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Phenomena of Counterion Binding, Polyion Complexation and Polyion Condensation

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Abstract: The article handles some aspects of polyelectrolyte research investigated during the last ten years at the university of Osnabrück. They are: counterion binding, polyion complexation, and polyion condensation. Fundamental features are presented that are common to the behaviour of most polyions. Another intention is to bridge a gap between synthetic and biological polyelectrolyte research. For this purpose, DNA condensation is reviewed in some detail.

INTRODUCTION

Polyelectrolytes are highly charged and flexible chain molecules. Due to this dual character they exhibit various interesting phenomena where counterion binding and polyion complexation are two of them.

The paper is organised as follows. Firstly, we classify the different modes of counterion binding, secondly we describe some fundamental features of polyanion/polycation complexation, and finally we give a short review about intramolecular polyion condensation. Experimental details and theoretical formalisms are not presented. In so far, the article is far away from any completeness. The primary intention is to break a bridge between polyelectrolyte physicochemistry and biopolymer research.

COUNTERION BINDING PHENOMENA

Polyions are charged polymer molecules which when they are dissolved in solution are surrounded by a cloud of small ions. The ions having the opposite sign of charge as the polyions are the counterions and the ions bearing the same type of charge are the coins, where the latter are present only when a low molecular salt is added.

Due to the electrostatic attraction between the counterions and the polyions, the counterions tend to be in close proximity to the polyions. Three types of binding can be distinguished:

(1) Assuming purely long-range interactions, the counterions are non-specific bound to a polyion. They are accumulated around the polyion skeleton occupying a certain territory of volume, V_p , within they are more or less completely hydrated and move along the polyion axis. The terminus for this feature is territorial or delocalised counterion binding[1)].

(2) If short-range interactions are dominant, the counterions bind to specific polyion sites. Thereby they loss a part of their hydration sphere. These co-ordinated or associated

counterions do not move. They are fixed by ionic and or covalent binding and therefore they are called site-bound.

(3) The hydrocarbon nature of polyions makes itself felt in the interactions of organic counterions. There are in addition to the electrostatic attraction strong non-ionic forces which stabilise the uptake of counterions. In some cases these van der Waals forces are stronger than the Coulomb forces. The counterions are then bound hydrophobic.

Mostly, the different modes of binding occur simultaneously. Consequently, it is difficult to separate them from each other. The degree of counterion binding, θ, which is defined as the number of bound counterions per polyion group, depends on the experimental method used. While NMR-techniques[2] are sensible in deducting site-binding, other methods such as dilatometry[3], dye-spectroscopy[4] or light scattering[5] , are more sensitive for territorial binding.

Other factors influencing the type and the degree of counterion binding are polyion architecture, counterion valence, and thermodynamic conditions such as temperature or solvent composition. Varying these physical parameters one finds features which are common to all polyelectrolytes. These effects are of fundamental meaning and shall be summarised now.

Polyion systems containing two or more counterion species simultaneously

We consider firstly situations where both univalent as well divalent counterions are present simultaneously. Two typical examples[5] are the systems PSS/Pb/H and PSS/Pb/Na whose binding isotherms are shown in Figures 1 and 2. There the degrees of counterion binding, θ_1, and θ_2, of the univalent and the divalent counterions are plotted versus c_2/c_p, where c_2 is the added Pb^{2+}-ion concentration and c_p the PSS repeat unit concentration. The H^+- and the Na^+-ion's concentrations, c_1 , were chosen in excess so that the ionic strength, I, is nearly identical with c_1. In detail it holds $c_p = 0.001$ M, $T = 25°C$, $I = 0.01$ M and $I = 0.02$ M, respectively. We observe: (1) If no Pb^{2+}-ions are present, θ_2 is zero and θ_1 takes on its saturation value which is of the order of 0.60. (2) When the amount of the added Pb^{2+}-ions increases θ_1 decreases while θ_2 increases. This indicates that Pb^{2+}-ions become bound while simultaneously univalent ions become released. The driving force for this ion exchange is to one part the higher valence of the Pb^{2+}-ions and to another part the entropy gain caused by the fact that the univalent ions are present in excess. A mathematical model which describes both effects is the two-parameter theory of Manning[1]. The solid lines in Figures 1 and 2 are calculated by this theory and we see the agreement with the experimental data is quite well.

