Optimization of the Hydrodynamic Bead Model for the Analysis of DNA Conformations in Solution

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The construction of detailed bead models with atomic resolution has been proposed to identify DNA and RNA conformations by hydrodynamic methods in solution. Comparison of model predictions with experimental values for the translational and rotational diffusion coefficients of a homologous series of double-helical B-DNA oligonucleotides has demonstrated the validity and consistency of this strategy when hydration of the molecules is properly accounted for. Further investigations of "hairpin" and quadruplex model oligonucleotides reveal the potential of this technique to differentiate between DNA conformations. This combination of model calculations with experimental hydrodynamic methods provides a powerful means to identify DNA or RNA conformations in solution, follow conformational changes under a wide variety of solvent conditions, or characterize intermolecular associations with other components of (bio)molecular importance.

Introduction

The complex biological function of DNA and RNA is closely related to the structural diversity of these molecules. Besides the classical double-helical A-, B-, and Z-DNA,[1] higher-ordered structures such as triple-helical and four-stranded conformations have been identified.[2,3] Moreover, branched nucleic acid molecules with three- and four-way helical junctions are of major importance in a number of DNA and RNA structural intermediates[4] as well as being used to construct 2D and 3D networks for various applications in the field of nanotechnology.[5] Another important topic in structural biology is to elucidate and characterize the interaction of DNA with proteins and small molecule ligands which have potential therapeutic effects, can act as sequence recognition agents, or modifiers, of protein-DNA properties.[6] For a comprehensive understanding of the structure−function relationship of DNA and RNA in natural and technical systems it is necessary to increase our knowledge about the conformational properties of these molecules with respect to inherent sequence effects and environmental parameters.

A wealth of conformational details have been elucidated by the high-resolution methods X-ray crystallography and NMR spectroscopy on short model oligonucleotides.[6,7] The existence of Watson−Crick base-pairing has been confirmed by X-ray diffraction,[8,9] and in the following years numerous crystal structures of specific DNA conformations and mismatches,[10] as well as complexes with small molecules and proteins, have been solved. Nevertheless, due to inherent limitations of these methods, it is desirable to have other techniques available that are able to cover the dilute regime and allow for the investigation of larger intermolecular interacting systems. Hydrodynamic methods (e.g., dynamic light scattering, ultracentrifugation) meet these requirements and can provide valuable information about conformational properties of (bio)molecules in solution. Because of their high sensitivity, modern techniques such as fluorescence correlation spectroscopy[11] are able to characterize the dynamics of single molecules in complex multicomponent systems.

Hydrodynamic methods have the great advantage that they are noninvasive, mostly rapid, and provide structural information directly from solution. In general, it is possible to determine the size and shape of molecules under almost any environmental conditions in dilute solution, a concentration regime which is not accessible by high-resolution techniques. Moreover, hydrodynamic measurements can be used to follow conformational changes as well as intermolecular interactions under the influence of external stimuli, e.g., temperature changes, ion composition, salt concentration, pH, etc. Especially with respect to intra- or intermolecular association processes, it is feasible to characterize DNA−DNA, DNA−RNA, or DNA−protein complexes, which are of major biological relevance.

The interpretation of experimental results depends substantially on accompanying model calculations. In general, no unique structural information is obtained from hydrodynamic experiments ad hoc, but model predictions have to be used to distinguish between alternative conformations. A review on classical hydrodynamic methods and model calculations to elucidate macromolecular structures in solution is given by Harding.[12] The most detailed representation of an anisotropic particle is obtained by the bead model, where the macromolecule is represented by a collection of spherical frictional centers.[13,14] Depending on the level of refinement, these elements may either represent individual atoms, specific molecular residues (e.g., nucleobases, sugars, amino acids, etc.), or arbitrary subunits.[15−18] Because hydration plays an important role for biological macromolecules,[19] water molecules have to be considered in the hydrodynamic calculations, either explicitly or indirectly by adjusting the radius of the frictional centers. For general purposes, Garcia de la Torre et al.[20,21] have developed a computer program that calculates transport coefficients and other static and dynamic properties of macromolecular assemblies.

On the basis of the crystal structure of B-DNA, we have built detailed hydrodynamic models of a homologous series of short DNA fragments. The calculated transport coefficients were

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compared with recent light scattering results to validate the model assumptions. We show that inclusion of water of hydration leads to very good agreement of the experimental data with model predictions. In a next step, this strategy has been applied to determine the size and shape of the two "hairpin" conformations and a series of quadruplex oligonucleotides in solution. Our results indicate that, in the future, this combination of experimental and theoretical hydrodynamic methods can provide valuable information about induced conformational changes and DNA−protein interactions under a wide variety of solution conditions which are generally not accessible to high-resolution techniques.

Hydrodynamic Theory

The hydrodynamic properties, namely rotational and translational diffusion coefficients for the oligonucleotides of different conformation, were calculated using the "bead" model, developed for small molecules[22,23] and rigid macromolecular assemblies.[13,14]

The concept behind this theoretical model assumes that the detailed molecular structure of any rigid molecule can be modeled by a collection of spherical subunits. These elements may either represent the individual atoms, specific molecular residues (e.g., the sugar moiety, the nucleobases, etc.), or arbitrary subunits of the molecule. Each of these spherical elements is considered as a source of local friction assigned with a frictional coefficient according to Stokes law. For stick boundary conditions, the frictional coefficient is given by

$$\zeta_i = 6\pi\eta_0 r_i \qquad \text{for translation} \tag{1}$$

and

$$\zeta_i = 8\pi\eta_0 r_i^3 \qquad \text{for rotation} \tag{2}$$

where η_0 is the solvent viscosity and r_i an effective radius of the corresponding ith spherical element.

The frictional force F_i experienced by the ith bead in the solvent is determined by the presence of all other molecular subunits. This hydrodynamic interaction can be described by the Oseen tensor

$$\mathbf{T}_{ij}^0 = (8\pi\eta_0 R_{ij})^{-1}\left(\mathbf{I} + \frac{\vec{R}_{ij}\vec{R}_{ij}}{R_{ij}^2}\right) \tag{3}$$

which was originally developed for continuum hydrodynamics[24,25] but has been later applied successfully on a molecular level.[26] R_{ij} is the distance between frictional elements i and j, $\mathbf{I}$ is the identity matrix, and η_0 corresponds to the viscosity of the medium. In this approach, it was assumed that the size of the frictional elements is much smaller than their distance, i.e., they are regarded as point sources of friction. Later, finite size effects of overlapping[27] and nonoverlapping beads[28,29] have been addressed. Considering translational−rotational coupling, the diffusion coefficients have been calculated from the generalized Einstein relationship[30,13]

$$\mathbf{D} = kT\mathbf{R}^{-1} \tag{4}$$

The generalized resistance tensor $\mathbf{R}$ is a 2×2 matrix comprised of four 3×3 matrixes

$$\mathbf{R} = \begin{bmatrix} \mathbf{R}_t & \mathbf{R}_{c,0}^T \\ \mathbf{R}_{c,0} & \mathbf{R}_{r,0} \end{bmatrix} \tag{5}$$

$\mathbf{R}_t$ and $\mathbf{R}_{r,0}$ are the rotational and translational friction tensor, respectively, and $\mathbf{R}_{c,0}$ and $\mathbf{R}_{c,0}^T$ describe the "rotational-translational" coupling.

Hydrodynamic Model Calculations. We have developed a computer program to calculate the rotational and translational diffusion coefficients of macromolecules of arbitrary size and shape. This program is based on the bead model procedure by Bloomfield and Garcia de la Torre.[13] The core of the program has been extended by some specific modules that enable a rapid generation of arbitrary DNA conformations from different sources. Details of the model calculations can be found in ref 31.

