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Structure Calculations for Single-Stranded DNA Complexed with the Single-Stranded DNA Binding Protein GP32 of Bacteriophage T4: A Remarkable DNA Structure†

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ABSTRACT: In this study it is established by calculation which regular conformations single-stranded DNA and RNA can adopt in the complex with the single-stranded DNA binding protein GP32 of bacteriophage T4. In order to do so, information from previous experiments about base orientations and the length and diameter of the complexes is used together with knowledge about bond lengths and valence angles between chemical bonds. It turns out that there is only a limited set of similar conformations which are in agreement with experimental data. The arrangement of neighboring bases is such that there is ample space for aromatic residues of the protein to partly intercalate between the bases, which is in agreement with a previously proposed model for the binding domain of the protein [Prigodich, R. V., Shamoo, Y., Williams, K. R., Chase, J. W., Konigsberg, W. H., & Coleman, J. E. (1986) *Biochemistry 25*, 3666–3671]. Both C2′endo and C3′endo sugar conformations lead to calculated DNA conformations that are consistent with experimental data. The orientation of the O2′ atoms of the sugars in RNA can explain why the binding affinity of GP32 for polyribonucleotides is lower than for polydeoxyribonucleotides.

The gene 32 protein (GP32) of bacteriophage T4 is a much studied example of the single-stranded DNA binding proteins (Chase & Williams, 1986), and it binds much more strongly to single-stranded DNA (ssDNA) than to double-stranded DNA (dsDNA) in a more or less nonspecific way (Jensen et al., 1976). The binding to polydeoxyribonucleotides is stronger than to polyribonucleotides (Newport et al., 1981). The binding is characterized by a high cooperativity, leading to a continuous covering of the DNA by the protein (Delius et al., 1972); estimates for the cooperativity factor range from 2×10^2 to 10^4 (Kowalczykowski et al., 1981; Lohman, 1984; Watanabe, 1989; Kuil et al., 1989). There has been much discussion about the size of the binding site (n) and values obtained range from 5 to 11 nucleotides per protein, but it has been argued that inactivation of a fraction of the protein has caused the lower values (Bobst et al., 1982; Scheerhagen et al., 1986b). Minimizing the contribution of inactive protein during the determination of the site size led to values for n close to 10 (Bobst et al., 1982; Scheerhagen et al., 1986b; Kuil et al., 1988). In addition, Watanabe (1989) reported that in the analysis of a titration experiment the cooperativity can easily be overestimated, which could explain the lower values of n. Reanalysis of titration curves yielded a value of $n = 9$ for the binding of GP32 to poly(rA), in agreement with our recent results (Kuil et al., 1989).

As was reviewed by Chase and Williams (1986), the protein is involved in replication, repair, recombination, and translation processes, and it protects ssDNA against nuclease attack. The protein is also involved in late transcription (Gauss et al., 1987).

The crystal structure of GP32 is not known and neither is that of the complex with ssDNA. Much of what is known about this complex structure stems from spectroscopic measurements. In Scheerhagen (1986) and Scheerhagen et al. (1989) a model for the complex is presented that is largely based on hydrodynamic and spectroscopic measurements (Scheerhagen et al., 1985a–c, 1986a,b; Scheerhagen, 1986). The DNA is held in a more or less rigid, regular conformation, at least over a region of several bases. The DNA strand is rather extended in the complex, and the base–base distance projected along the overall complex axis is between 4.3 and 5.4 Å. From hydrodynamic measurements using complexes with small DNA fragments (about 100 bases), it was concluded (Scheerhagen et al., 1985b; Scheerhagen, 1986) that the local complex axis (e.g., at the level of one protein) is not parallel to the overall complex axis, which implies that the true base–base distance is larger than the distance projected on the overall axis. This can reflect either flexibility of the complex or a tertiary structure. Since at that time the interpretation of the hydrodynamic measurements in terms of flexibility seemed difficult, Scheerhagen chose to model the complex as a regular superhelix, where the local complex axis at every position in the complex has the same orientation with respect to the overall axis (superhelix axis). Recently, hydrodynamic studies (Kuil et al., 1988, 1990) were undertaken with larger fragments of single-stranded DNA with the aim of obtaining more specific data about the flexibility of the GP32–single-

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stranded DNA complex. It appeared that the flexibility of the complex is comparable to and probably even higher than that of double-stranded DNA. If this flexibility is taken into account, it can be concluded that the base–base distance projected on the local complex axis is close to 5.3 Å. It appeared not to be necessary to assume a superhelical structure for the GP32–single-stranded DNA complex, although it remains a good possibility both for this complex and also for complexes of other single-stranded DNA binding proteins. The radius of the complex is about 17 Å (Kuil et al., 1990).