More complex becomes the situation when the two counterion species are of the same valence. Theory considers counterions as point charges and predicts no difference. However, experimentally different binding affinities are found. Depending on the polyion type and the solvent used, ion binding series can be marshalled. The central result is: Polyions that have the same functional groups behave similar. That is, all poly(sulfonates), such as poly(styrene sulphate), poly(vinyl sulphate), or dextran sulphate, show comparable binding affinities. We have[6] $Cs^+ > Rb^+ > K^+ > Na^+ > Li^+$, while the opposite series is found for poly(carboxylates) and poly(phosphates). In other words, a sulfonate polyion binds Cs^+-ions stronger than Rb^+-ions, K^+-ions stronger than Na^+-ions, and Li^+-ions lowest, while carboxylate and phosphate polyions behave just opposite. However, this holds only when the solvent is water. In aqueous/organic solvent mixtures the binding affinities are slightly different. The reason is that

the small ions are solvated. While alkali-ions conserve their full hydration sphere when they are bound to poly(sulfonates) they become partly dehydrated when they are bound to poly(carboxylates) or poly(phosphates). The latter bind alkali ions predominantly by site-binding while poly(sulfonates) bind them predominantly territorial.

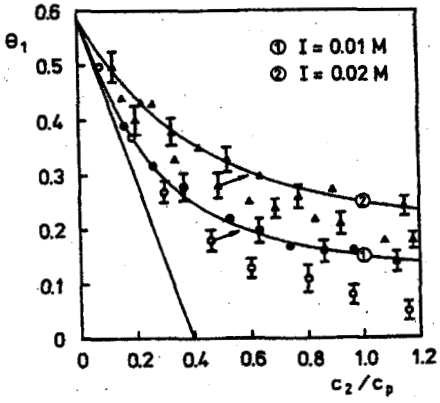

Fig.1: Counterion binding isotherms of the systems PSS/Pb/H , I = 0.01 M (O), I = 0.02 M (Δ) and PSS/Pb/Na, I = 0.01 M (●), I =0.02 M (▲). Straight line: simple condensation theory. Upper lines: Manning's two-variable theory. c_p =10^{-3} M.

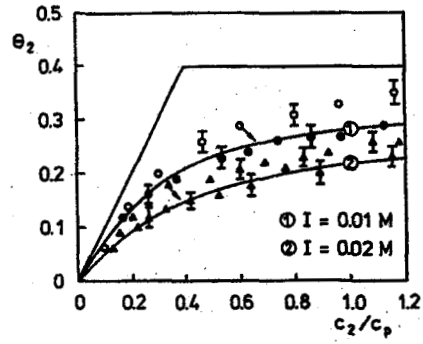

Fig. 2: Degree of Pb^{2+}-ion binding, θ_2 , versus the ratio c_2/c_p of the added Pb^{2+}-ion concentration to the PSS repeat unit concentration. Symbols as in Figure 1.

Polyions of different charge densities

Another parameter which influences the degree of counterion binding is the polyion charge density, i.e. the average distance, b, between two neighboured charges along the polyion chain. The higher the linear charge density the larger is the degree of counterion binding. According to Manning[1] there exists a critical charge density where below this value no binding takes place at all. We have proofed this prediction using poly(acrylamide/acrylic acid copolymers) (PAM-Y), varying the degree of hydrolysis, Y. Some typical results are shown in Figure 3 where the degree of counterion binding, θ_1 ,of Na^+-ions and K^+-ions, respectively, is plotted versus c_1/c_p. Obviously, the accord between theory and experiment is quite well. At a given ratio c_1/c_p, θ_1 decreases as Y, which is a measure for the charge density, decreases. However, for $Y = 30$, θ_1 should theoretically be zero, but this is not found. The probable reason is that Manning's theory describes territorial counterion binding whereas poly(carboxylates) bind most of their counterions by site-binding.

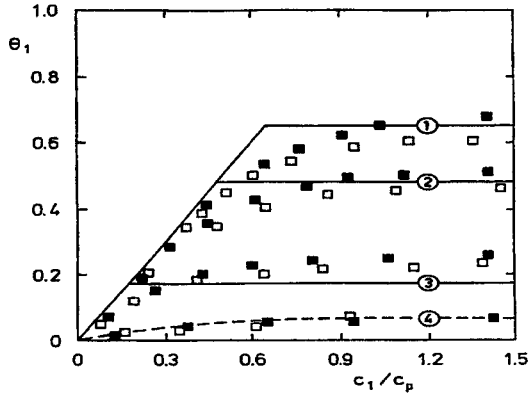

Fig. 3: Degrees of Na^+-ion (■) and K^+-ion (□) binding, θ_1, versus the ratio c_1/c_p. The polyions are poly(acrylic acid) (PAA) and poly(acrylamide/acrylic acid copolymers) (PAM-Y), having the degrees of hydrolysis $Y = 100$ (1), $Y = 68$ (2), $Y = 42$ (3), and $Y = 30$ (4). Solid lines: counterion binding theory. $c_p = 10^{-3}$ M.