The general outline of the program comprises the following steps: (i) building of model structures as an assembly of spherical subunits; (ii) determination of the solvent accessible surface area according to the method of Lee and Richards[32] and recalculation of bead radii; (iii) calculation of the frictional coefficients for each subunit and setup of the $3N \times 3N$ super matrix using the proper hydrodynamic interaction tensor; (iv) determination of the frictional tensors $\mathbf{R}_t$, $\mathbf{R}_{r,0}$, $\mathbf{R}_{c,0}$, and $\mathbf{R}_{c,0}^T$; (v) inversion of the 6×6 resistance matrix $\mathbf{R}$ to obtain the diffusion tensors (eq 4); (vi) computation of the center of diffusion and recalculation of the diffusion tensors; (vii) determination of the diffusion coefficients and relaxation times according to eqs 6 and 7.

The translational diffusion coefficient is calculated from the diagonal elements of the diffusion tensor

$$D_t = \frac{1}{3}Tr(\mathbf{D}_t) \tag{6}$$

In a depolarized light scattering experiment, the rotational correlation time is determined by the reorientation of the main symmetry axis of the cylinder symmetric oligonucleotides

$$\tau_r = \frac{1}{6D_\perp} \tag{7}$$

where $D_\perp = D_r^{xx} = D_r^{yy}$.

The input file that provides bead models of arbitrary DNA conformations for the hydrodynamic calculations has been generated in three different ways: (1) from high-resolution structural information in the Brookhaven data bank; (2) using the program HYPERCHEM,[33] which allows the user to build arbitrary single-stranded, double-helical, and "hairpin" conformations; (3) our own computer program to generate four−stranded quadruplex molecules from planar tetrameric units.[34]

Because we have included explicitly a shell of hydration in our model calculations, each oligonucleotide was placed into a SPC/E water bath[35] using GROMOS87.[36] The minimum distance between water oxygen atoms and the DNA surface was chosen to be 2.3 Å. Sodium ions were distributed around the DNA molecule to neutralized the negative charges on the phosphate groups. The dimensions of the simulation box vary with the size of the model oligonucleotide, but the water shell extends for at least 10 Å. Each system was equilibrated using the GROMOS87 molecular mechanics force field.[36] Quadruplex structures were allowed to equilibrate together with the surrounding water molecules. The B-DNA and "hairpin" conformations from HYPERCHEM were position restrained using a harmonic potential.

Although hydrodynamic bead models have experienced significant refinements within recent years, they still lack a rigorous theoretical treatment. We have included the coupling of rotational and translational motion,[37,13] which is necessary

to obtain reliable rotational diffusion coefficients from the diffusion tensor. In this respect, it is important to choose the proper hydrodynamic interaction tensor. As pointed out in the theoretical section, they have been developed for point sources, identical overlapping beads, and nonoverlapping subunits of different size. In large macromolecular assemblies like our oligonucleotides, a number of frictional subunits have only very limited or no contact with the solvent, because they are surrounded by other beads of the macromolecule. Hence, it is expected that their hydrodynamic contribution depends on their position within the macromolecule. A number of studies on small molecules suggest to use an effective hydrodynamic radius for each spherical subunit that is dependent on the solvent accessible surface area of each individual bead in the molecule to the solvent. We have used the model by Lee and Richards[32] to determine the contact surface area (CSA), which is obtained by rolling a probe sphere over the surface of the macromolecule, where the finite probe size can be chosen in such a way to reflect the molecular dimensions of the solvent molecules (e.g., 1.4 Å for water). The effective hydrodynamic radius of the beads can be calculated according to

$$r_{\mathrm{eff}} = (\mathrm{CSA}/4\pi)^{1/2}$$

This radius is used to compute the frictional coefficient according to eqs 1 and 2. Thus, any overlap of neighboring subunits results in a decrease of the hydrodynamic radius, and completely buried spherical elements within the macromolecule have a zero hydrodynamic radius and therefore no frictional effect.

Results

The potential of our hydrodynamic model calculations has been verified for a homologous series of double-helical oligonucleotides by comparing the model predictions with experimental data. The translational and rotational diffusion coefficients of the short DNA fragments were recently determined by polarized and depolarized dynamic light scattering.[38,39] In a next step, this new approach was applied to model oligonucleotides of different conformation. We have increased the complexity of the DNA structures going from the classical double-helical B-DNA to single-stranded molecules in a "hairpin" conformation and to four-stranded tetraplex conformations.

A. Test of the Hydrodynamic Model Calculations for Double-Helical Model Conformations. We have calculated translational and rotational diffusion coefficients for a series of double-helical oligonucleotides in the B-DNA conformation (with a length of 8, 12, 16, and 20 nucleotides per strand). For a most detailed representation of the molecular structure of the DNA fragments, each atom was considered as a spherical source of friction. The coordinates were generated from energy equilibrated structures obtained from HYPERCHEM,[33] and the bead size was determined by the van der Waals radii of the corresponding atoms. To account for the effect that frictional centers within the interior of the macromolecule with limited exposure to the solvent contribute less to the hydrodynamic properties, we have used the CSA concept[32,40] to calculate an effective hydrodynamic radius. The bead size is now rescaled by the solvent accessible surface area of each atom. Although this model is lacking a theoretical justification, it has been successfully applied to small molecules (J. Töhl, W. Eimer, unpublished results),[22,41] proteins,[17] and t-RNA.[18] To calculate bead friction coefficients, we have used stick boundary conditions in Stokes' law (see eqs 1 and 2).

TABLE 1: Comparison of Hydrodynamic Model Calculations Using the Original and Modified Oseen Tensor for a Homologous Series of Unhydrated B-DNA and Two "Hairpin" Oligonucleotides

model DNA	original Oseen tensor	modified Oseen tensor	exptl data
	D_t^{20} [10^{-6} cm^2/s]		
13 mer hairpin	2.12	2.12	
17 mer hairpin	1.83	1.84	1.46
8 mer duplex	1.96	1.96	1.53
12 mer duplex	1.60	1.61	1.34
16 mer duplex	1.38	1.38	1.14
20 mer duplex	1.22	1.22	1.09
	τ_r^{20} [ns]		
13 mer hairpin	1.40	1.40	1.90
17 mer hairpin	2.31	2.30	4.20
8 mer duplex	1.79	1.78	3.22
12 mer duplex	3.77	3.76	6.39
16 mer duplex	6.80	6.79	11.7
20 mer duplex	11.3	11.2	16.2

First of all, we have tested the influence of the hydrodynamic interactions tensors on the final transport properties. In the original Oseen tensor, the individual subunits are considered as point sources of frictional resistance. Nevertheless, we found that a modification of the bead radii using the CSA concept has an influence on the calculated diffusion coefficients for various double-helical B-DNA conformations. Similar observations have been made by Hellweg et al.[42] who have determined the translational diffusion coefficient for the MoFe protein from *Acetobacter vinelandii* as well as for the rotational correlation time of small organic molecules by Mikosch[41] and Töhl (unpublished results).