Recently, we showed that a regular DNA (polynucleotide) conformation in the complex can explain the linear dichroism spectra of complexes formed between GP32 and ssDNA or homopolynucleotides (van Amerongen et al., 1988; van Amerongen & van Grondelle, 1989) and that for such a regular structure the base orientations must be characterized by a strong tilting and twisting, where the values of tilt (TL) and twist (TW) are −40° and 30°, respectively, or −40° and 150° with an uncertainty in both angles of about 15°. Following the Cambridge convention for nomenclature (Dickerson, 1989), from now on TL will be called the inclination, η, and TW is replaced by $-\omega$ (ω is propeller twist). Although the linear dichroism measurements do not prove that a regular DNA structure indeed exists in the complex, such a structure would be in agreement with the possibility discussed by Prigodich et al. (1986) that the binding site of the protein contains a binding pocket formed by regularly placed aromatic residues, which are in close contact with the DNA bases. A partial intercalation of these aromatic residues between the bases can account for the large increase in the base–base distance and such a structure would imply a base–base distance of about 6.5–7.0 Å (optimizing van der Waals interactions), which must be larger than the base–base distance projected on a local complex axis. The model is in accordance with the fact that five tyrosines, two phenylalanines (Prigodich et al., 1986), and one or two tryptophans (Khamis & Maki, 1986; Casas-Finet et al., 1988) are in close contact with the bases and the fact that the size of the binding site is about 10.

Assuming that a regular ssDNA structure exists in the complex, it is important to establish which structures are in agreement with the known experimental facts about the base–base distance and the orientations of the bases with respect to the complex axis. In this study we have investigated which ssDNA conformations are consistent with those data, taking into account known bond lengths and valence angles between chemical bonds. We discuss the possible structures in terms of the ability of the bases to stack with aromatic residues. In addition, steric contacts of bulky groups such as the O2′ in RNA are considered. It turns out that only a limited class of DNA/RNA structures remains. These structures are dramatically different from DNA/RNA conformations known so far.

Methods

To describe the position and orientation of a base with respect to a neighboring base, six parameters are needed, three for the relative position and three for the relative orientation. When all the bases in the single DNA strand are regularly placed with respect to each other, two bases form the elementary unit of a regular helix and six parameters suffice to describe all base positions and orientations. A convenient way to characterize such a helix (as far as the bases are concerned) is with the use of six base parameters as used by Scheerhagen et al. (1986a), which are a logical extension of the five parameters given by Arnott (1970) to describe the structure of dsDNA. We shall follow the Cambridge convention (Dickerson, 1989) for the nomenclature and we shall give the names as used by Scheerhagen et al. (1986a) in brackets. The neighboring bases in a single strand are rotated with respect to each other around the helix axis with an angle called twist (Ω) [rotpb] and the distance between them, projected on the helix axis, is called rise (Dz) [ax]. The definitions for dx, dy, η, and ω are given in Figure 1. Once one has chosen six base parameters, these need not necessarily correspond to a possible helix, since the bases must be connected with each other by a sugar–phosphate backbone, i.e., a chain closure must exist. The relative positions of the atoms in such a chain are completely determined by the bond lengths, the valence angles, and dihedral angles. The sugars usually adopt only a few different conformations, of which the C3′endo and C2′endo conformations [see, e.g., Arnott and Hukins (1972)] occur most frequently and are rather extreme representatives of the possible sugar conformations (Berman, 1981; Saenger, 1984). We used both conformations for our calculations. When the sugar–phosphate chain alters its conformation, the valence angles and bond lengths remain essentially unaltered (not considering the sugar conformation) [see Arnott et al. (1976) and Zhurkin et al. (1978)]. If the sugar conformation [from Arnott and Hukins (1972)] and the valence angles and bond lengths [from Arnott et al. (1976)] are known, the structure

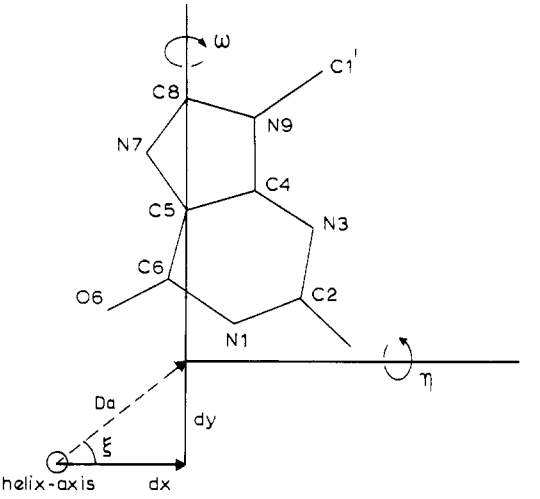

FIGURE 1: Parameters to describe the base orientation and position with respect to the helix axis. The helix axis is perpendicular to the plane of the figure. In this case guanine is shown. For all other bases the intersection point of the η- and ω-axes has the same distance to the C8 (purines) or C6 atom (pyrimidines), which lies on the ω-axis, and the orientation of the η- and ω-axes with respect to the glycosidic bond is the same.