Counterion binding at different temperatures

The central parameter of polyelectrolyte theory is the charge density parameter $\xi = e^2 / (4\pi \varepsilon_0 \varepsilon k_B T b)$. Here, e is the elemental charge, ε the dielectric constant of the solvent, k_B the Boltzmann-constant, and T the temperature. The influence of the average polyion charge distance, b, was examined just before. The dielectric constant, ε, and the temperature, T, are coupled with each other. For most solvents ε decreases with increasing T. As a consequence the product εT is more or less independent on T. Since $\theta_1 = 1 - \xi^{-1}$, this means that also the degree of counterion binding, θ_1 , should be temperature independent. Own measurements do not confirm this prediction. In Figure 4 the degrees of counterion binding of the system PSS/Pb/H are plotted versus T and it is seen that both θ_1 and θ_2 decrease slightly as T is

increased. Most marking is this effect for the effective degree of dissociation, $\theta_e = 1 - \theta_1 - 2\theta_2$. While at $T = 10°C$ only 5% of the PSS groups are dissociated the degree of dissociation is increased to 20% at $T = 60°C$.

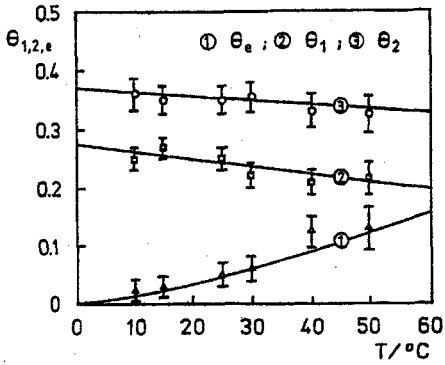

Fig. 4: Temperature dependence of the degrees of counterion binding, θ_1 and θ_2, and the degree of polyion dissociation, θ_e, for the system PSS/Pb/H. Parameters: $c_2/c_p = 1.18$ and $I = 0.01$ M.

Counterion binding in mixed solvents

It is possible to vary the solvent dielectric constant, ε, by holding the temperature constant. This is achieved by working with aqueous/organic solvent mixtures. For instance, in water/methanol or water/acetone the dielectric constant, ε, decreases as the amount of the organic solvent is increased while in mixtures of water/formamide or water/N-methylformamide just the opposite is the case. According to counterion binding theory[1] θ is inverse proportional to ε. That is, the degree of counterion binding, θ, should increase when ε decreases and decrease when ε increases. Two typical examples are presented in Figures 5 and 6. For the system PSS/Mg/Na/water/formamide the results of measurement are in line with theory. θ_2 is the smaller the larger ε is. However, for the system dextran sulphate/Cd/Na/water/acetone the opposite effect is observed. θ_2 decreases when the acetone content is increased.

Several further measurements on other polyion / mixed solvent systems give rise to the fundamental result that there is no correlation at all between the degree of counterion binding and the solvent dielectric constant. This is caused by the fact that in mixed solvents solvent/solvent interactions and selective solvent/polyion adsorptions are much more dominant than counterion/polyion interactions.

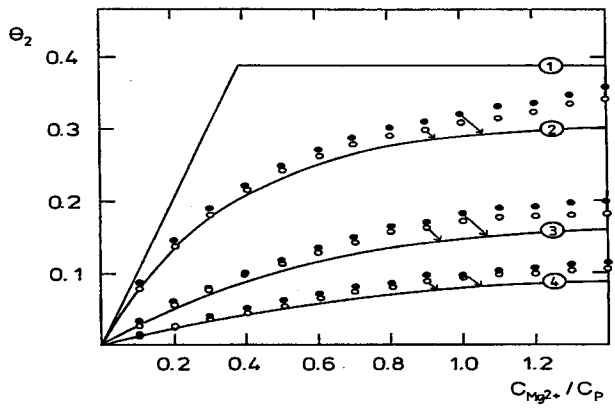

Fig. 5: Counterion binding isotherms of the system PSS/Mg/Na at $T = 25°C$. Solvents: (O) 45 wt.-% water and 55 wt.-% N-methylformamide (NMF); (●) 53 wt.-% water and 47 wt.-% formamide. In both mixtures ξ is 2.21. Solid lines: (1) simple condensation theory; (2) - (4) two-variable theory, where $I = 0.01$ M, 0.04 M, and 0.08 M (from the top to the bottom); $c_p = 10^{-3}$ M.