Calculated translational diffusion coefficients and rotational correlation times for a homologous series of unhydrated double-helical B-DNA oligonucleotides using the original and the modified Oseen tensor, respectively, are compared in Table 1. Using effective bead radii according to the CSA concept, both hydrodynamic interaction tensors provide the same hydrodynamic transport coefficients. Doing the calculations with the original van der Waals radii, the translational diffusion coefficients for the oligonucleotides vary by about 6% while the rotational correlation times differ by as much as 11% (data not shown). By applying the solvent accessible surface area concept, the effective bead dimensions are reduced significantly and an overlap of spheres becomes unlikely. Hence, the difference in the original and modified Oseen hydrodynamic interaction tensors becomes negligible. On the basis of this result, we have used exclusively the original Oseen tensor in our further hydrodynamic model calculations, because only this tensor is valid for rotational diffusion.[43]

A comparison of the theoretical predictions with experimental data from polarized and depolarized dynamic light scattering on short oligonucleotides[38,39] is presented in Table 1. It becomes clearly evident that the hydrodynamic model calculations overestimate the measured translational and rotational diffusion coefficients well beyond the experimental error. As demonstrated below, this effect is attributed to a neglect of the hydration shell that has a significant influence on the translational and rotational motion of the oligonucleotides in aqueous solution.

Combination of Molecular Groups to Hydrodynamic Subunits. Our primary intention is to generate molecular structures with the highest resolution possible (atomic resolution, at the best) for the hydrodynamic model calculations. Nevertheless, in some cases, e.g., in order to calculate the transport coefficients for large molecular assemblies such as DNA−protein complexes,

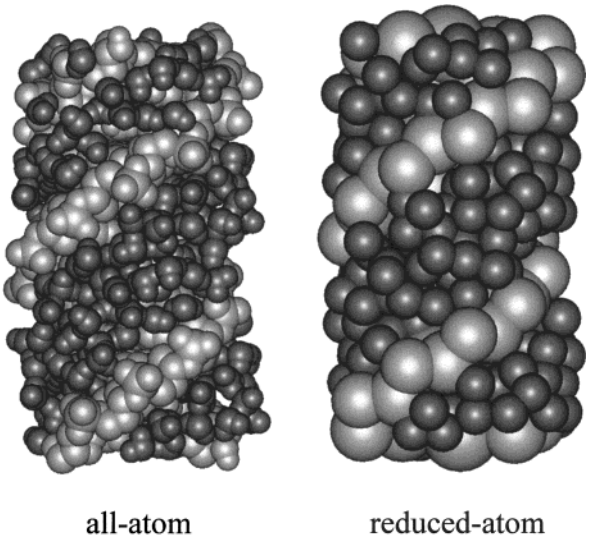

Figure 1. All-atom and reduced-atom hydrodynamic model for the 12 mer d[CG]$_6$ in a double-helical B-DNA conformation.

it will be desirable to reduce the number of frictional subunits in the bead model calculations without losing accuracy in the model predictions of the transport coefficients.

For this purpose, we have combined groups of atoms into new, now larger frictional elements and recalculated the transport coefficients of the model oligonucleotides. For the individual nucleotides within the DNA molecules, we have chosen the ribose moiety, the phosphate group, and the purine and pyrimidine bases, respectively. The location of the new frictional centers is determined by the center of gravity of the molecular subunit, and the bead radius was calculated from the total volume of the individual atoms of each residue. In the same way, we have assigned the water molecule a single bead. The radius of this sphere was calculated from the van der Waals radii of the oxygen and hydrogen atoms to 1.91 Å. With this procedure, in the reduced model the number of frictional elements could be significantly diminished (e.g., from 754 to 70 beads for the unhydrated dodecamer and from 1516 to 321 subunits for a hydrated structure). For comparison, the all-atom and reduced hydrodynamic model structures for the 12 mer duplex oligonucleotide are displayed in Figure 1. It becomes clearly evident that the molecular shape of the DNA fragment is well represented by the reduced model.

To test the sensitivity of the hydrodynamic model calculations with respect to these modifications, we have determined transport coefficients for unhydrated and hydrated DNA structures for both the all-atom and the reduced model. The surrounding water molecules were generated by placing the oligonucleotides in a SPC/E water box using GROMOS87.[36] Equilibrated structures were obtained by energy minimization (see Experimental section). As will be justified (see shell model below), for the hydration shell we have considered all water molecules where the oxygen atoms had a distance of less than 4 Å from the DNA molecule. This value is considered to represent the first hydration shell.[19] The results from the hydrodynamic calculations for the double-helical and hairpin molecules are given in Table 1S of the Supporting Information.

The translational and rotational diffusion coefficient for both models are in excellent agreement, indicating that the substantial reduction in the number of hydrodynamic subunits has no significant influence on the accuracy in the model predictions. Consideration of water molecules in the calculations strengthens this observation. Our results are consistent with previous investigations on proteins, which reveal that the position of the frictional centers is more important than their size as long as the surface structure of the diffusing molecules is preserved.[17] This strategy provides a promising way to perform hydrodynamic model calculations on large DNA or RNA molecules and their associates such as DNA−DNA or DNA−protein complexes.

Consideration of a Shell of Hydration for the Transport Properties. It is known from hydrodynamic experiments that hydration plays an important role for the dynamics of biopolymers.[19,44,45] To mimic the amount of water that is bound to DNA for the time period characteristic for their rotational and translational motion, we have constructed three different hydration models: the cylinder, the shell, and the energy model. These three models differ in the way water molecules are selected as bound water, which is included in the hydrodynamic calculations. The difference in the hydration pattern is displayed in Figure 2. In the following, we will discuss these models separately and compare the results with experimental data.

The Cylinder Model. We start from an oligonucleotide of defined length in the classical B-DNA conformation,[33] which is first energy minimized in a SPC/E waterbox as discussed above. The bead models for the hydrodynamic calculations are generated by selecting only those water molecules from the box that are located within a certain distance from the main axis of the DNA conformers. The size of the hydration shell is determined by the cutoff radius, r_{cut}, defining the dimension of a cylinder whose symmetry axes coincide with the helical axis of the oligonucleotides. Obviously, the water molecules start to fill the grooves of the DNA first, and with increasing r_{cut} they cover the surface of the macromolecule. To account for water that is bound to the ends of the molecules, the length of the cylinder increases with the cutoff radius. This strictly geometric model creates a cylindrically symmetric distribution of water molecules around the oligonucleotides (see Figure 2a).

The change in the computed rotational and translational diffusion coefficients for the double-helical model oligonucleotides of different length with increasing thickness of the hydration layer is shown in Figure 3. For low values of r_{cut}, no water molecules are considered (the space is solely filled by the atoms of the oligonucleotide), and hence we obtained a constant value for the transport properties. Beyond a distance of about 8 Å from the helix axis, the first water molecules are included in the hydrodynamic calculations leading to a continuous decrease of the diffusion coefficients with increasing thickness of the hydration shell. The number of water molecules considered in the model calculations and the change in the transport coefficients with increasing cutoff radius is presented for the double-helical dodecamer in Table 2S of the Supporting Information.

