 K and $C_{I^-} = 10^{-3}$ M, this distributes molecular iodine almost equally between iodine and triiodide. Individual binding of any such species onto the polymer will displace the equilibrium between the free species. This bias has been recorded in determination of the enthalpy of binding with amylose, which ranges from -40 to -87 kJ mol^{-1} [14]. Seemingly contradictory results (reviewed in ref. 14) stem mainly from the van't Hoff plot of the apparent equilibrium constant as a function of temperature. For example, Cronan and Schneider [15] calculated two ΔH values by van't Hoff referring the equilibrium constant to the free iodine and the free triiodide species respectively. It is easily shown that the difference (about -17 kJ mol^{-1}) is equal to the enthalpy of triiodide formation and is clearly associated with the way the equilibrium constants were expressed. Calorimetric determination of the formation of I_3^- species in aqueous solution from I_2 and I^-, in fact, gives a value of -19.66 kJ mol^{-1} [14].

Once these discrepancies are reconciled, one finds that the enthalpy of complexation is quite large (-71.6 kJ mol^{-1}). Furthermore, it is still a function of the degree of complexation, as would be expected for a cooperative process [16]. Under those reaction conditions which leave the

spectroscopic properties unchanged, the enthalpy of complexation appears to be constant and close to the interaction energy between iodine atoms (-65.5 kJ mol^{-1}). This supports the interpretation that a strong cooperative interaction between iodine units, stemming from the large polarizability of iodine molecules, is responsible for almost all the energetics of the process, the contribution of the interaction with the polymeric site proper being vanishingly small. The main conclusion is that no specific interaction exists in the blue starch–iodine complex, inasmuch as the properties of the complex reside mainly in the ability of iodine–triiodide species to stack one upon the other. This interpretation is reinforced by the existence of many other substrates that can complex triiodide–iodine, while the only data available for site-binding are those of complexes of I_3^- with cyclodextrins, the six- and the seven-member cycles, with ΔH values of -35.56 and -18.0 kJ mol^{-1}, respectively [17].

This section can be concluded by stressing a common feature of these cases, i.e. the formation of complexes between Ca^{2+} ions and D-ribose, and between iodine–triiodide species and amylose respectively, are not to be considered as specific in the sense that new types of interactions are established between the charged ligand and the carbohydrate moiety. Rather, this interaction can be energetically traced back to already existing dominant contributions, namely the conformational equilibria of D-ribose in the former case, and the I–I stacking energy in the latter case. As to the iodine amylose interaction, a large conformational disorder–order transition of the polysaccharide is promoted by iodine binding. However, by contrast with the Ca^{2+}/D-ribose case, this change of conformation is not the main driving contribution to binding, because the conformational free energy change is at least one order of magnitude less than that associated with the "self-interaction" between the iodine species.

ION BINDING TO ALGINATES AND PECTINS

Polyelectrolytic background for ion binding

Ion–polyelectrolyte binding modes can be operationally defined as (i) non-localized binding of diffusable ions in the polymeric domain; (ii) localized binding of the "outer sphere" complex type, statistically close to the polymeric fixed charges; and (iii) site-binding of ions forming "specific" complex-like structures. While binding of the third type is analogous to that of monomeric species, interaction of the other types depends solely on the polyelectrolytic nature of the substrate. From the thermodynamic point of view, however, this useful modelling definition may cause some confusion because it ignores the fact that all types of interaction must satisfy the requirements given by the charge density of the polymer. It is, in fact, a general result of the polyelectrolyte theories that counterion–polyion inter-

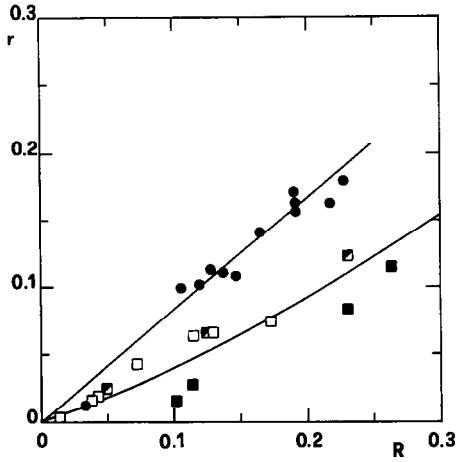

Fig. 4. Binding isotherms of Ca^{2+} to polygalacturonate (●) and alginate (■, ◩, □, polymer concentration = 0.4×10^{-2}, 0.93×10^{-2} and 1.4×10^{-2} equivalent l^{-1}, respectively). r and R denote the bound and the total cation-to-polymer molar concentrations, respectively. Data were obtained through dialysis at 25°C.

action is a direct function of the ionic strength and the "structural" charge density of the polymer. The definition of the "structural" charge density is not without problems and has recently been re-formulated on a statistical basis for semi-flexible polyelectrolytes [18].