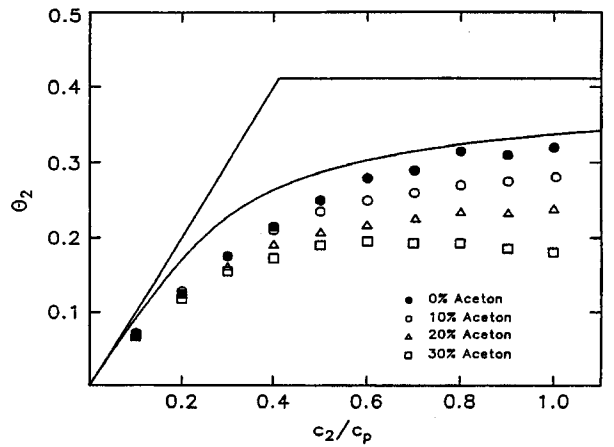

Fig. 6: Counterion binding isotherms of the system dextran sulphate/Cd/Na/water/acetone at T = 25°C. Upper line: simple condensation theory; lower line: two-variable theory. $c_p = 2 \cdot 10^{-3}$ M.

Effects of polyion architecture

There exist different polyion architectures. We distinguish between linear and branched polyions where the branched molecules can be subdivided into combs, stars or trees. Since the electrostatic potential is a function of the polyion charge distribution, this is the case also for the degree of counterion binding. However, a comparison between linear and branched polyions is only reasonable if the molar mass and the average number of charges per molecule are equal. Additionally, the repeat units should be of identical chemical structure. Polyelectrolytes for which this conditions are approximately fulfilled are pullulan sulphates and dextran sulphates, where the first are linear while the latter are comb-like branched. Figure 7 shows some results of measurement. There the degree of counterion binding, θ, is plotted versus the branching index $g = \langle R^2 \rangle_{z,\text{linear}}^{1/2} / \langle R^2 \rangle_{z,\text{branched}}^{1/2}$, where $\langle R^2 \rangle_{z,\text{linear}}$ and $\langle R^2 \rangle_{z,\text{branched}}$ are the z-average mean square radii of gyration of a linear and a branched macromolecule which both are uncharged and which both have the same molar mass. That is, the higher g the smaller is the degree of branching. Since θ decreases as g is increased dextran sulphate binds the more counterions the stronger it is branched. Thus, we can speculate: The stronger a polyion is branched the higher is its degree of counterion binding.

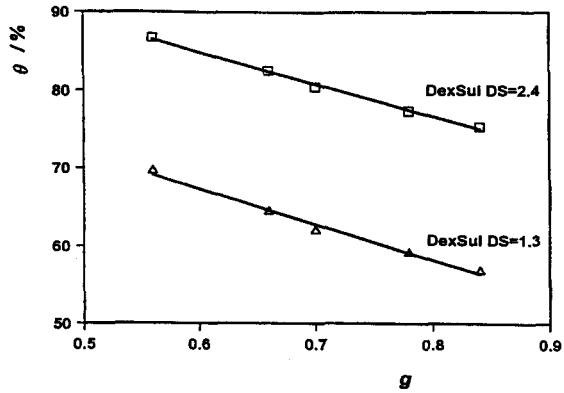

Fig. 7: Plot of the degree of Na^+-ion binding, θ, of dextran sulphate versus the branching index , g, at two different degrees of sulphating, $DS = 1.3$ (Δ), and $DS = 2.4$(□).

INTERPOLYMER COMPLEXES

The characteristics of polyions is governed not alone by their chemical structure but also by the formation of polyion clusters[7]. These accumulations are ordinarily observed as phase separation effects, such as precipitation, gelation, coacervation, emulsion, or liquid-crystallisation. A special class are polyelectrolyte complexes formed between oppositely charged polyanions and polycations. They are called interpolymer complexes and are of definite interest due to the fact that they involve the loss of the properties of the original components and give rise to the development of materials with completely new properties. For instance, the water permeability of polyelectrolyte complexes is more than 10 times higher than that of reconstituted cellophane and also considerably higher than that of crosslinked poly(2-

hydroxyethyl-metha-acrylic acid) hydrogels. Also the dielectric constants of complexes are extremely high and their dispersion properties are very different from those of conventional polymers.

Frequently, interpolymer complexes are insoluble, but also soluble complexes are known. The solubility depends to one part on the complex stoichiometry and to another part on the solvent quality. An interesting phenomenon is observed when a low molecular salt is added. It exists a critical salt concentration, $c_{s,d}$, at which soluble complexes become unstable[8)] and dissociate back to their single strands, the polyanions and polycations. This effect is of general nature. It happens both for synthetic as well for biological systems. Here, we discuss two examples. They are PAA/IONENE and CT-DNA/IONENE, where IONENE is the shortening of poly((dimethylimino)ethylene-(dimethylimino)-methylene-1,4-phenylene-methyldichloride).