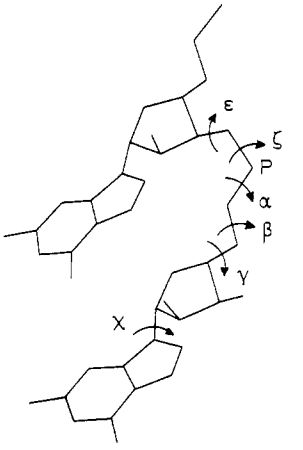

FIGURE 2: Nomenclature of the dihedral angles.

of the sugar–phosphate backbone is completely determined by five dihedral angles, α, β, γ, ϵ, and ζ (see Figure 2). When in addition a sixth dihedral angle χ is known, describing the orientation of the bases with respect to the sugars, the complete helix is determined and thus also the six base parameters [for definitions of the dihedral angles see, e.g., Saenger, (1984)]. The reverse is not true. Given the base parameters, the dihedral angles are not completely determined, but there are at most a finite number of possible combinations (Zhurkin et al., 1978).

We will now inspect for which choice of base parameters (three of which have been determined experimentally) a chain closure is possible. We only take into account geometrical constraints (bond lengths, valence angles, and sugar conformation). Energy minimization and molecular dynamics calculations are not possible (yet) since the interacting residues in the protein, and therefore their interactions with the DNA chain, are not known to a sufficient extent. To perform the structure calculations, we have written a program based on an algorithm of Zhurkin et al. (1978), which was used to calculate regular double-stranded DNA conformations. We extended the algorithm in a straightforward way to use it for the six base parameters necessary to describe the single-stranded DNA helix. When these parameters are given together with the sugar conformation, the program calculates whether the bases can be connected via a sugar–phosphate backbone, and if so, it gives the four dihedral angles χ, α, β, and γ. These angles together with the base parameters and sugar conformations are sufficient to draw the DNA molecule. It is impossible (in view of computer time needed for the calculations) to vary all base parameters and the sugar conformation in small steps, so we have used the following procedure. For Dz a value of 5.5 Å was taken, which is somewhat larger than the value reported by Kuil et al. (1990) but is within the error interval. We used $\eta = -40°$ and $\omega = -30°$ or $-150°$ (van Amerongen & van Grondelle, 1989). Ω was varied from $+30°$ (right-handed helix) to $-30°$ (left-handed helix) in steps of 1°. In the proposed structure of Scheerhagen et al. (1989) the value of Ω was taken to be 15°. The values of dx and dy were varied as outlined below. First, Da and ξ are defined by dx = Da cos ξ and dy = Da sin ξ. The angle ξ determines the orientation of the bases with respect to the helix axis (see Figure 1). When $\xi \approx 60°$ the bases point toward the inside of the helix when viewed from the sugar along the glycoside bond, and when $\xi \approx 240°$ they point away from the helix axis. To decide which values of Da and ξ (or dx and dy) may correspond to realistic structures, we considered the following experimental results. Otto et al. (1987) have found that four to six tyrosines are located on the outside of GP32 and that these residues are involved in the binding of the DNA, which is in good agreement with the findings of Prigodich et al. (1986) that in the complex five tyrosines are in close contact with the bases. This strongly suggests that the DNA is wound around the protein in such a way that the bases can make multiple contacts with the aromatic amino acids. Therefore, we believe that the bases do not point to the outside of the helix (when ξ is close to 240°), in which case these stacking interactions are not very likely. On the other hand, it is also unlikely that they point to the inside ($\xi = 60°$), since it is known that in T4 DNA the cytosines are modified at the C5 position, leading to 5-(hydroxymethyl)cytosine and an α-glucosylated derivative of 5-(hydroxymethyl)cytosine; these modifications should not interfere with the binding process (Kelly et al., 1976). We assume that realistic values of ξ are found much closer to 150° or 330° than to 60° or 240°,