To compare the model predictions with experimental data, we have included in Figure 3 the rotational and translational diffusion coefficients as obtained by polarized and depolarized dynamic light scattering.[38,39,60] The experimental values for the transport coefficients of all double helical conformers indicate that a significant amount of water sticks to the surface of the DNA molecules, at least on a time scale characteristic for the diffusional motions. It is important to emphasize that all data for the homologous series of double-helical B-DNA give a consistent picture, suggesting that a hydration shell extending to (11.5 ± 1.0) Å from the main axis of the DNA conformers has to be included in the hydrodynamic calculations to represent the experimental diffusion coefficients. Representative hydro-

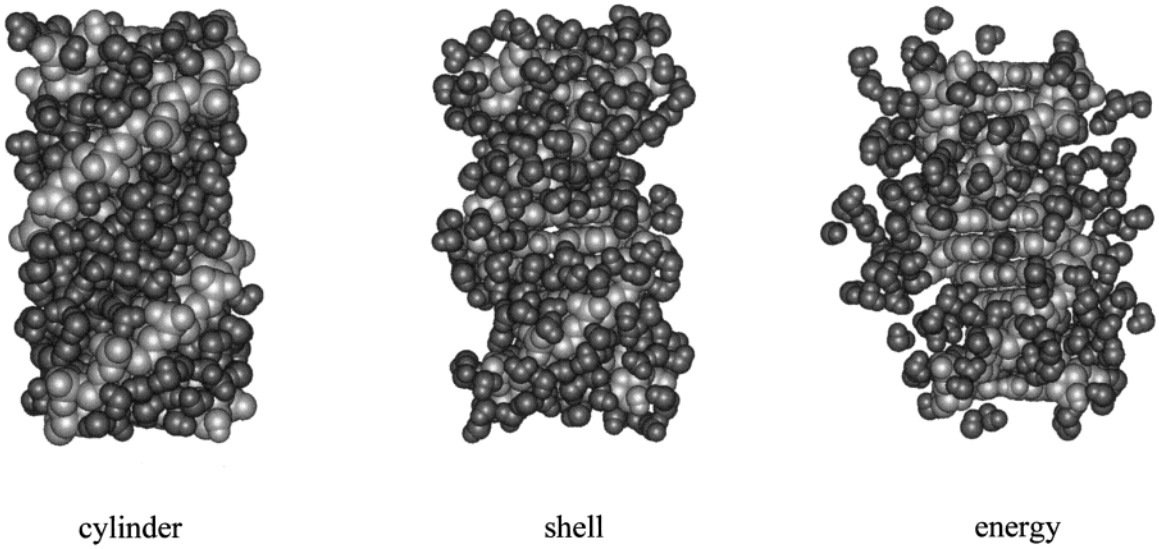

Figure 2. Bead model for the hydrated 12 mer double-helical oligonucleotide. The hydration layer is built according to the (a) cylinder model, (b) shell model, (c) energy model, considering the same number of water molecules.

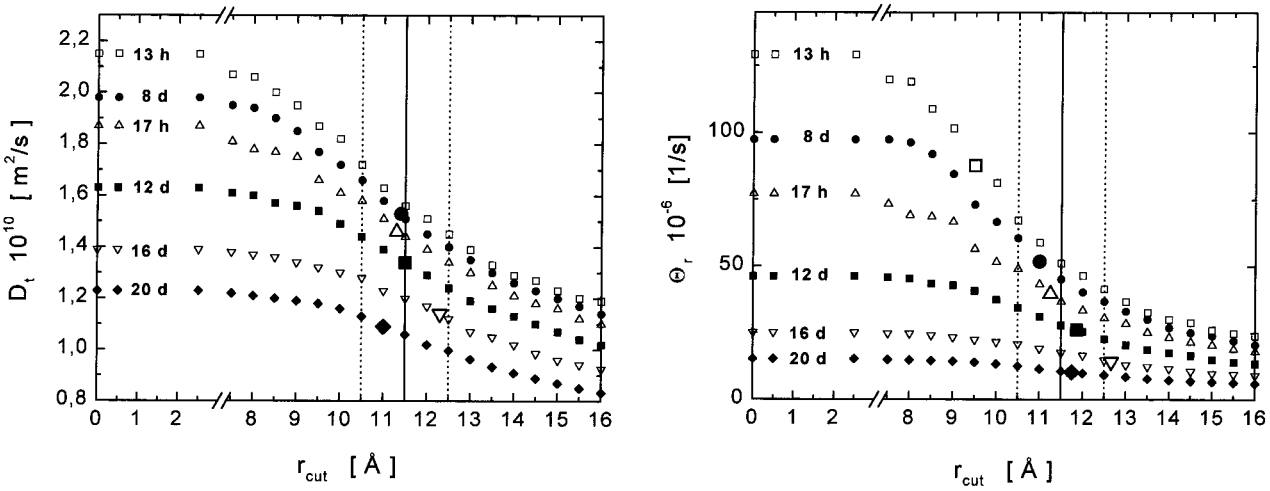

Figure 3. Hydrodynamic model calculations of the translational (a) and rotational (b) diffusion coefficient for double-helical [d] and hairpin [h] conformations. The numbers indicate the length of the model compounds in nucleotides per strand. Water of hydration is included according to the cylinder model. The larger symbols represent experimental data from polarized and depolarized dynamic light scattering.[38,39,60]

dynamic model structures with different amounts of bound water are displayed in Figure 4.

The Shell Model. What we call the shell model is an alternative geometrical way to select a hydration layer to be considered in the hydrodynamic calculations. In this model, we pick all water molecules whose oxygen atoms are located within a certain distance, *d*, from the DNA surface. An example is presented in Figure 2b where we have chosen all water molecules positioned less than 3.6 Å (206 H_2O molecules) apart from the dodecamer. This distance is considered to comprise the first hydration shell.[19] It becomes clearly evident that the shell model provides a less smooth surface for the hydrated oligonucleotides. The change in the diffusion coefficients with increasing hydration layer thickness is displayed in Figure 5. In our model, we assume a minimum distance of 2.3 Å between the oxygen atoms of the water molecules and the nearest DNA atoms. Therefore, the calculated diffusion coefficients do not change for $d < 2$ Å, while beyond this distance they gradually decrease. The experimental data from the homologous series of the double-helical DNA fragments are consistent with a hydration shell extending for (3.6 ± 0.8) Å beyond the oligonucleotide surface. This value comes very close to the thickness of the first hydration layer.

The Energy Model. Alternatively to geometric considerations, we have selected the hydration layer based on energy criteria, i.e., the water−DNA interaction energy. After energy minimization of the hydrated DNA structures, water molecules below a certain energy threshold value are considered in the hydrodynamic calculations. The threshold values for the nonbonding average water−DNA interaction energy was varied from −15 kcal/mol and −1 kcal/mol, corresponding to an average of less than one water molecule up to about 30 water molecules per nucleobase. A typical hydration pattern is shown in Figure 2c. The energy criterium provides a rather diffuse orientation of the water molecules around the DNA helix which are preferentially located in the vicinity of the phosphate backbone, probably due to a strong electrostatic contribution to the total interaction energy.

For the different DNA conformers we observed a more gradual decrease of the transport properties compared to the geometric hydration models discussed before. This effect is related to the rather widespread distribution of "bound" water molecules around the DNA. A comparison of the theoretical predictions of this model with experimental results leads to a very different degree of hydration for the model oligonucleotides (data not shown). Moreover, data for translational and rotational