Complex formation

Complexes are simply prepared by mixing the original polyanion and polycation solutions in appropriate proportions. Thereby, it is usual to call the longer polyion the host and the shorter counterpart the guest. That is, in our case DNA and PAA, respectively, are the hosts while IONENE is the guest. For simplicity, we keep the host mol repeat concentration, c_H, constant while the guest mol repeat concentration, c_G, is varied. Therefore, the molar fraction $X_G = c_G / c_H$ is the characteristic system parameter.

The easiest way to observe and characterise polyelectrolyte complexes is UV-VIS spectroscopy. Figure 8 presents a typical scan for the system PAA/IONENE. The transmission is plotted versus the logarithm of the concentration, c_s, of the low molecular salt NaCl, where the IONENE fraction, X_G, is varied. We distinguish three different regions. In region A, where $c_s < 10^{-2}$ mol/dm^3, the complexes are water-soluble and of a nonstoichiometric composition. That is, the solution is clear and the transmission is nearly 100%. In region B, where 10^{-2} mol/dm^3 < c_s < 10^{-1} mol/dm^3, the polyelectrolyte complex charges are neutralised nearly completely and as a consequence of increasing ionic strength the complexes fall out and the solution becomes turbid. Finally, in region C, where $c_s > 10^{-1}$ mol/dm^3, no complexes will be formed at all. Due to energetic reasons it is more favourable that the complexes dissociate back into their components, the polyanions and polycations. This phenomenon is called salting in effect, while the transition from region A to B is the salting out effect.

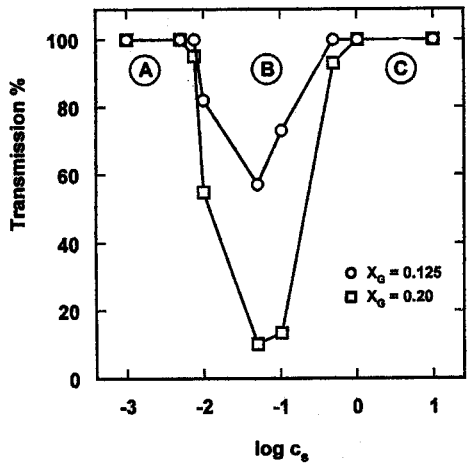

Fig. 8: Transmission spectra of the system PAA/IONENE at $X_G = 0.125$ and $X_G = 0.20$. $c_{PAA} = 10^{-3}$ M.

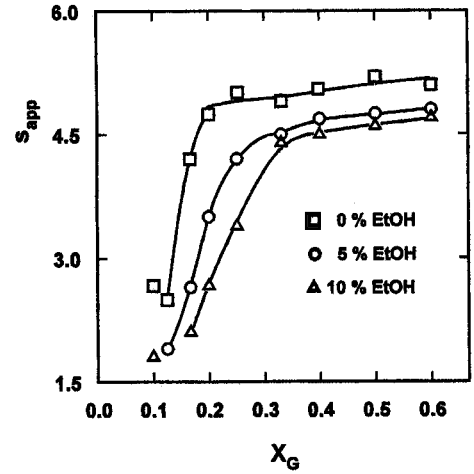

Fig. 9: The apparent sedimentation coefficient, s_{app} , of the system PAA/IONENE/water/ethanol versus the IONENE fraction, X_G. $c_{PAA} = 10^{-3}$ M.

The stoichiometry of soluble polyelectrolyte complexes can be determined by ultracentrifugation. This method has the great advantage that it separates insoluble from soluble complexes. An example shows Figure 9. There the apparent complex sedimentation coefficient, s_{app} , of PAA/IONENE is plotted versus the IONENE fraction X_G. Firstly the

sedimentation coefficient rises with X_G and then it reaches it's saturation value. Since s_{app} is a measure for the complex molar mass, the complexes firstly become larger and then their molar mass remains constant. The X_G-value at which saturation takes place is the saturation degree of complexation, φ_{sat}. For PAA/IONENE/water φ_{sat} is 0.2. That is, 20% of the PAA charges are occupied with IONENE-charges while 80% remain unoccupied. It is also seen that the degree of complexation, φ_{sat}, increases with increasing ethanol content. That is , the complex stability is the higher the smaller the dielectric constant, ε, of the solvent.