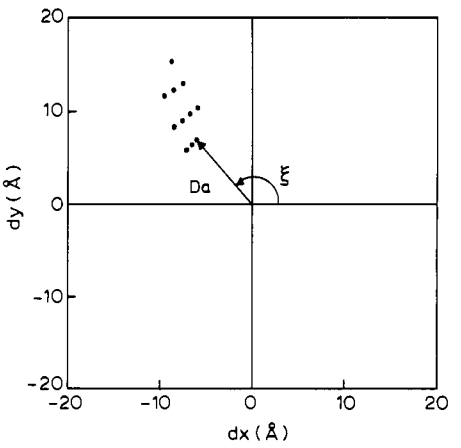

FIGURE 3: Possible values of dx and dy when Dz = 5.5 Å, $\eta = -40°$, $\omega = -30°$, and the sugar has a C3′endo puckering. When Da = 9 Å, $\omega \approx 13°$ and when Da = 18 Å, $\Omega \approx 8°$.

respectively. When ω is close to $-30°$, ξ will be near 150° and when ω is close to $-150°$, ξ will be near 330°. We will demand that for acceptable structures ξ is within either one of the invervals $120° < \xi < 180°$ and $300° < \xi < 360°$, dependent on the value of ω; ξ was varied within these intervals in steps of 5°. From recent hydrodynamic experiments (Kuil et al., 1989b) it is estimated that the radius of the complex is about 17 Å, which is in reasonable agreement with the results of Cohen and Chiu (1983), who obtained a global picture of a crystallized GP32*I dimer and observed that a monomeric protein has a diameter close to 20 Å. This GP32*I is a major proteolytic fragment of GP32 that contains the binding site. In our analysis we consider values of Da ranging from 9 to 21 Å to be the most likely ones, and we varied Da in steps of 3 Å within this interval. For all combinations of the base parameters and the sugar conformation it was calculated whether a chain closure was possible. The same procedure was repeated, but now with Dz = 5.2 Å, to study the effect of a small variation in Dz. Finally, for Dz = 5.5 Å, the above mentioned values for η and ω were varied by adding or subtracting 15° [reflecting the uncertainty in the LD results (van Amerongen & van Grondelle, 1989)]. Only the combinations (−25°, −15°) and (−25°, −165°) were not considered, since they are excluded by the LD results. All calculations were performed for both the C2′endo and C3′endo sugar conformations.

Results and Discussion

We shall discuss the results of structure calculations starting from sets of experimental data for Dz, η, and ω.

$\eta = -40°$, $\omega = -30°$, and Dz = 5.5 Å. Taking a C2′endo sugar conformation and varying Da, ξ, and Ω as indicated under Methods, we do not find any possible DNA structure. Only when a C3′endo puckering of the sugar is taken can a chain closure be obtained for the values of dx and dy indicated in Figure 3. For each of these possible solutions, Ω can be found within a certain range and in general Ω is small. The solutions are characterized by values of ξ between 120° and 140°. For Da = 9 Å, the solutions correspond to $\xi \approx 135°$. When Da gets larger, ξ becomes slightly smaller ($\xi = 120°$ at Da = 18 Å) and the bases point somewhat more inward to the helix axis. Parameters for possible structures are given in Table I (entry 2) for the two extreme values of Da. All conformations corresponding to these solutions resemble each other very much. They all appear to be right-handed, with $\Omega \approx 13°$ at Da = 9 Å and gradually decreasing to 8° when Da is raised to 18 Å. This reflects the fact that more bases

