

SOME ASPECTS OF THE ENTHALPY OF DILUTION OF BIOLOGICAL POLYELECTROLYTES

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It is shown that calorimetric experiments on the dilution process of polyelectrolytes, i.e. on the excess enthalpy of electrostatic origin, can give information on the conformational state of the polymer. Analytical expressions are obtained relating the enthalpy of dilution to the charge density on the polymer, from Manning's theory. The experimental data reported on some ionic biopolymers are interpreted on the basis of the theoretical prediction of the electrostatic contribution to the enthalpy of dilution in water of a polyelectrolytic salt and of a weak polyacid.

The procedure outlined provides a means of calculating the actual charge per unit length and of disclosing possible non-electrostatic effects arising from conformational transitions.

Keywords: biological polyelectrolytes, biopolymers

Introduction

Experimental work has been carried out in our laboratory on the solution properties of biological polyelectrolytes. The main interest is in defining the solution parameters which govern on the conformation of ionic polysaccharides. We show here that calorimetric experiments on the dilution process can give information on the conformational state of the polymer.

A polyelectrolyte solution contains the salt of a polyion, typically neutralized by small ions (counterions). The chief distinction between a polyelectrolyte solution and an analogous small-electrolyte aqueous solution lies in the classification of electrolytes into strong and weak. Thus, very dilute aqueous solutions of small

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electrolytes, e.g., sodium acetate, exhibit only dissociated ions, but a substantial fraction of sodium ions are still bound to the polyacrylate polyion at corresponding normalities. Polyelectrolyte salts can, therefore, be classified as true weak electrolytes [1, 2]. An accurate description of the stoichiometry and mode of counterion binding to the polyion has been a central goal of both experimental and theoretical studies of polyelectrolyte solutions [1-6].

In order to relate enthalpy data (dilution, mixing, dissociation, etc.) with the charge density on the polymer we have previously used the well-known counterion condensation theory which is based on a simple chain model in a linear extended conformation [5-7]. In addition, on the basis of the dissociation behaviour of a weak polyacid as a function of its concentration, we have derived [8] an expression for the total enthalpy of dilution of the same polyacid in the free-acid form. We return here to the problem of the calorimetric determination of the enthalpy of dilution and discuss the conformational features which may be derived therefrom.

Additional ideas about the claimed specificity of the counterion/polyelectrolyte interactions have been discussed elsewhere when dealing with the competitive equilibria between monovalent and divalent cations [9] and with ion interaction with carbohydrate monomers and polymers [10].

Theory

The task of computing the ionic contribution to the free energy associated with each polyion has already been accomplished [1-3] and extension to other thermodynamic functions has been made [6] including the relaxation of the chain flexibility [11].

As a starting point the equation for the (excess) electrostatic enthalpy of the polyelectrolyte solution as a function the dimensionless linear charge density parameter, ξ , and of the polyion concentration C (with a total of n_e moles of charged groups), which enter in the Debye screening parameter (defined below), is given as [6, 7]:

$$H^{el} \approx \left(\frac{\partial \frac{A^{el}}{T}}{\partial \frac{1}{T}} \right)_{n_i, b} = -\frac{1}{2} n_e R T \left(1 + \frac{d \ln D}{d \ln T} \right) \xi \left\{ 2 \ln \left(1 - e^{Kb} \right) + \frac{Kb}{e^{Kb} - 1} \right\} \quad (1)$$

with $\xi = 1_B/b$, where b (Å) is the average distance between charges on the linear polyelectrolyte and $1_B = e^2/DkT$ is the Bjerrum length (Å), e is the elementary charge, D the bulk dielectric constant of the medium, T is the absolute tempera-

ture, k is the Boltzmann constant. The value of K , which is the reciprocal of the Debye length, is defined for each ionic strength, I , as $K^2 = 8\pi 1_B I$.

The expression for the molar (electrostatic) enthalpy change upon mixing n_e moles of polymeric salt with pure solvent to change the polymer concentration from C_i to C_f , is:
for $\xi < 1$

$$\Delta_{\text{dil}} H = -\frac{1}{2} R T \left(1 + \frac{d \ln D}{d \ln T} \right) \xi^m \cdot \left\{ 2 \ln \frac{1 - e^{-K_f b}}{1 - e^{-K_i b}} + \frac{K_f b}{e^{K_f b} - 1} - \frac{K_i b}{e^{K_i b} - 1} \right\} \quad (2)$$

where $m = 1$ and $K^2_{i,f} = 8\pi 1_B I_{i,f}$, where $I_{i,f}$ is the ionic strength of the medium. For salt-free solutions, $I_{i,f}$ is equal to $1/2 C_{i,f}$.