Some experimental data on the binding of divalent cations to acidic polysaccharides, namely alginates and pectins, can now be presented and interpreted. These polymers are very well known for their gelling properties under conditions in which ordered chain aggregation (crystallite-like) is favoured by the screening of charged groups.

Their polyelectrolytic character makes it necessary, however, to point out that the so-called "binding" of counterions is a peculiarity stemming from the charge density of the polyelectrolyte, making it difficult to factorize from the raw data the contribution of specific binding (if any). This is shown in Fig. 4, where the results of equilibrium dialysis of calcium ions with polysaccharide can only discriminate the higher affinity of calcium for pectin with respect to that for alginate. It is not uncommon that the Gibbs energy term suffers from this ambiguity, by contrast with other thermodynamic functions such as enthalpy.

Thermodynamics of binding

Leaving aside derivation of the thermodynamic functions associated with the process of "ion binding", let us briefly state that:
— in some modelling of the solution component (in dilute conditions) a polyelectrolyte approach, based on the counterion condensation theory

developed by Manning [4], predicts a positive enthalpy change on mixing the polymer with a simple salt;
— under the same circumstances, a positive volume change and a negative entropy change are also predicted for the merely electrostatic interaction of the point charges with the linear charged polyelectrolyte.

The enthalpy of binding (or complexation) has often been evaluated from the temperature dependence of the binding constant. Direct calorimetric evaluation of the enthalpy of binding has already been given for different cations and polysaccharides. Scrutiny of the dependence of the experimental enthalpy changes as a function of the degree of complexation (or of the ion-to-polymer molar ratio) will readily disclose cooperativity (binding and/or state transition). We have already stressed that the shape of the enthalpy change upon mixing with ions can easily monitor the occurrence of a chain conformational transition [19,20]. The key for understanding this occurrence resides in the possibility of comparison with the quantitative behaviour of the enthalpy change, as predicted for a non-cooperative process.

The theoretical derivation of the "binding" Gibbs energy associated with the mixing of a polyelectrolyte univalent salt with a divalent cation is the subject of another paper [21]. The derivation of the Gibbs energy, enthalpy and volume change on mixing a polyelectrolyte with monovalent ions has already been discussed for other cases, e.g. for carrageenans with Cs^+ in ref. 20.

Here we wish to stress that, given the polyelectrolytic model in the premise, all thermodynamic derivatives are easily calculated. In addition, it can be shown that, although they depend in a rather peculiar way on the concentration of the charged species [19], their ratio is independent of concentration within the limit of infinite dilution (manuscript in preparation): in other words, polyelectrolytes do obey an entropy–enthalpy linear correlation of the Barclay–Butler type previously mentioned. However, these relations are not limited to ΔH and ΔS. For example, the plot of the theoretical (purely electrostatic) values of the volume changes ΔV versus the corresponding enthalpy changes ΔH is described by $\Delta H/\Delta V = 0.795$ kJ ml^{-1}.

Our data of enthalpy change versus volume change on mixing alginate and pectate with different divalent cations are shown in Fig. 5. The structural characteristics of alginate and of pectin (essentially polygalacturonate) have already been reported [22]. The theoretical trend of the purely polyelectrolytic binding is also reported. In the upper part of the figure, the positive enthalpy of mixing underlines a process in which the electrostatic interactions drive the binding process; however, it is also clear that the volume change, as experimentally observed, may be larger than that which would be calculated on the basis of theory alone. It simply suffices to recall that no provision in the theory is made for the volume change upon