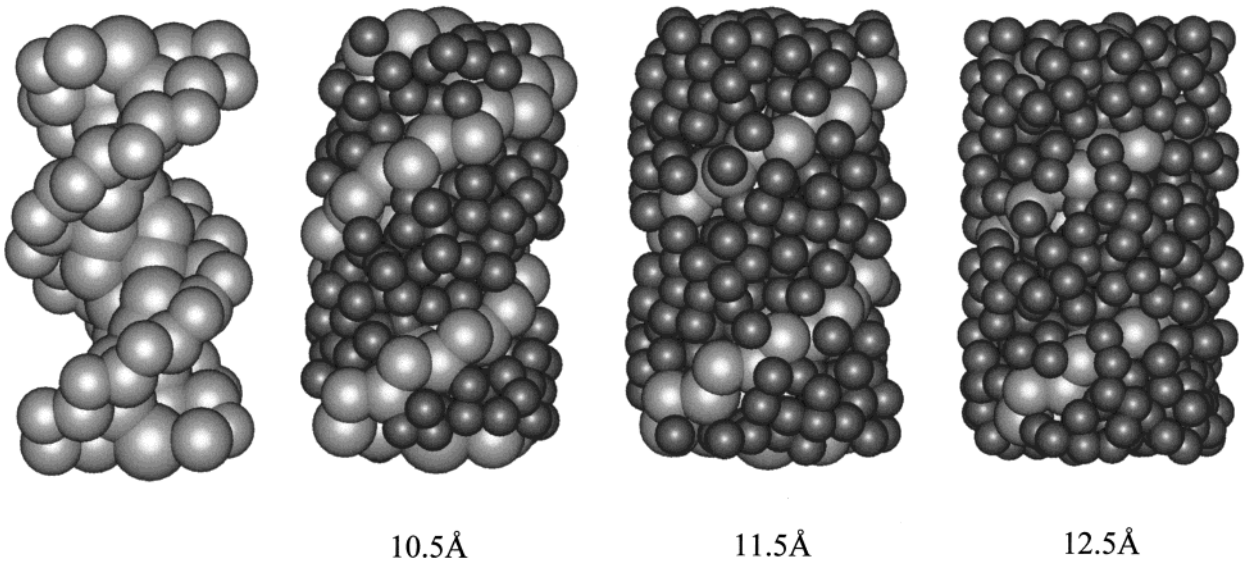

Figure 4. Bead model for the hydrated 12 mer double-helical oligonucleotide with increasing amount of water molecules in the hydration shell according to the cylinder model.

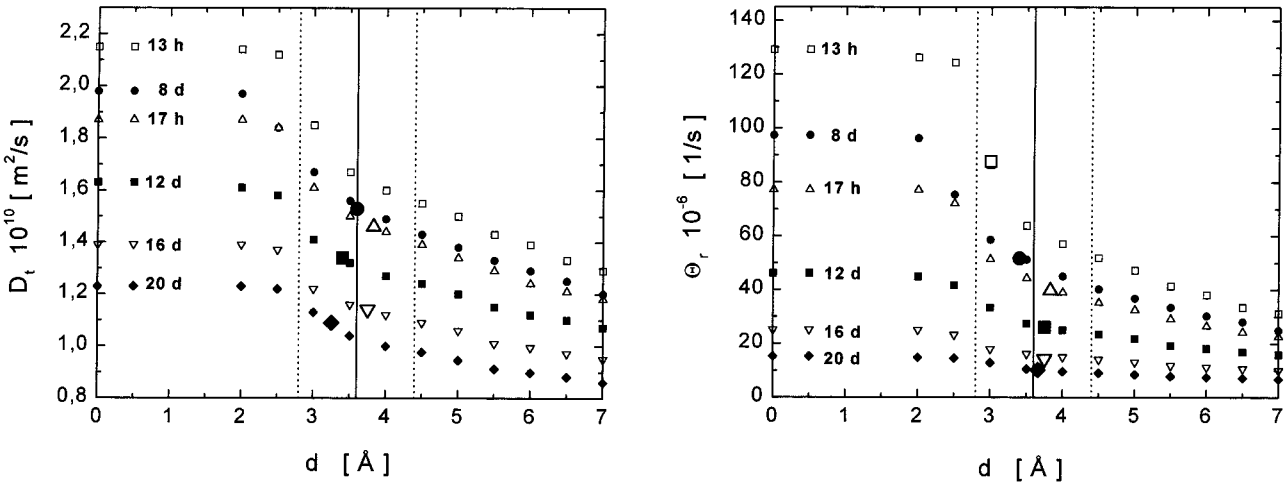

Figure 5. Hydrodynamic model calculations of the translational (a) and rotational (b) diffusion coefficient for double-helical and hairpin conformations. The numbers indicate the length of the model compounds in nucleotides per strand. Water of hydration is included according to the shell model. The larger symbols represent experimental data from polarized and depolarized dynamic light scattering.[38,39,60]

diffusion coefficients do not give a concise picture. While the translational diffusion coefficients from polarized dynamic light scattering correspond to an average number of 2−4 water molecules per nucleotide, the rotational diffusion coefficients suggests a significantly higher degree of hydration. This inconsistency clearly indicates that the energy model in its present form is not appropriate to provide reliable predictions for the transport properties of the model compounds.

Comparison of the Hydrodynamic Models. First of all, our investigation clearly illustrates that water of hydration has a significant influence on the rotational and translational motion of double-helical DNA conformers in solution. Applying the cylinder or the shell model, we obtained a concise picture for the degree of hydration for the different model compounds, when comparing the experimental diffusion coefficients with hydrodynamic model predictions. The amount of water sticking to the surface of the oligonucleotides to match the experimental data however depends on the hydrodynamic model.

A comparison of the three model approaches is presented in Figure 1S of the Supporting Information, exemplary for the 20 mer double-helical DNA conformation. For a constant degree of hydration, the energy model predicts a much lower diffusion coefficient than both geometric models. This effect is due to the very diffuse orientation of the hydration layer around the DNA molecule. The cylinder and the shell model provide very similar transport coefficients at low and high coverage with water. In the intermediate regime, the cylinder model predicts larger diffusion coefficients because it returns a more symmetric and dense water layer (see Figure 2).

From the cylinder model, the average degree of hydration for all oligonucleotides is estimated to 12.8 water molecules per nucleobase. This value is in good agreement with various experiments, indicating that the first hydration shell contains around 10 water molecules per nucleotide.[1,19,46] Corresponding results from recent computer simulations[19,47] come to the same conclusion. This number of water molecules for the first hydration layer agrees with the critical level of hydration necessary for the stabilization of DNA.[47] Moreover, the distribution of water molecules in the cylinder model coincides with the idea of a spine of hydration, located preferentially in the grooves of double-helical B-DNA.[1,7] With respect to hydrodynamic model calculations, the cylinder model has the disadvantage that it is applicable only to cylinder symmetric molecules but cannot be used for molecules of anisotropic shape.

The shell model calculations are consistent with an average of 8.5 water molecules per nucleotide. This number is still in good agreement with the values described in the literature. As displayed in Figure 2b, the spine of hydration is not as well recovered as in the cylinder model. However, the shell model can be applied to oligonucleotides and any other molecules of arbitrary shape, and it can provide valuable information about changes in the hydration pattern, for example upon binding of drug molecules to the DNA.

For the energy model, the consistency between hydrodynamic predictions for rotational and translational diffusion coefficients and the experimental data is not quite satisfactory. Furthermore, we observed a wide spread in the amount of hydration for the double-helical DNA conformers of different length. In its present form, the energy model does not provide good predictions for the transport properties of oligonucleotides in solution. However, the selection of water molecules according to their interaction energy with the DNA depends strongly on the quality of the force field. Molecular dynamics simulations probably give better equilibrated structures and hence might improve the selection criteria for the hydration layer.

In summary, a comparison of experimental results from dynamic light scattering with hydrodynamic model calculations provides clear evidence for the influence of solvation on the diffusional motion. At this stage, the geometric models are superior for hydrodynamic calculations, because they provide consistent predictions for the transport properties of the model B-DNA oligonucleotides in aqueous solution. Which model to choose depends on the symmetry of the molecular system.

The potential of the refined hydrodynamic model calculations will be demonstrated in the following.

B. Application of the Hydrodynamic Model Calculations. *Hydrodynamic Dimensions of "Hairpin" Conformations in Solution.* Oligonucleotides in a "hairpin" conformation comprise of self-complementary sequences at both ends of the molecule which form a double-helical stem region by refolding. A short noncomplementary sequence in the center of the molecules provides the interconnecting loop region. These conformers are quite common features of secondary RNA structures.[48] For model building of the 17 mer d[CGCGCGTTGTTCGCGCG] "hairpin" molecule, we started from a double-helical hexamer $d[CGCGCG]_2$ in the B-DNA conformation. For the 17 mer, the connecting loop region consists of five nucleotides which were added as a single-stranded pentamer d[TTGTT] to one of the 3'-ends of the double-helix. A loop was formed by connecting the 5'-end of the extended strand to the free 3'-end of the complementary double-helical strand. Geometry optimization of the "hairpin" conformation was achieved by alternating energy minimization and short MD simulation runs (quenched dynamics procedure). Removal of the two complementary base pairs from the stem region gives the 13 mer d[CGCGTTGTTCGCG].