When the structural parameter ξ is greater than unity then thermodynamic equilibrium is reached only by condensation of a fraction $r = (1 - \xi^{-1})$ of counterions, the effective value of ξ being permanently reduced to the critical value of unity (this is the central issue of the counterion condensation theory for univalent charges). Therefore, the total (excess) free energy of polyelectrolytic origin contains also a 'mixing' term contributed by the associated counterions, free counterions, co-ions and solvent; however, this latter term is assumed to be of entropic nature only and, therefore, its reduced form is assumed to be independent of temperature (i.e. $\partial \Delta G_{\text{mix}}/RT / \partial T \equiv 0$). To evaluate the enthalpy of dilution, the same form of Eq. (2) for $\Delta_{\text{dil}} H$ will therefore hold, having $m = -1$ for $\xi > 1$. Moreover, $I_{i,f}$ will be given by $I_{i,f} = 1/2 C_{i,f} (1 - r) = 1/2 \xi^{-1} C_{i,f}$.

In the limit of very dilute solutions and, more generally, when $Kb \ll 1$, then the terms within the brackets reduce to

$$\Delta_{\text{dil}} H = -\frac{1}{2} R T \left(1 + \frac{d \ln D}{d \ln T} \right) \xi^m \cdot \ln \frac{C_f}{C_i} \quad (3)$$

where, as before, $m = 1$ for $\xi < 1$ and $m = -1$ for $\xi > 1$.

The simplified Eq. (3) shows why the enthalpy of dilution data are usually reported as a function of the logarithm of the polyelectrolyte concentration, C . Furthermore, it shows that the normalized value of the slope (that is the constant part of Eq. (3)) is equal to $1058 \text{ J} \cdot \text{min}^{-1}$ (for univalent charges at 298 K in water, $\partial \ln D / \partial \ln T = -1.372$ [12]), hence

$$\frac{\partial \Delta_{\text{dil}} H}{\partial \log C} = -\frac{1}{2} R T \left(1 + \frac{d \ln D}{d \ln T} \right) \xi^m \quad (4)$$

Equation (4) simply relates the enthalpy of dilution to the conformational structure of the polyelectrolyte through its charge density parameter ($\xi = 7.135/b$ in water at 298 K, for univalent ions). However, it must be mentioned that one single

value of the slope given by Eq. (4) may correspond to both ξ and ξ^{-1} values, the ambiguity being removed only when $\xi = \xi^{-1} = 1$.

Results and discussion

Theoretical behavior

Calculations of $\Delta_{dil}H$ according to Eq. (2) (in water at 298K) have been made for various values of ξ in the concentration range from 0.1 to 0.0001M. Figure 1 is the three-dimensional representation of Eq. (2) as a function of ξ and $\log C$. The general trend of the curves is similar for all ξ values, showing a decreasing slope at higher concentrations, and approaching linearity at low ionic strength. The linear portion of the curves represent the $\Delta_{dil}H$ changes calculated according to Eq. (3). The linearization of the exponentials containing terms in Kb is a good approximation for polymer concentration which are much less than ca. $2 \cdot 10^{-3} \text{ mol} \cdot \text{l}^{-1}$. The value of the right hand term of Eq. (4) is shown as a function of ξ in Fig. 2; the minimum with discontinuity at $\xi = 1$ is distinctive, as is the linear dependence on ξ for $0 < \xi < 1$. Moreover, one can easily understand why an ambiguity arises in the determination of ξ from experimental enthalpy of dilution data for all ξ values different from $\xi = 1$.

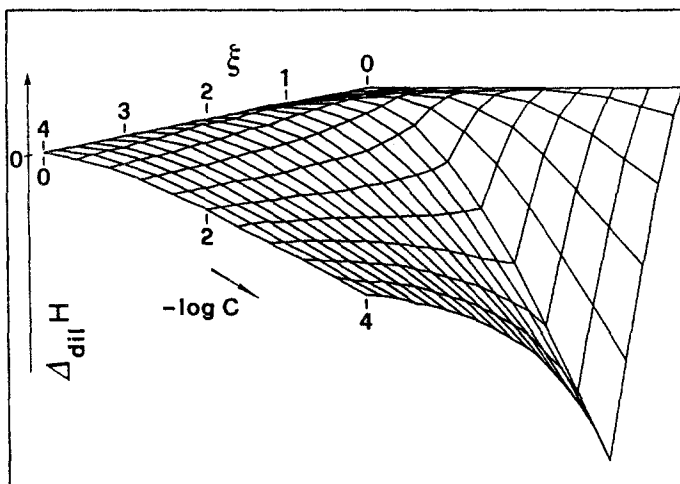


Fig. 1 Three-dimensional representation of the electrostatic enthalpy change for the dilution process of a polyelectrolyte, $\Delta_{dil}H$, as a function of the logarithm of the polyelectrolyte concentration, $\log C$, and of the charge density parameter, ξ , of the polyelectrolyte (in water at 298K, for univalent charges). The steepest curve of $\Delta_{dil}H$ corresponds to $\xi = 1$