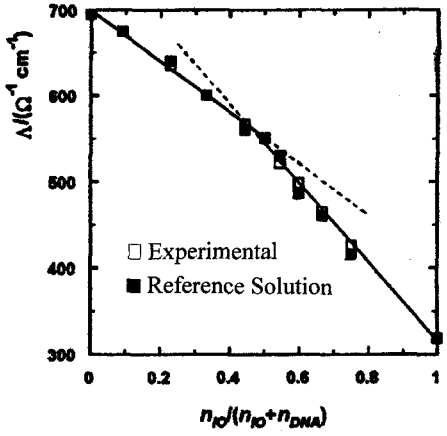

Fig. 10: Plot of the specific conductivity, Λ, of the system CT-DNA/IONENE versus the IONENE fraction at $T = 25°C$. $c_{NaCl} \approx 10^{-3}$ M; $c_p = 2.16 \cdot 10^{-3}$ M.

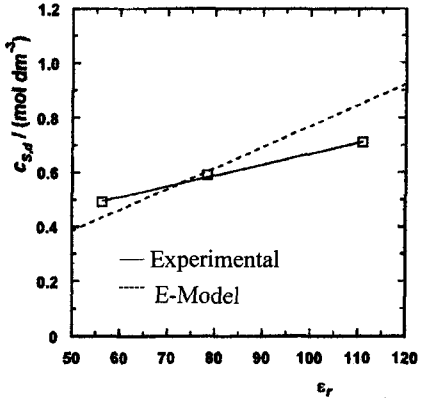

Fig. 11: Plot of the dissociation concentration, $c_{s,d}$, of the system CT-DNA/IONENE versus the solvent dielectric constant.

Another possibility to determine the complex stoichiometry are conductivity measurements. An example shows Figure 10. There the specific conductivity, Λ, of the system CT-DNA/IONENE is plotted versus the IONENE mol fraction $x_{IO} = n_{IO}/(n_{IO} + n_{DNA})$. The NaCl concentration is 10^{-3} M and the entire polymer concentration is $2.16 \cdot 10^{-3}$ M. The open squares are the data of the polyion mixtures and the filled squares represent the reference solutions having the same ionic concentration but containing no polyions. The break at $x_{IO} = 0.5$ points to a complex stoichiometry of 1:1. However, this stoichiometry depends on the type and the concentration of the added low molecular salt used. For the alkali- and earth alkali salts at low concentration the degree of complexation, φ, increases with increasing c_s. At mediate salt concentration φ reaches its saturation value 1 and at very high salt concentrations φ converges to zero. That salt concentration at which φ becomes 0.5, when c_s is high, is the complex dissociation concentration, $c_{s,d}$. There half of the complex units are complexed while the other are not. Some typical examples are given in table 1. For the earth alkali salts $c_{s,d}$ is nearly half as large as for the alkali salts. That is, the higher the valence of the ions the lower is $c_{s,d}$. Other factors such as the ion type, the polyion's molar mass, architecture, or temperature are rather negligible. The only exception is the dielectric constant of the solvent. It exists a strong correlation between $c_{s,d}$ and ε. As shown in Figure 11, $c_{s,d}$ is proportional to ε, an effect which can be explained quite well theoretically[8].

Table 1: Dissociation concentrations, $c_{s,d}$, of the system CT-DNA/IONENE

salt	**LiCl**	**NaCl**	**KCl**	**CsCl**	**$CaCl_2$**	**$SrCl_2$**
$c_{s,d}$ / (mol/l)	0.49	0.56	0.52	0.67	0.23	0.23

POLYION CONDENSATION

A DNA when free in solution possesses a conformation of an expanded coil. In living organisms, however, DNA is packed in a hundreds of time more compact form. This strong packing maintains stability and ensures an efficient replication of the genome. Conventionally, this *in vivo* effect is called condensation, but it has been shown that DNA compactisation can be produced also *in vitro*. This was firstly observed in 1971 by Lerman[9] who discovered that the rate of sedimentation of DNA in highly dilute solutions increased strongly when concentrations of added neutral polymers such as polyethylene oxide and salts like NaCl arrived certain critical levels. He was also the one who gives this *in vitro* collapse its name Ψ-condensation where Ψ (spoken ***psi***) stands for ***p***olymer- and ***s***alt-***i***nduced. However, this phenomenon is much more general. Compactisation is observed not only for DNA but also for RNA and other natural and synthetic polymers[10-12]. It is found[13] that there exists a coexistence between the compacted state and the random coil state. Therefore, the general terminus is coil-globule transition.

Here, we want give a short review on the recent progress in the understanding of DNA compactisation. The topics are morphologies of collapsed DNA, condensing agents, and intermolecular attractive forces.