The calculated transport coefficients for the "hairpin" oligonucleotides are displayed in Figures 3 and 5 for the cylinder and shell hydration model, respectively. First of all, a comparison with model calculations for double-helical structures of similar strand length (12 mer and 16 mer, respectively) predicts that "hairpin" and B-DNA conformations are easily distinguishable by their transport coefficients. This conclusion is impressively validated by the experimental dynamic light scattering data.

Furthermore, the experimental results for the 17 mer indicate that the hydration shell to be considered in the hydrodynamic model calculations is of the same thickness as for our model B-DNA fragments. This observation is not that surprising since the stem region of the "hairpin" molecule exists in a double-helical B-DNA conformation. Nevertheless, it is worth noticing that for the loop region the same hydration pattern as for the stem gives good agreement between experimental data and model calculations. This conclusion holds for both the cylinder and the shell model, respectively.

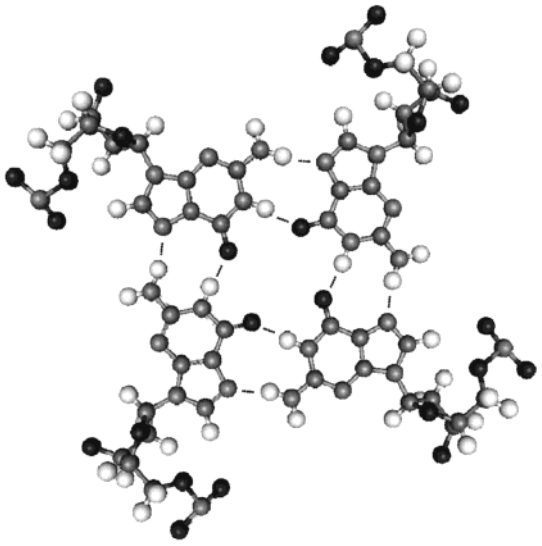

Figure 6. Molecular representation of a G-tetramer as the basic building block for quadruplex G-DNA.

Within the cylinder model, the rotational diffusion coefficient for the 13 mer "hairpin" oligonucleotide is significantly smaller than predicted from the hydrodynamic model calculations assuming the same extent of hydration as for the longer double-helical and "hairpin" DNA fragments (see Figure 3b). In our model building, we consider water of hydration at both ends of the oligonucleotides by increasing the length of the cylinder in the same way as the cutoff radius. For short DNA, molecules like the 13 mer water at both ends make up a large portion of the entire hydration shell, probably overrating the influence of these water molecules on the diffusional motion. Correspondingly, for the shell model which provides a more detailed hydration layer in the loop region this end effect is less pronounced, as seen in Figure 5b.

Size and Shape of Quadruplex G-DNA in Solution. Oligonucleotides that contain short (2−4 nucleotides) guanine-rich regions can organize into four-stranded helical structures in solution.[3,49,50] The basic structural motif of these quadruplex conformations is a square-planar arrangement of four guanine bases as shown in Figure 6. This tetrameric unit is stabilized by four hydrogen bonds between the guanine nucleobases. Vertical stacking of these tetraplexes in the presence of monovalent cations, with a high specificity for potassium, results in the formation of four-stranded helices that have been observed for 5'-guanosine monophosphate[51,52] and various model oligonucleotides.[3,53]

We have generated a homologous series of quadruplex structures from planar tetrameric units that consist of either guanine or thymine nucleotides. The molecular geometry of these basic building blocks was obtained from high-resolution X-ray crystallography data[54,55] and NMR spectroscopy.[56] Details of the model generation are described in ref 34. The sequence of the 8 mer quadruplex $d[TTGGGGTT]_4$ is found in telomeric ends of Tetrahymena.[57] The longer model compounds were built by successive addition of G_4- and T_2-units, respectively. Hydrated structures have been obtained in the same way as

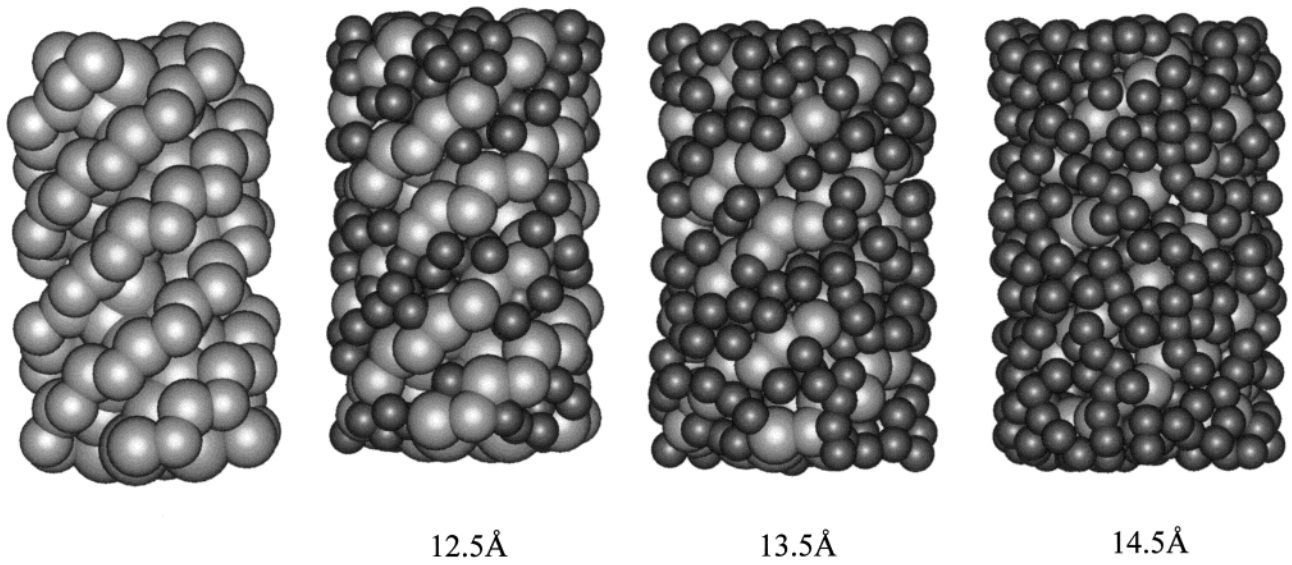

Figure 7. Bead model for the hydrated 12 mer d[TTGGGGTTGGGG]$_4$ quadruplex structure with an increasing amount of water molecules in the hydration shell according to the cylinder model.