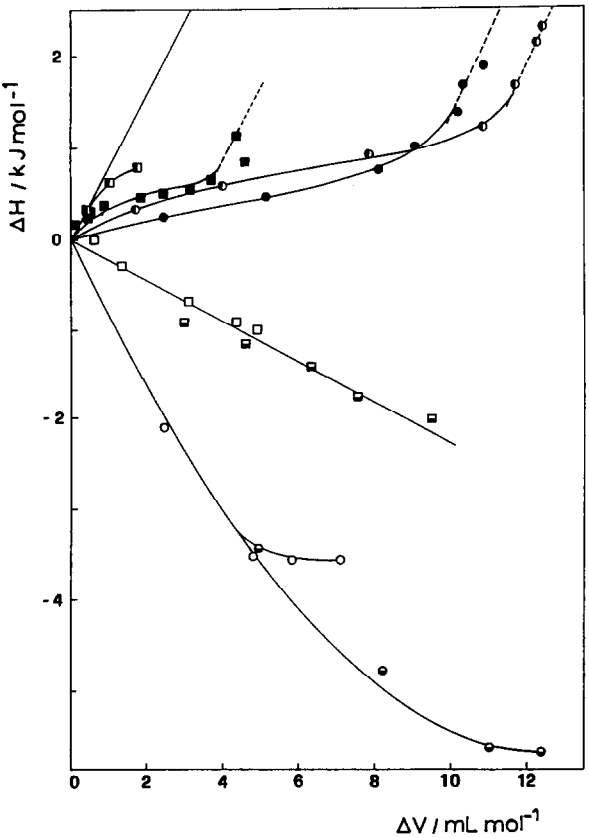

Fig. 5. Plot of enthalpy change as a function of volume change on mixing polygalacturonate (circles), or alginate (squares) with the divalent cations: (○, □) Ca^{2+}; (◒, ⬓) Pb^{2+}; (●, ■) Cd^{2+}; and (◐, ◧) Zn^{2+}. Experiments at 25°C with an LKB batch-type microcalorimeter, and with a modified Carlsberg-type dilatometric system.

desolvation. One may further notice that only at a higher cation-to-polymer ratio does the binding process seem to take place with a mechanism resembling the mere polyelectrolytic behaviour. The most simple explanation is that at a low cation to polymer ratio, the binding process removes a large amount of electrostricted water, while at higher ionic strength it may occur with little solute desolvation. The provisional broken lines are parallel to the theoretical curve predicted by the equation. A tentative explanation is that desolvation of the divalent counterions and the polymer chain takes place mainly in the initial part of the binding curve, while in the last portion purely electrostatic interaction occurs. Such a behaviour in the concentrate regime deserves further investigation.

The data in the lower part of the figure are characterized by a negative favourable enthalpy of mixing. Partial account is also taken of the fact that the alginate sample has about one-third of the "specific binding sites" with respect to that of the pectin sample; it is known, in fact, that only the

guluronate sequences of alginate and the galacturonate sequences of pectins are involved in complexation. The negative enthalpy of mixing with these divalent counterions has been ascribed by us to a polymeric conformational transition [19]. This is also clearly shown (at least for pectate) by the levelling off of the enthalpy change while the volume change (and binding) is still increasing.

Site specificity

In this presentation of the thermodynamic results, the only evidence for a process involving some peculiar interaction may be the favourable enthalpy. However, this arises principally from the conformational rearrangement of the disaccharidic polymer units. The overall value of about -4 kJ mol^{-1} is undoubtedly too small to infer a contribution of specific interactions between the cation and the coordinating hydroxy-carboxylate groups. The existence of this specificity must be proved from other experimental evidence.

Due to the dissymetry of the chelants, the most efficient way to demonstrate specifity is to record an induced circular dichroism in the wavelength range of absorption of the cation. Experiments to characterize this phenomenon have been carried out using as "cationic chromophores", among others, the ionic species Cu^{2+}, Pb^{2+} and Ruthenium Red.

The octahedral coordination geometry of the cation Pb^{2+} gives rise with simple symmetric ligands to complexes in which the symmetry is maintained and the absorption band (centred at 208 nm in water) does not become optically active. The orbital symmetry is broken either by an external magnetic field (magnetic circular dichroism) or by a chiral ligand. If the ligand does not interact symmetrically with the cation, i.e. different distance and/or orientation, then chiral absorption bands with different transition wavelengths are observed. Such a broadening of absorption and extrinsecation of c.d. bands is found for the alginate and pectate complex with Pb^{2+} ions [22]. Not only are at least three bands observed in the circular dichroism spectra of these complexes, but their sign also reflects strongly the opposite chirality of the D-galacturonate moiety of pectate with respect to the L-guluronate moiety of alginate (Fig. 6). An additional difference, the presence of a fourth band in the case of polygalacturonate, may be tentatively ascribed to the fact that in L-guluronate the hydroxyl group on carbon 2 is axial, while it is equatorial in D-galacturonate (Fig. 7 shows the structure of the two dimeric uronates). Other polyuronates show further differences.