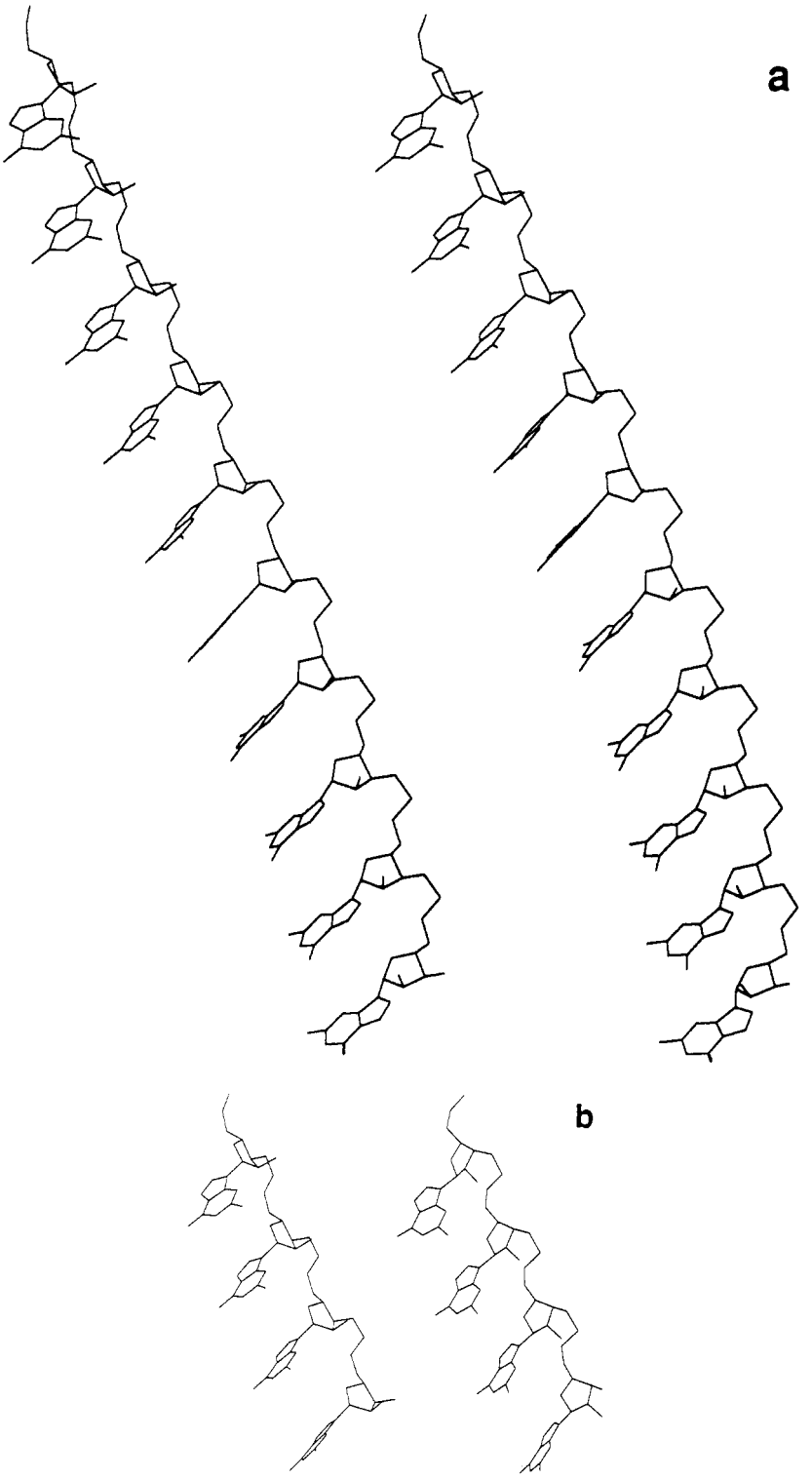

FIGURE 4: (a) Stereo picture of a regular single-stranded DNA (RNA) helix with base parameters Dz = 5.5 Å, $\eta = -40°$, $\omega = -30°$, $\Omega = 11°$, dx = −8.5 Å, and dy = 8.5 Å and a C3′endo puckering of the sugar. The DNA possibly adopts this conformation in the complex with GP32. (b) Parts of a regular helix with the sugar in a C3′endo (left) or a C2′endo conformation (right). In the case of the C3′endo puckering the base parameters are as in (a) and the shown part corresponds to the upper part of the helix in (a). The O2′ atom points toward the inner part of the helix. For the other helix the base parameters are Dz = 5.5 Å, $\eta = -55°$, $\omega = -15°$, $\Omega = 11°$, dx = −12.3 Å, and dy = 8.6 Å. The O2′ atom now points more to the outside of the helix and in this figure it is located behind the phosphate. Both structures are good possibilities for the ssDNA structure in the complex with GP32. The base parameters are similar and the main difference concerns the orientation of the O2′ atom.

are needed in a helix turn when the helix diameter is increased. The dihedral angles do not change much. In all cases χ is between 213° and 216°, which denotes an anti orientation of the bases with respect to the sugar, whereas $173° < \alpha < 193°$, $97° < \beta < 114°$, and $-167° < \gamma < -169°$. In the following, only the values of χ will be given explicitly, which we consider to be the most important dihedral angle, as it determines the relative orientation of two large groups with respect to each other, the sugar and the base. The orientations that are usually observed are called anti when $177° < \chi < 252°$, high anti when $252° < \chi < 287°$, and syn when $-3° < \chi < 87°$ (Berman, 1981). Note that the intervals for χ are different

Table I: Parameters for Possible DNA Structures[a]