Morphologies of collapsed DNA

DNA compactisation can be observed by a number of different techniques. The most important methods are electron microscopy[13], laser light scattering[11-12], sedimentation[9], viscometry[14], fluorescence microscopy[15-16], and circular dichroism[17-20]. A typical example is presented in figure 12. There the translational diffusion coefficient distribution, w(D), of CT-DNA (M_w = 2.2 10^3 kg/mol) is plotted as a function of the condensing agent concentration of poly(ethylene glycol) (PEG) at T = 25°C, $c_{DNA} = 0.3$ kg/m^3, $c_{NaCl} = 0.5 \cdot 10^3$ mol/m^3, and $M_{w,PEG} = 4$ kg/mol. At low PEG-concentration, w(D) is unimodal and the diffusion coefficients, D, are low, i.e. they are of the order of 10^{-2} s/m^2. According to the Einstein-Stokes relation, $R_h = k_B T / 6\pi\eta_0 D$, the diffusion coefficient can be recalculated into the hydrodynamic radius, R_h. The result is that R_h is large when D is small and vice versa. This indicates that at low c_{PEG} all DNA-molecules are in the coil state. As c_{PEG} increases, w(D) becomes bimodal. A second peak occurs where the D-values become very large. They lie in the range of 0.1 s/m^2 to 1 s/m^2 so that the corresponding hydrodynamic radius becomes very small. This state is the collapsed state. Increasing the PEG-concentration further the coil state diminishes totally and all DNA molecules become collapsed. This transformation is reversible. If we remove the condensing agents, i.e. the PEG-molecules, the DNA condensates expand back to their coil state.

The most frequent morphology of collapsed DNA in aqueous solution is that of a toroid, but also spheroids, rods, and beaded fibres can be observed. Whether toroids or a mixture of different structures are formed, depends on such specifics such as the base pair sequence, temperature, pH, or the concentration and the type of the condensing agent. In this aspect, it is interesting that toroids can be formed by blind-end fusion of rods as well as by single continuous circumferential winding[21].

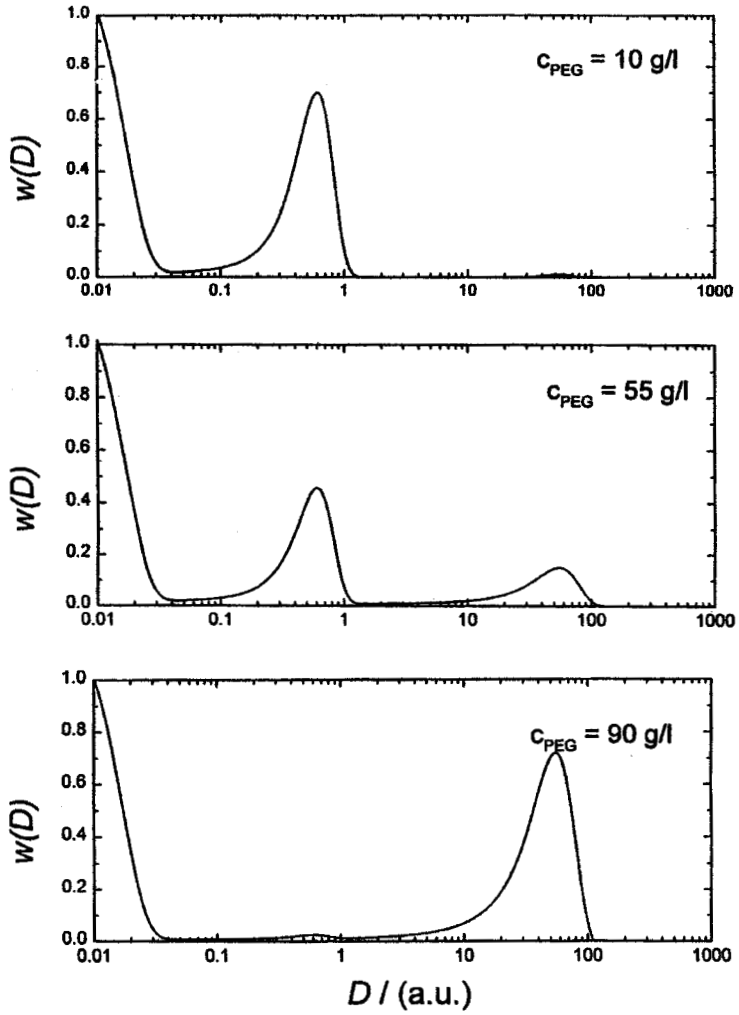

Fig. 12: Translational diffusion coefficient distribution, w(D), of the system CT-DNA/water/NaCl at various PEG-concentrations. c_{NaCl} = 0.5 M; c_{DNA} = 0.3 g/l. $M_{w,DNA}$ =2.2·10^6 Da; $M_{w,PEG}$ = 4000 Da.