The complexity of the Pb^{2+} binding process with polyuronates emerges, however, on inspection of the intensity change of the circular dichroism bands as a function of the cation/polymer ratio. While some bands change in a Langmuir-like behaviour typical of a continuous monotonic binding

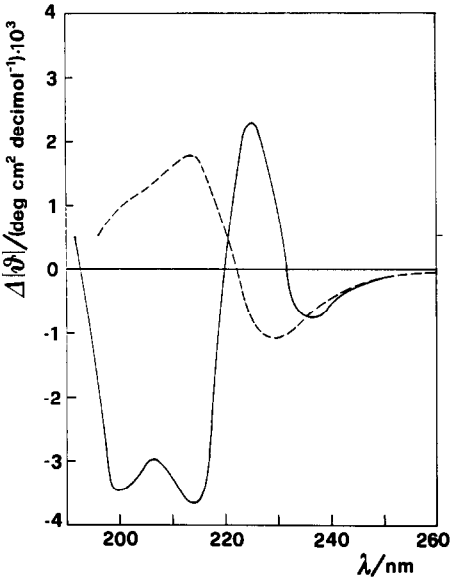

Fig. 6. Circular dichroic bands of the Pb^{2+} complexed by polygalacturonate (———) and alginate (------), at about half saturation.

process, others do not appear at all until a given cation concentration is reached. The apparent cooperativity of these interactions may indeed simply arise from the existence of two modes of binding: in the first, cations bind to the polyuronate inducing a conformational transition by screening

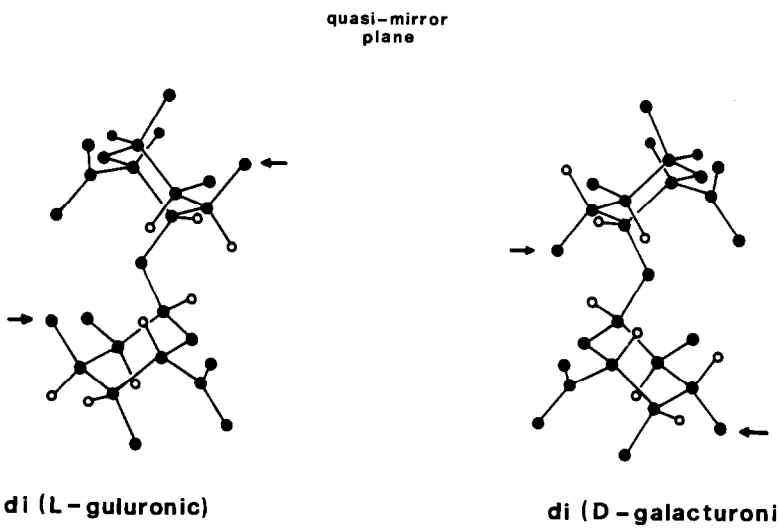

Fig. 7. Quasi-mirror images of the digalacturonate and the diguluronate structures. The reported conformations do not necessarily correspond to the minimum of the conformational energy.

the fixed charges. When a sufficient number of screened carboxylates has been achieved, ordered chains may dimerize and give rise to another c.d. band on the cationic chromophore. Support for such an interpretation comes from the change in the molecular weight of the polymer in the dispersed pre-gelling phase [23,24].

The interaction with Ruthenium Red is still more complex. This is a positively charged complex of ruthenium ions with oxygen and ammonia, $Ru_3O_2(NH_3)_{14}$ having a total of six positive charges screened by counter-anions. In addition to its apparent "specificity" claimed in view of the resulting flocculation with some classes of highly charged polyanions, its most interesting features stem from its complexes with acidic polysaccharides [25]. In fact, the extrinsic circular dichroism bands exhibited by bound Ruthenium Red species can only arise from the structural dissymetry of the whole complexing chain, because each spectrum seems peculiarly linked to the biopolymer secondary structure.

CONCLUSIONS

Interaction of ions with carbohydrate monomers and polymers may give rise to new conformational and/or chemical species. The establishment of chemically "specific" interactions can thus be claimed. In other cases, however, such interaction is a mere "amplifier" of already existing effects and properties, either of the ionic component, e.g. I_2–I_3^- amylose, or stemming from the carbohydrate ligand, e.g. D-ribose.