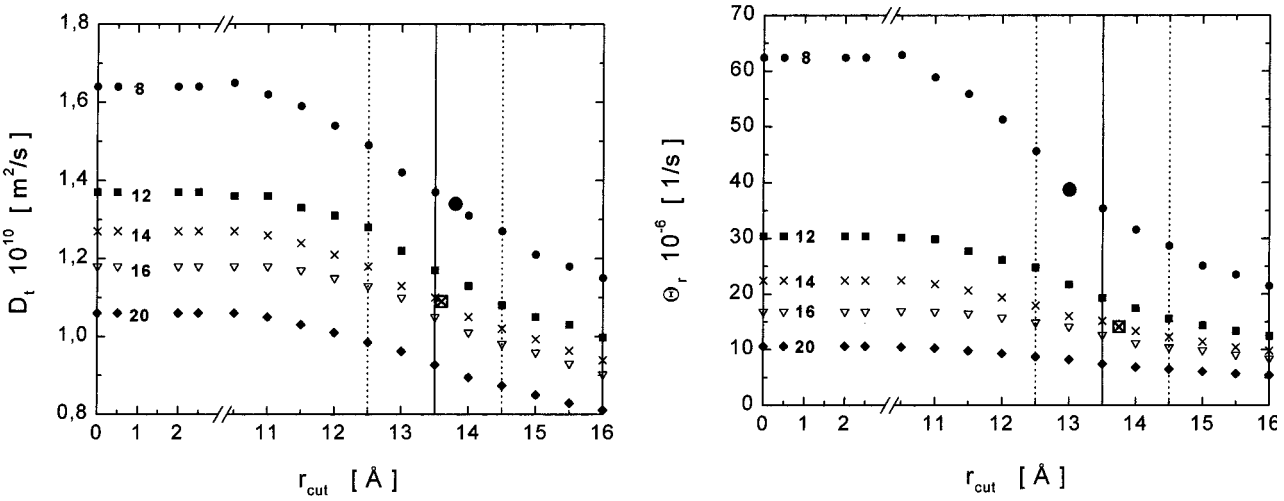

Figure 8. Comparison of calculated and experimental transport coefficients for a homologous series of model quadruplex oligonucleotides of different length. The numbers indicate the length of the model compounds in nucleotides per strand. Water of hydration is included according to the cylinder model. The larger symbols represent experimental data from polarized and depolarized dynamic light scattering.[31,59]

described for the double-helical and "hairpin" model systems. Examples for the 12 mer quadruplex d[TTGGGGTTGGGG]$_4$ with different hydration layers are presented in Figure 7.

The calculated rotational and translational diffusion coefficients for the quadruplex model structures with a length from 8 to 20 nucleotides per strand are displayed in Figure 8. The hydration layers were build according to the cylinder model. Experimental data have been included for d[$T_2G_4T_2$] and d[$(T_2G_4)_2T_2$], oligonucleotides that are known to exist in a quadruplex conformation in aqueous solution in the presence of NaCl.[31] A comparison of the experimental transport properties with model predictions suggests that the hydrodynamic radius of the quadruplex molecules amounts to (13.5 ± 0.8) Å, a value that is 2 Å larger than found for double-helical DNA (see above). This difference accounts precisely for the difference in the molecular dimensions of unhydrated double-helical B-DNA[1] and the quadruplex conformation.[58] That means, the thickness of the hydration layer that has to be considered in the hydrodynamic model calculation is identical for both DNA conformations and corresponds to a single shell of water molecules. However, the average amount of water molecules per nucleotide is reduced significantly from 12.8 to 7 in the case of the four-stranded helical G-DNA. This effect is attributed to much shallower grooves found in quadruplex conformations which accommodate less water than the deep grooves of B-DNA.

We have performed complementary hydrodynamic model calculations for the quadruplex oligonucleotides using the shell model. The results are displayed in Figure 2S of the Supporting Information. The experimental data are consistent with hydrodynamic model predictions considering a hydration layer thickness of (3.6 ± 0.5) Å. This finding agrees very well with the results for the B-DNA model compounds presented above. As consistently extracted from the cylinder model, the experimental results suggest that a first hydration shell has to be included to describe the diffusional motion of oligonucleotides in double-helical B-DNA or the quadruplex conformation. The number of water molecules included in the shell model calculations however diminishes from 8.5 per nucleotide (for the double-helical conformation) to 5.2 per nucleotide (for the quadruplex structures) for the same layer thickness. From these results we may conclude that, due to a smaller groove depth, less water is necessary to stabilize quadruplex G-DNA in aqueous solution. To our knowledge, an experimental verification of this hypothesis is still pending.

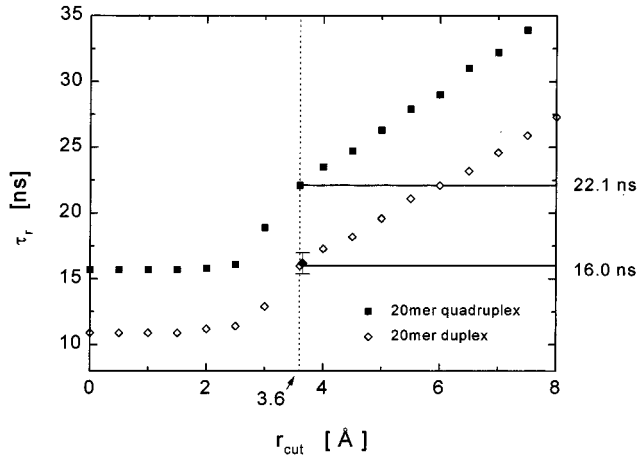

Figure 9. Comparison of calculated rotational diffusion coefficients for a double helical and quadruplex oligonucleotide with a length of 20 nucleotides per strand. A typical error from a depolarized dynamic light scattering experiment is included.

Finally, we demonstrate that double-helical and quadruplex conformations can be easily distinguished by experimental methods in combination with hydrodynamic model calculations. The predicted rotational relaxation times for a duplex and quadruplex structure with a length of the 20 nucleotides per strand are displayed in Figure 9. These transport properties can be measured by polarized and depolarized dynamic light scattering, respectively.[59] The theoretical values for a given degree of hydration differ significantly. As it becomes evident from Figure 9, the differences in the rotational relaxation times for oligonucleotides of the same length but different conformation are well outside the experimental error, characteristic for a depolarized dynamic light scattering experiment.

Conclusion

Diffusional motion is very sensitive to the size and shape and hence to the conformation of oligonucleotides in solution. In the present work, we have constructed a detailed bead model of DNA to calculate the transport coefficients of short DNA fragments of different conformation. The validity and quality of the hydrodynamic calculations have been demonstrated by comparing model predictions with experimental data for the translational and rotational diffusion coefficients of a series of short double-helical B-DNA. In this context, the importance of hydration for the molecular dynamics in aqueous solution has been clearly elucidated.

In the next step, we have successfully applied this approach to a series of model oligonucleotides in "hairpin" and quadruplex conformations to characterize their diffusional behavior in aqueous solution. It was demonstrated that the different DNA structures can be easily identified from their transport properties. The strategy proposed for the model building of the DNA and RNA conformations is equivalently applicable to other macromolecules such as proteins or intermolecular assemblies of smaller molecules such as micellar systems.

The combination of bead model calculations together with experimental methods provides a powerful means to obtain detailed information about structures and dynamics of macromolecular systems directly in solution. The experiments can be performed under a wide variety of environmental conditions, and a characterization of conformational transitions induced by changes in the ionic strength, or through the influence of external stimuli, is easily accomplished. Moreover, intermolecular associations such as DNA−DNA or DNA−protein complexes can be investigated. Therefore, hydrodynamics provide valuable complementary information to the high-resolution techniques of X-ray crystallography and NMR spectroscopy in the complex (bio)molecular world.