entry	Dz (Å)	η (deg)	ω (deg)	sugar puckering	helix handedness	Da (Å)	ξ (deg)	Ω (deg)	χ (deg)
1	5.2	−40	−30	C3′endo	right	9	150 ± 30	16 ± 7	215 ± 19
						21	145 ± 25	8 ± 5	230 ± 19
				C2′endo		9	135 ± 15	14 ± 3	272 ± 13
						21	130 ± 10	9 ± 2	273 ± 14
2	5.5	−40	−30	C3′endo	right	9	135 ± 5	13	214 ± 1
						18	120	8	216
3	5.5	−25	−30	C3′endo	right	9	150 ± 30	14 ± 7	232 ± 16
						21	150 ± 30	7 ± 3	239 ± 20
				C2′endo		9	150 ± 30	19 ± 7	288 ± 15
						21	135 ± 15	8 ± 2	290 ± 16
4	5.5	−55	−15	C3′endo	right	9	150 ± 30	15 ± 7	196 ± 17
						21	140 ± 20	7 ± 3	196 ± 15
				C2′endo		9	140 ± 20	18 ± 6	250 ± 14
						21	135 ± 15	8 ± 2	252 ± 11
5	5.5	−40	−15	C3′endo	right	9	150 ± 30	15 ± 10	210 ± 17
						21	150 ± 30	6 ± 4	210 ± 16
				C2′endo		9	150 ± 30	19 ± 11	264 ± 21
						21	150 ± 30	8 ± 4	270 ± 20
6	5.5	−25	−150	C3′endo	right	9	300–305	25 ± 5	72 ± 9
						12	300	19 ± 2	78 ± 5
					left	9	355–360	−27 ± 3	43 ± 5
						12	360	−22 ± 3	43 ± 6
				C2′endo	left	9	360	−25 ± 3	109 ± 6*
7	5.5	−55	−165	C3′endo	right	9	340 ± 10	15 ± 13	111 ± 12*
						21	335 ± 5	8 ± 2	117 ± 10*
				C2′endo		9	330	28 ± 2	167 ± 4*
8	5.5	−40	−165	C3′endo	right	9	310 ± 10	20 ± 10	97 ± 20
						21	305 ± 5	9 ± 3	102 ± 15
					left	9	355–360	−25 ± 5	68 ± 7
						18	360	−12 ± 1	57 ± 1
				C2′endo	right	9	300	28 ± 2	159 ± 7*
						12	300	24 ± 4	160 ± 6
					left	9	355 ± 5	−23 ± 7	139 ± 26
						12	360	−19 ± 3	130 ± 4

[a] In this table possible structures are given for different choices of Dz, η, ω, and the sugar puckering. When certain values are not given, they are the same as on the previous line. Upper and lower values for Da are given and for one structure in this table, larger values than Da = 9 Å are not possible. Invervals are given where the values of ξ, Ω, and χ should lie. When only a single value is presented, the interval is smaller than 1°. Conformations indicated with an asterisk have an uncommon value for χ (determining the orientation of the base with respect to the sugar).

here than those given by Berman, as a different definition for χ has been used. In Figure 4a a representative example of the calculated helices (entry 2 of Table I) is given. Note that in all figures part of an RNA molecule instead of a DNA molecule is given. This meant to show the orientation of the O atom of the ribose. A striking feature of the structure in Figure 4a is the fact that neighboring bases have a small angle with respect to each other and there is a large distance between them. This conformation seems to be very well suited for having interactions with partly intercalated aromatic residues of GP32. The fact that only small values for Ω are found is in agreement with the conclusion in Scheerhagen et al. (1986a) that at least Ω or the distance of the bases to the helix axis should be low. The negative value of dx does not seem to be in agreement with conclusion from CD measurements and calculations for poly(rA) complexed with GP32 that dx should be positive (Scheerhagen et al., 1986a). However, we have recently shown (van Amerongen & van Grondelle, 1989) that poly(dA) seems to be a better model system for ssDNA in the complex, and for poly(dA) a negative value for dx is not in disagreement with the CD spectra (Scheerhagen et al., 1986a).

η = −40°, ω = −150°, and Dz = 5.5 Å. The same calculations as above were performed with ω = −150° but no possible structures were found.

η = −40°, ω = −30°, and Dz = 5.2 Å. When the value of Dz is reduced to 5.2 Å, more structures are possible than in the case of Dz = 5.5 Å (see Table I, entry 1). For the C3′endo puckering these structures are remarkably similar to those found with Dz = 5.5 Å, but more variation is now possible. When Da = 9 Å, then ξ = 150 ± 30° and Ω = 16 ± 7° (for the right-handed helices), whereas for Dz = 5.5 Å these values were 135 ± 5° and 13°, respectively. The bases are again in the anti conformation with respect to the sugars, but the variation in χ is also larger: χ = 215 ± 19° (in contrast to 215 ± 1°). So when Dz is lowered, the main features of the predicted structures remain the same, but larger variations in the parameters are possible. For this lower value of Dz, conformations with a C2′endo puckering are also allowed. These structures are characterized by similar average values for ξ and Ω, but the variations around these average values are smaller; for instance, for Da = 21 Å, ξ = 130 ± 10° and Ω = 9 ± 2°. In this case another value of χ is necessary (273 ± 14°) to obtain a chain closure, which corresponds to a high anti conformation.