In addition to chemical interactions that are strongly related to the chemical identity of the species, there are often physical interactions, resulting in a modulation of effects. The long-range non-specific electrostatic interactions between ions and ionic polysaccharides are a notable example. Theoretical tools are increasingly being provided to handle single cases, in the search for a correct interpretation of apparently complicated phenomena, and in prediction of new modes of interaction.

ACKNOWLEDGEMENTS

The work was carried out with financial support from the Consiglio Nazionale delle Ricerche (C.N.R.), Rome, and the Ministero dell'Università e della Ricerca Scientifica e Tecnologica (M.U.R.S.T.). The technical assistance of Mr. J. Cumani is also acknowledged.

REFERENCES

1 S.-C. Tam and R.J.P. Williams, Struct. Bonding (Berlin), 63 (1985) 103.
2 See, for example, A. Cesàro, F. Delben and S. Paoletti, Int. J. Biol. Macromol., 12 (1990) 170.

3 A.D. French and J.W. Brady (Eds), Computer Modeling of Carbohydrate Molecules, ACS Symp. Ser. No. 430, Washington, 1990.
4 See, for example, G.S. Manning, Acc. Chem. Res., 12 (1979) 443.
5 S.J. Angyal, Adv. Carbohydr. Chem. Biochem., 47 (1989) 1.
6 D. Cremer and J.A. Pople, J. Am. Chem. Soc., 97 (1975) 1354.
7 S.J. Angyal, Angew. Chem. Int. Ed. Engl., 8 (1969) 157.
8 F. Franks, P.J. Lillford and G. Robinson, J. Chem. Soc. Faraday Trans. 1, 85 (1989) 2417.
9 R. Urbani and A. Cesàro, Polymer, 32 (1991) 3013.
10 F. Franks, J.R. Hall, D.E. Irish and K. Norris, Carbohydr. Res., 157 (1986) 53.
11 R.E. Lenkinski and J. Reuben, J. Am. Chem. Soc., 98 (1976) 3089.
12 A.M. Alvarez, N. Morel-Desrosiers and J.-P. Morel, Can. J. Chem., 65 (1987) 2656.
13 W. Banks and C.T. Greenwood, Starch and Its Components, Edinburgh University Press, Edinburgh, 1975, Chapt. 3.
14 A. Cesàro, E. Jerian and S. Saule, Bipolymers, 19 (1980) 1491.
15 C.L. Cronan and F.W. Schneider, J. Phys. Chem., 73 (1969) 3990.
16 T.L. Hill, An Introduction to Statistical Thermodynamics, Addison-Wesley Inc., New York, 1960, Chapt. 14.
17 J.A. Thoma and L. Stewart, in R.L. Whistler and E.F. Paschall (Eds.), Starch Chemistry and Technology, Vol. 1, Academic Press, New York, 1965, Chapt. 9.
18 A. Cesàro, S. Paoletti, R. Urbani and J.C. Benegas, Int. J. Biol. Macromol., 11 (1989) 66.
19 S. Paoletti, A. Cesàro, F. Delben, V. Crescenzi and R. Rizzo in P. Dubin (Ed.), Microdomains in Polymer Solutions, Plenum Press, New York, 1985, 159–189.
20 S. Paoletti, F. Delben, A. Cesàro and H. Grasdalen, Macromolecules, 18 (1985) 1834.
21 S. Paoletti, J.C. Benegas, A. Cesàro, G. Manzini, F. Fogolari and V. Crescenzi, Biophys. Chem., 41 (1991) 73.
22 A. Cesàro, F. Delben and S. Paoletti, J. Chem. Soc. Faraday Trans. I, 84 (1988) 2573.
23 A. Gamini, G. Civitarese, A. Cesàro, F. Delben and S. Paoletti, Makromol. Chem. Macromol. Symp., 39 (1990) 143.
24 S. Paoletti, A. Cesàro, F. Delben and A. Ciana, in M.L. Fishman and J.J. Jen (Eds.), Recent Advances in the Chemistry and Function of Pectins, Symp. Ser. No. 31, Washington, 1986, Chapt. 7, pp. 73–87.
25 E. Murano, S. Paoletti, A. Cesàro and R. Rizzo, Anal. Biochem. 186 (1990) 120.