Remarkably, the size and the shape of the condensed particles are quite insensitive to the DNA molar mass. Toroids of T7 or lambda phage DNA, where the single molecules have a molar mass of the order of 10^7 Da, have the same size as toroids of plasmid DNA, having a much smaller molar mass[22]. The reason is that small DNAs build associates to form a condensate while large DNAs build monomolecular structures.

Condensing agents

A DNA molecule occupies in it's collapsed state a 10^4 - 10^8 times less volume than when it is in it's coil state. To achieve such a high packing density the DNA molecules must be highly ordered, and the repulsive electrostatic forces between the repeat units must be overcome. *In vivo*, multivalent cations, such as polyamines fulfil this task[23-24], where ATP serves as the energy source. *In vitro*, condensation takes place also, but the valence of the condensing agent must be larger than a certain critical value.

Univalent cations are generally ineffective. This can be explained by the counterion binding theory. According to Bloomfield[25] nearly 90% of the DNA charge must be neutralised for condensation to occur, but this is not possible by univalent cations.

Also divalent cations mostly do not induce condensation, although there are some exceptions. Mn^{2+} can produce toroidal condensates of supercoiled plasmid DNA[26] , but not of linearized plasmid. In the diaminoalkane series $NH_3^+(CH_2)_nNH_3^+$, compounds with $n = 3$ and $n = 5$ cause condensation of T4 DNA molecules, while those with $n = 2$, 4, and 6 do not[28]. That is, also the linker length and the agent's hydrophobicity play a role.

Cations with a valence $z > 2$, generally produce DNA compactisation, where mostly those with higher charge induce condensation at lower concentration. For instance, spermine^{4+} is more effective than spermidine^{3+}.

Despite of these morphological differences, toroids and rods have similar distributions of dimensions. That is, the distribution of toroid circumferences is similar to the distribution of rod lengths where additionally the distribution of the toroid thickness, i.e. the outer - inner radius difference, is similar to the rod diameter distribution[28]. This suggests that the energy of gentle bending in toroids is similar to the energy if infrequent sharps are bent in rods.

Of general interest is also the internal structure of a DNA toroid. X-ray diffraction studies reveal a very close packing with a DNA helices center-to-center distance of nearly 27 A°. Thereby, the DNA remains in its B-form as revealed by CD-measurements. Within itself, a DNA toroid is a periodic structure, which is formed by the repetition of virtually identical elemental structural units. While in former days, the spool model[29] was suggested, today a model is favoured where the DNA is coiled with a constant radius of curvature into series of equally sized contiguous loops that are approximately coplanar but concentric[30]. The centres of the loops lie on a helical path around the toroid axis, so that the overall strands remember to a helix with an extremely shadow pitch. In this aspect, the investigations of Marx and Ruben[28] are very illustrative. On the surface of a frozen hydrated spermidine-DNA toroid single DNA helices appear to cross over each other and diverge as they progress around a toroid.

Intermolecular forces

Polyion condensation arises from a complex interplay of interactions. They are among others an entropy loss upon collapse of the expanded worm-like coil, stiffness which sets limits on tight curvature, and correlated counterion fluctuations which screen of short range electrostatic attractions. Very interesting are in this aspect hydration forces. According to Rau and Parsegian[31], polyvalent ligands bound to DNA double helices appear to act by reconfiguring the water between the macromolecular surfaces to create attractive long-range hydration forces. One should also mention Manning`s theory[32]. He predicts a force which is repulsive for distances either much less than or of the order of the Debye length, but which is attractive at intermediate distances. Thereby attraction results from the enhanced translation entropy of the territorial bound counterions as they share the space between the polyions.

CONCLUSIONS

Our understanding of counterion binding, polyion complexation and polyion condensation has increased during the last decade. There are a number of new insights due to the application of new techniques, the deeper theoretical understanding of intermolecular forces, and the extension over a wider range of agents, solvent conditions and polyion types used. However, it remains also much to be done.

One aspect is the correlation between counterion binding and polyion complexation. Experimentally, it is found that the degree of complexation is inverse proportional to the degree of counterion binding, but up to now it can not explained theoretically.

Another point is the relation between intramolecular complexation and condensation. It is sketchily understood why large DNAs form monomolecular condensates while small DNAs build multimolecular associate. The kinetic process may be very different and it is absolutely unclear whether and which role the polyion morphology plays.

It will be not likely to solve quickly these and other problems, but due to its practical and scientific intellectual significance one can be sure that the research of polyions will continue many more decades.

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