η = −40°, ω = −150°, and Dz = 5.2 Å. As was observed for Dz = 5.5 Å, taking a value of ω = −150° does not lead to possible conformations.

Variations in η and ω. So far, conformations with values of η = −40° and ω = −30°/−150° have been considered, and we only find right-handed helices with ω = −30°. The orientations of the bases are probably best characterized by 120° < ξ < 180° (negative dx, positive dy), 9 Å < Da < 21 Å, and 6° < Ω < 23°. Most of the predicted structural parameters do not depend strongly on sugar puckering. There are more possible solutions for a C3′endo puckering than for a C2′endo puckering.

Below it will be examined what consequences variations of 15° in the values of η and ω will have for the allowed structures, assuming a value of Dz = 5.5 Å. The combinations of

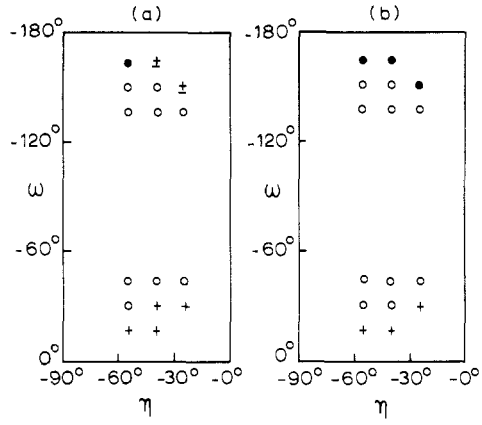

FIGURE 5: Possibility of chain closure with various parameters. For all indicated combinations of η and ω, which are in agreement with LD results, it has been calculated whether chain closures do exist between the bases. A value of Dz = 5.5 Å has been taken. The symbols used mean (○) no chain closure possible, (●) chain closure possible but the value of χ is unusual, (+) chain closure possible, leading to right-handed helices, and (±) chain closure possible, leading to both right- and left-handed helices. (a) C3′endo puckering; (b) C2′endo puckering.

ω and η that have been examined are given in Figure 5. The corresponding base parameters are given in Table I. In Figure 5 it can be seen that regular ssDNA structures do not exist for all combinations of η and ω that are in agreement with the LD results. In general, no structures are possible when the bases are far from a perpendicular orientation with respect to the helix axis, characterized by η and ω values both far from 0° (and for ω also far from −180°).

When η and ω are close to −40° and −30°, respectively, only right-handed helices exist, both with C2′endo and C3′endo puckering for the sugar. For all these structures the base parameters are similar. Ω is rather small and is dependent on the value of Da. Larger vales of Da lead to lower values of Ω. The bases adopt anti or high anti orientations with respect to the sugars. The possible combinations of η and ω are even more restricted than was already concluded from the LD measurements (van Amerongen & van Grondelle, 1989). The structure given in Figure 4a, with the sugar in a C3′endo conformation, can be considered as a good representative of all possible regular structures with η and ω close to −40° and −30°, respectively. We note that in this structure the O2′ atom of the ribose points toward the helix axis (where we believe the protein is located), which might sterically interfere with the binding process; this could possibly explain the lower binding affinity of GP32 for ribonucleotides than for deoxyribonucleotides (Newport et al., 1981). In Figure 4b a part of a helix is shown in which the sugar puckering is C2′endo. The other parameters correspond to those in entry 4 of Table I and are in fact not too much different from those in Figure 4a. It can be seen that with C2′endo puckering the O2′ atom is turned away from the inside of the helix, and it is closer to the phosphate backbone of the polynucleotide. This might correspond to an energetically unfavorable structure.

ω values in the range −150 ± 15° with η = −40 ± 15° have also been considered. For the C2′endo puckering only a few structures are possible, characterized by η, ω combinations that place the bases not too far from a perpendicular orientation with respect to the helix axis (entries 6–8 in Table I). However, for these structures the base orientation with respect to the sugars is unusual, as it cannot be classified as anti, high anti, or syn, and we consider the corresponding structures to be unlikely. For the same reason we discard the structure with η = −55° and ω = −165° and a C3′endo puckering. There