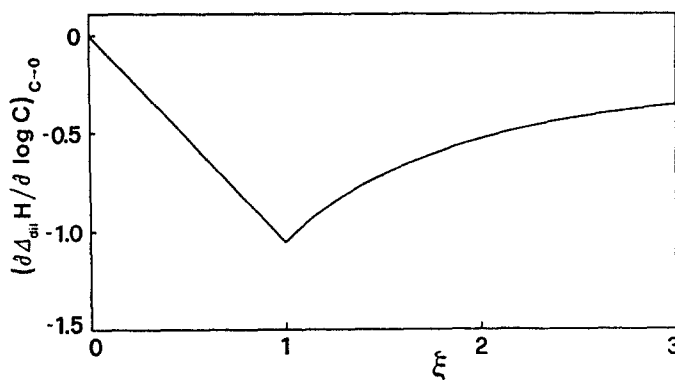


Fig. 2 Limiting value of the slope [Eq. (4)] as a function of the charge density parameter ξ

Experimental $\Delta_{dil}H$ values of linear ionic biopolymers

The enthalpies of dilution were experimentally measured with an LKB 10700 isothermal microcalorimeter at 298 K. Various experiments were carried out in order to cover a suitable range of concentration and to calculate the enthalpy change, $\Delta_{dil}H$, from an initial concentration C_0 to the final concentration C . The results reported in Fig. 3 all show a negative trend as predicted by the theory.

Table 1 Structural charge density (ξ_{sr}) and polyelectrolytic charge density (ξ_{calor}) of some polysaccharides

Polymer	ξ_{sr}	slope / J·mol ⁻¹ [Eq. (4)]	ξ_{calor}	
			if $\xi < 1$	if $\xi > 1$
C6-ox-cellulose <b ^o > = 5.15	1.38	640	0.60	1.65
C6-ox-amylose <b ^o > = 3.70	1.93	750	0.70	1.41
Carboxymethyl-amylose <b ^o > = 3.70	1.93	1100		1
k-carrageenan <b ^o > = 10.3	0.69	720	0.68	1.47
alginate ^a <b ^o > = 4.35	1.64	610	0.58	1.73
EPS-YE-2(S1) <b ^o > = 8.0	0.89	850	0.90	1.11
EPS-TA-1 <b ^o > = 4.70	1.52	800	0.76	1.32

^a from Ref. [5].

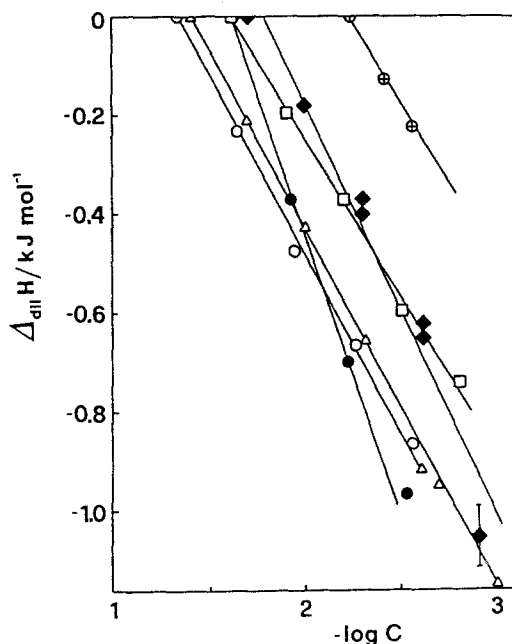


Fig. 3 Enthalpy of dilution data (at 298 K, in water) of some linear ionic polysaccharides:

C6-ox-cellulose (□), C6-ox-amylose (●), carboxymethylamylose (○), *k*-carrageenan (Δ), galactoglucon from *Rhizobium meliloti* EPS-YE-2(S1) (◆), polysaccharide from *Rhizobium trifolii* EPS-TA-1 (⊕), at concentration below 10^{-2} M

Provided that the conformational features of the polyelectrolytes do not significantly change at a local level, the linear slope of the curves should give the value of ξ of that of ξ^{-1} . These 'thermodynamically' derived structural parameters are shown in Table 1, together with the values inferred from the regular (helical) conformation of the polymers, as deduced from the X-ray diffraction studies or from conformational calculations. For the polymers reported in Fig. 3, only small discrepancies between the two sets of values are observed. One should note, however, that in some cases either the rigid structure may not be preserved in solution or the theoretical approach may be obviously inappropriate for the system studied (for example for CMA having long side chains).