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Supporting Information Available: Figure 1S showing diffusion coefficients for the 20 mer duplex conformation and Figure 2S showing transport coefficients for a series of model quadruplex oligonucleotides. Table 1S giving a comparison of hydrodynamic model calculations for the atomistic all-atom and the reduced-atom model DNA conformations and Table 2S giving details of the hydrodynamic model calculations for the double-helical dodecamer. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Saenger, W. In *Principles of nucleic acid structure in Springer advanced texts in Chemistry*; Cantor, C. R., Ed.; Springer-Verlag: New York, 1983.
(2) Frank-Kamenetskii, M. D.; Mirkin, S. M. *Annu. Rev. Biochem.* **1995**, *64*, 65.
(3) Williamson, J. R. *Annu. Rev. Biophys. Biomol. Struct.* **1994**, *23*, 703.
(4) Duckett, D. R.; Murchie, A. I. H.; Clegg, R. M.; Bassi, G. S.; Giraud-Panis, M.-J. E.; Lilley, D. M. J. *Biophys. Chem.* **1997**, *68*, 53.
(5) Seeman, N. C. *Annu. Rev. Biophys. Biomol. Struct.* **1997**, *26*, 225.
(6) Neidle, S. *Biopolymers* **1997**, *44*, 105.
(7) Beerman, H. M. *Biopolymers* **1997**, *44*, 23.
(8) Rosenberg, J. M.; Seeman, N. C.; Kim, J. J. P.; Suddath, F. L.; Nicholas, H. B.; Rich, A. *Nature* **1973**, *243*, 150.
(9) Drew, H. R.; Wing, R. M.; Takano, T.; Broka, C.; Tanaka, S.; Itakura, K.; Dickerson, R. E. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 2179.
(10) Brown, T.; Hunter, W. N. *Biopolymers* **1997**, *44*, 91.
(11) Schrof, W.; Klinger, J.; Heckmann, W. Horn, D. *Colloid. Polym. Sci.* **1998**, *276*, 577.
(12) Harding, S. E. *Biophys. Chem.* **1995**, *55*, 69.
(13) Garcia de la Torre, J.; Bloomfield, V. A. *Q. Rev. Biophys.* **1981**, *14*, 81.
(14) Garcia de la Torre, J. **1989**, 3.
(15) Byron, O. *Biophys. J.* **1997**, *72*, 408.
(16) Tirado Garcia, M. M.; Jimenez Rios, M. A.; Garcia Bernal, J. M. *Int. J. Biol. Macromol.* **1990**, *12*, 19.
(17) Venable, R. M.; Pastor, R. W. *Biopolymers* **1988**, *27*, 1001.
(18) Antosiewicz, J.; Pörschke, D. *J. Phys. Chem.* **1989**, *93*, 5301.
(19) Westhof, E. In *Water and Biological Macromolecules*; Westhof, E., Ed.; The Macmillan Press, Ltd.: London, 1993.
(20) Garcia de la Torre, J.; Navarro, S.; lopez Martinez, M. C.; Diaz, F. G.; Lopez Cascales, J. J. *Eur. Biophys. J.* **1994**, *67*, 530.
(21) Garcia de la Torre, J.; Harding, S. E.; Carrasco, B. *Eur. Biophys. J.* **1999**, *28*, 119.
(22) Pastor, R. W.; Karplus, M. *J. Phys. Chem.* **1988**, *92*, 2636.
(23) Pastor, R. W.; Zwanzig, R. *J. Chem. Phys.* **1989**, *90*, 5729.
(24) Oseen, C. W. In *Hydrodynamik*; Akademische Verlagsgesellschaft: Leipzig, 1927.
(25) Burgers, J. M. In *Second report on viscosity and plasticity*; Nodemann Publishing Co.: Amsterdam, 1983.
(26) Yamakawa, H. In *Modern theory of polymer solutions*; Harper & Row: New York, 1971.
(27) Rotne, J.; Prager, S. *J. Chem. Phys.* **1969**, *50*, 4831.
(28) Yamakawa, H. *J. Chem. Phys.* **1970**, *53*, 436.
(29) Garcia de la Torre, J.; Bloomfield, V. A. *Biopolymers* **1977**, *16*, 1747.
(30) Brenner, H. *J. Colloid Interface Sci.* **1967**, *23*, 407.
(31) Niermann, M. Ph.D. Thesis, Universität Bielefeld, 1996.
(32) Lee, B.; Richards, F. M. *J. Mol. Biol.* **1971**, *55*, 379.
(33) van Gunsteren, W. F.; Berendsen, H. J. C. In *Molecular simulation (GROMOS) library manual*; University of Groningen: Groningen, 1986.
(34) Töhl. J. Ph.D. Thesis, Universität Bielefeld, 1996.
(35) Berendsen, H. J. C.; Grigera, J. R.; Straatsma, T. P. *J. Phys. Chem.* **1987**, *91*, 6269.
(36) van Gunsteren, W. F.; Berendsen, H. J. C. In *Groningen molecular simulation (GROMOS) library manual*; University of Groningen: Groningen, 1986.

(37) Garcia Bernal, J. M.; Garcia de la Torre, J. *Biopolymers* **1980**, *19*, 751.
(38) Eimer, W.; Williamson, J. R.; Boxer, S. G.; Pecora, R. *Biochemistry* **1990**, *29*, 799.
(39) Eimer, W.; Pecora, R. *J. Chem. Phys.* **1991**, *94*, 2324.
(40) Richards, F. M. *Annu. Rev. Biophys, Bioeng.* **1977**, *6*, 151.
(41) Mikosch, W.; Dorfmüller, T.; Eimer, W. *J. Chem. Phys.* **1994**, *101*, 11044.
(42) Hellweg, T.; Eimer, W.; Krahn, E.; Schneider, K.; Müller, A. *Biochim. Biophys. Acta* **1998**, *1337*, 311.
(43) Yoshizaki, T.; Yamakawa, H. *J. Chem. Phys.* **1980**, *73*, 578.
(44) Saenger, W. *Annu. Rev. Biophys. Biophys. Chem.* **1987**, *16*, 91.
(45) Berman, H. R. *Curr. Opin. Struct. Biol.* **1991**, *1*, 423.
(46) Falk, M.; Hartman, K. A., Jr.; Lord, R. C. *J. Am. Chem. Soc.* **1962**, *84*, 3843.
(47) Subramanian, P. S.; Beveridge, D. L. *J. Biomol. Struct. Dyn.* **1989**, *6*, 1092.
(48) Brion, P.; Westhof, E. *Annu. Rev. Biophys. Biomol. Struct.* **1997**, *26*, 113.
(49) Williamson, J. R.; Raghuraman, M. K.; Czech, T. R. *Cell* **1989**, *59*, 841.
(50) Wang, Y.; Patel, D. J. *Biochemistry* **1992**, *31*, 8122.
(51) Eimer, W.; Dorfmüller, Th. *J. Phys. Chem.* **1992**, *96*, 6790.
(52) Pinnavaia, T. J.; Marshall, C. L.; Mettler, C. M.; Fisk, C. L.; Miles, H. T.; Becker, E. D. *J. Am. Chem. Soc.* **1978**, *100*, 3625.
(53) Sen, D.; Gilbert, W. *Nature* **1990**, *344*, 410.
(54) Zimmerman, S. B.; Cohen, G. H.; Davies, D. R. *J. Mol. Biol.* **1975**, *92*, 181.
(55) Laughlan, G.; Murchie, A. I. H.; Norman, D. G.; Moore, M. H.; Moody, P. C. E.; Lilley, D. M. J.; Luisi, B. *Science* **1994**, *265*, 520.
(56) Wang, Y.; Patel, D. J. *J. Mol. Biol.* **1993**, *234*, 1171.
(57) Wang, Y.; Patel, D. J. *Structure* **1994**, *2* (12), 1141.
(58) Fisk, C. L.; Becker, E. D.; Miles, H. T.; Pinnavaia, T. J. *J. Am. Chem. Soc.* **1982**, *104*, 3307.
(59) Bolten, M.; Niermann, M.; Eimer, W. *Biochemistry*, in press.
(60) Haber-Pohlmeier, S. Ph.D. Thesis, Universität Bielefeld, 1995.