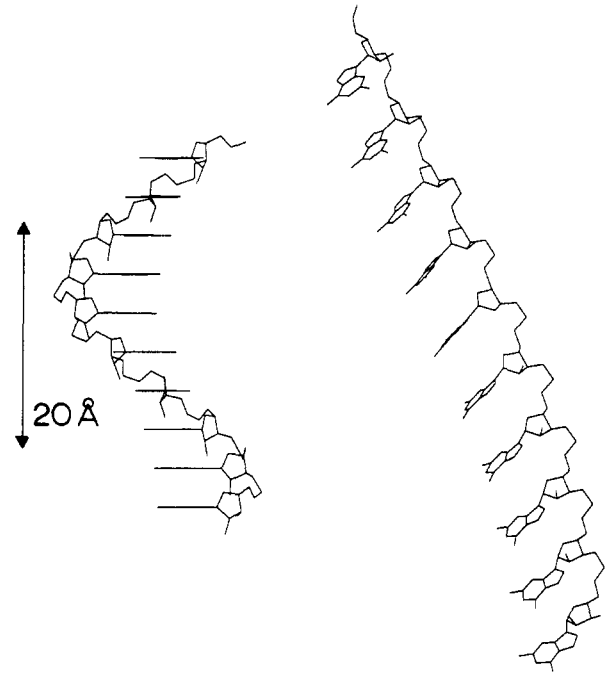

FIGURE 6: Comparison of the structure of a single DNA (RNA) strand as it is present in double-stranded DNA (B-conformation) (left) and in the complex with GP32 as proposed (right).

are solutions for the η, ω combinations (−40°, −165°) and (−25°, −150°), corresponding to both right- and left-handed helices, with the bases in a syn orientation with respect to the sugars. However, for the right-handed helices the sugars are located between the helix axis and the bases and therefore stacking interactions with the aromatic residues are not possible. For the left-handed helices this is not the case, but now the O atom of the ribose points away from the complex axis and it is also not in close contact with the sugar–phosphate backbone. It is not clear how in such a structure the presence of the O atom can lower the binding affinity of GP32 to polynucleotides, and for this reason we consider this structure less likely at the present stage.

Conclusions

In conclusion, if a regular ssDNA or RNA structure in the complex with GP32 is assumed, the arrangement of the polynucleotide is probably close to one of the structures given in Figure 4. This proposed conformation is based on all the known spectroscopic and hydrodynamic features of this DNA–protein complex. In comparison with the structure proposed by Scheerhagen et al. (1989), which was based on less experimental results and which was presented as a "possible conformation", the following detailed differences and agreements can be distinguished. The bases are clearly tilted with respect to the helix axis as was proposed by Scheerhagen et al. (1989), but the values of η and ω have now been determined more accurately. The base–base distance projected on the helix axis (5.5 Å) is smaller than the somewhat arbitrarily chosen value of 6.0 Å in the model of Scheerhagen et al. (1989). We assume that the DNA is wound around the protein, leading to a larger diameter for the DNA helix in our case. It is not necessary to assume a superhelix to explain the hydrodynamic data, as they can be explained by flexibility. The low value for Ω is a feature of both models. To illustrate the large changes in the ssDNA structure induced by GP32, the conformation of one strand is given in Figure 6, both as present in dsDNA (B-conformation) and in the complex witth GP32 as proposed above (see Figure 4a). Remarkable features

are the unwinding of the ssDNA in the complex, corresponding to a low value for Ω, the large base–base distance, and the specific base orientations. The values of η and ω are in agreement with the linear dichroism results (van Amerongen et al., 1988; van Amerongen & van Grondelle, 1989). The tilting of the bases explains the circular dichroism spectra of the poly(rA)–GP32 and poly(dA)–GP32 complexes (Scheerhagen et al., 1986a) and, in addition, is in accordance with the positive birefringence signals measured for GP32–ssDNA complexes (Scheerhagen et al., 1985a). The diameter of the complex agrees with hydrodynamic data (Kuil et al., 1990) but also with the diameter of GP32*I (Cohen & Chiu, 1983), assuming that ssDNA is wound around the protein. It is known that GP32 has a lower binding affinity for polyribonucleotides than for polydeoxyribonucleotides (Newport et al., 1981). From a structural point of view, the O2′ atom of the ribose could interfere in two different ways with the binding process, either by coming into close contact with the protein or with the phosphate backbone. For a C3′endo puckering it would probably come into close contact to the protein, but for a C2′endo puckering the O2′ atom approaches the phosphate backbone. More detailed calculations are required to decide if such a conformation is energetically unfavorable. In the proposed structure, modification at the C5 position of cytosine will not disturb the binding process as is required (Kelly et al., 1976). The large value of Dz is in accordance with recent hydrodynamic data (Kuil et al., 1988, 1990). Such a large value can explain the observed hyperchromism of ssDNA in the complex with GP32 [see, e.g., Scheerhagen et al., (1986a)]. This large base–base distance, where the bases make only a small angle with respect to each other, is in excellent agreement with the model proposed by Prigodich et al. (1986), where a large number of aromatic residues form a regular hydrophobic binding pocket for the bases, making stacking interactions possible. Therefore, it seems very likely that these stacking interactions cause the different binding affinity of GP32 toward single- and double-stranded DNA, whereas ionogenic interactions increase the binding constant.

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