The other biopolyelectrolytes, as shown in Fig. 4, exhibits completely different behaviour: not only because of the nonlinearity, but especially for the enthalpy of dilution data ranging toward positive values (for DNA a limiting value of about $13 \text{ kJ}\cdot\text{mol}^{-1}$ is reached). Denaturation by dilution (due to the decrease of the ionic strength in the medium) is well known for DNA and is important in the preparation of nucleic acid solutions in the absence of added salts. However, direct calorimetric measurements have not been reported to date. The partial denaturation of the native double-helical structure takes place with a cooperative

character upon dilution in the absence of added electrolytes. This view is supported by other experiments, the most simple one the hyperchromicity upon dilution.

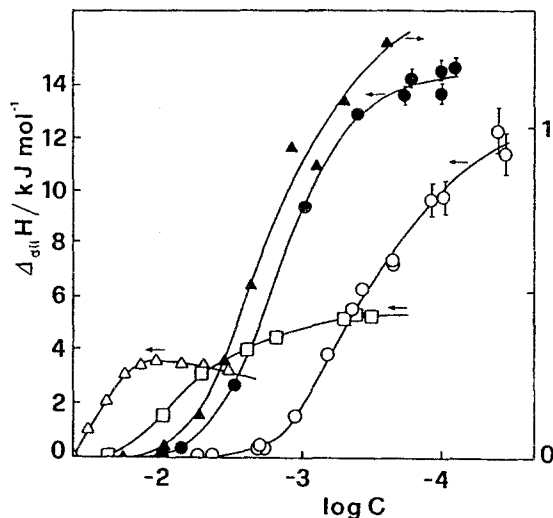


Fig. 4 Enthalpy of dilution data (at 298K in water) of some biopolyelectrolytes showing conformational changes upon ionic strength dilution: DNA-calf thymus at 298K (O) and at 308K (●), t-RNA (□), EPS-TA-1 (Δ), and, on left-scale, xanthan (▲)

Some polysaccharides are believed to maintain conformational regularity in solution, as inferred both from chain dimension and rigidity and from 'thermal denaturation' experiments. One undiscussed example is the exocellular polysaccharides from *Rhizobium trifolii* strain TA-1 (EPS-TA-1) [11], which undergoes a conformational transition induced by decreasing the ionic strength or by increasing temperature. Another well known example is given by the polysaccharide from *Xanthomonas campestris* (Xanthan), for which a double helical structure is claimed, although some debate has been produced in the literature about the molecular conformation adopted in solution and the total integrity of the (native ?) structure. The results presented in Fig. 4 clearly underline that the interaction existing at higher polymer concentrations are not preserved upon dilution.

Conclusions

The enthalpy of dilution provides a measure of the energy changes in the interaction of solvated solute species upon dilution and, as outlined above, it includes contributions due to changes in the non-electrostatic interactions between

all the species in the solution. The nonionic contribution to the enthalpy of dilution of a hydrophilic polymer in water has been reported to be negligible [5]. Therefore, when deconvoluted up from the electrostatic interactions, enthalpy of dilution data provide useful information on nonelectrostatic interactions arising from conformational effects.

The experimental data reported confirm this statement for a number of polyelectrolytes known to assume more or less extended conformation which persists in the whole range of concentration investigated. However, the data also give some insight on the energetics of the conformational transition of those regular biopolymeric conformations which can be destroyed by decreasing ionic strength upon dilution.

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Zusammenfassung — Es wurde gezeigt, daß kalorimetrische Versuche an Lösungsprozessen von Polyelektrolyten, d.h. die Überschußenergie elektrostatischen Ursprunges, Informationen über den Konformationszustand des Polymers liefern können. Ausgehend von der Theorie von Manning wurden analytische Ausdrücke entwickelt, welche die Lösungsenthalpie mit der Ladungsdichte am Polymer in Verbindung bringen. Die experimentellen Daten für einige ionische Biopolymere wurden auf der Grundlage der theoretischen Voraussage des elektrostatischen Beitrages zur Lösungsenthalpie eines polyelektrolytischen Salz und einer schwachen Polysäure in Wasser interpretiert.