Raman Spectra of the Model B-DNA Oligomer d(CGCGAATTCGCG)$_2$ and of the DNA in Living Salmon Sperm Show That Both Have Very Similar B-Type Conformations†

William L. Kubasek,‡ Yang Wang,‡ Gerald A. Thomas,‡ Thomas W. Patapoff,‡ Karl-Heinz Schoenwaelder,§ Johan H. Van der Sande,§ and Warner L. Peticolas*,‡

Department of Chemistry and Institute of Molecular Biology, University of Oregon, Eugene, Oregon 97403, and Department of Medical Biochemistry, Faculty of Medicine, The University of Calgary, Calgary, Alberta T2N4N1, Canada

Received May 19, 1986; Revised Manuscript Received August 22, 1986

ABSTRACT: Raman spectra were obtained from aqueous solutions of the deoxyoligonucleotide d-(CGCGAATTCGCG)$_2$ (I), which has been suggested as a model for B-type DNA conformation. These spectra were compared with the Raman spectra of the aqueous solutions of several DNAs of natural origin taken under identical solution conditions. Since the model sequence has a high percent GC (66%), the Raman spectrum was compared with the Raman spectrum of the DNA from *Micrococcus lysodeikticus* (72% GC), and the spectra of the two different DNAs were found to be rather similar in both 50 mM salt and 6 M salt solutions. Computer-aided band-shape analysis of the backbone vibrational region of the Raman spectra shows the existence of several bands corresponding to different furanose ring puckers. This appears to indicate a heterogeneity of furanose ring pucker in both the model dodecamer and the native DNA. Significant differences were found in the intensity of the conformational marker band at 810 cm^{-1}, which indicates corresponding differences in furanose ring pucker heterogeneities in these two high GC content DNAs. The Raman spectrum of the dodecamer (I) was used to analyze the Raman spectrum of the DNA inside the head of living intact salmon sperm. Sperm spectra were taken with both our conventional Raman spectrograph and a newly developed intracavity laser Raman microscope system. Although the DNA in the sperm head is required by packing considerations to be in a highly compact and condensed state, the Raman spectra of the intact sperm are almost identical with that of the model dodecamer (I) if the difference in base composition is taken into account. A comparison of the Raman spectra of purified DNA from salmon sperm and DNA in the intact sperm head shows some differences in the Raman intensities of the base vibrations (a different Raman hypochromism), indicating that there is a difference in the base stacking in the DNA packed in the sperm head and the free DNA. It is concluded that the furanose ring conformations in the purified salmon sperm DNA in solution are so varied that packing the DNA into the sperm head is carried out without significantly changing the DNA average backbone conformation.

The nature and magnitude of the changes in DNA structure necessary for gene regulation and chromatin packaging have been the subject of intensive research. In efforts to characterize the sequence specificity of DNA conformation, DNA ligand interaction, and DNA dynamical processes, many workers are applying a number of physical techniques. At the present time the only B-type DNA whose conformation is known exactly is the dodecamer d(CGCGAATTCGCG)$_2$ (I), whose three-dimensional structure has been described in the crystalline state by Dickerson and Drew (1981). This dodecamer represents the most reasonable choice for use as a standard for the Raman spectroscopic determination of DNA conformation in solution and in living cells. In the past in this laboratory we have developed a method for the determination of the secondary structure of DNA that was based on a comparison of solution DNA Raman spectra with the Raman spectra of fibers of DNA taken under conditions where X-ray fiber diffraction patterns show the DNA to be in the canonical B form (Erfurth et al., 1972, 1975). This latter method has been used and extended by a number of laboratories (Goodwin & Brahms, 1978; Thomas & Peticolas, 1983; Benivides & Thomas, 1984; Wartell & Harrell, 1986).

Since oligomer I contains a rather high G-C content (66%), we began our studies with a detailed comparison of the Raman spectra of the oligomer taken under a variety of conditions and that of the natural DNA obtained from *Micrococcus lysodeikticus*, which has a similar G-C content (72%). Since recent work has shown this native DNA is in the B family (Wartell & Harrell, 1986), we did not expect much difference in the Raman spectra of the native DNA and the model oligonucleotide, both taken in 50 mM pH 7 salt solution. On the other hand, DNA packed into a specific arrangement in a living cell might well have a conformation considerably different from that in solution. In particular, the packing of DNA into sperm heads is of considerable interest because of the possibility that the packing or condensation of DNA may cause it to go into a different conformation. In many species the process of spermatogenesis is accompanied by drastic increases in nucleic acid packing, or condensation. This is brought about by the replacement of the histones of the nucleosome particles with protamines after the last step in meiosis. These protamines effectively neutralize the negative charges of the DNA backbone while occupying substantially less volume. It seems reasonable to consider the possibility

† We gratefully acknowledge the support of NIH Grants GM 15547 and GM 33825 for the support of the microscope project and NSF Grant DMD 8417199 for some general support.

‡ University of Oregon.

§ The University of Calgary.

0006-2960/86/0425-7440$01.50/0

that such a high degree of condensation might introduce or necessitate a change in the average backbone geometry of the DNA helix in the sperm head compared to the same DNA molecule in simple aqueous solution, where it is free of ligands. Possible conformational distortions in the packed DNA conformation could include large global conformational changes or localized conformational variability in the helical backbone. Any such changes of the order of a few percent or more should appear as anomalies in the Raman spectrum obtained from living intact sperm heads.

It is possible to envisage the packing of DNA into the sperm head with little change in the average conformation of the backbone of the DNA. The accommodation of DNA condensation without measurable change in the average backbone or furanose ring conformation requires that the DNA helix be extremely flexible and possess a wide distribution of furanose ring conformations even in the purified form in solution. There is considerable evidence now that this is the case. Recently, a comparison was made of the Raman spectra of crystals of the dinucleotide pTpT with the Raman spectrum of the same dinucleotide in solution and with the Raman spectra of native DNAs in solution (Thomas & Peticolas, 1983). The pTpT dinucleotide is unique in that it is the only DNA fragment that has been crystallized with all of its furanose rings in the C2′-endo conformation. The comparison of the Raman spectra of the dinucleotide pTpT in solution and in the crystal with the Raman spectra of native DNAs in solution shows that the Raman marker band at 835 cm^{-1} for the C2′-endo conformation is 3–7 times stronger for pTpT in the crystal where both furanose rings are held rigidly in the C2′-endo conformation than they are in native DNAs. This includes native DNAs in solution and in fibers. From the comparison of these Raman spectra, it was concluded that only a small fraction of the furanose rings of native DNAs have the C2′-endo conformation. This conclusion was bolstered by the fact that in solution, the dimer pTpT shows no intensity at all in the 835-cm^{-1} band characteristic of C2′-endo ring pucker. In solution this dimer is almost certainly not a duplex structure, and there is no evidence for any specific ring pucker in the Raman spectrum for small single-stranded oligomers.

The conclusion that DNA in aqueous solution has only a small fraction of furanose rings with C2′-endo ring pucker appears to be in good agreement with the X-ray determined conformation of I by Dickerson and Drew (1981), who find that there is considerable variation in ring pucker among the furanose rings of the monomers in I in the crystalline state with only a third of the furanose rings having the C2′-endo ring pucker. Thus, both Raman spectroscopy on crystals and solutions (Thomas & Peticolas, 1983) and X-ray diffraction (Dickerson & Drew, 1981) on crystals indicate that we must reject the old idea that all of the furanose rings in B-form DNA have the same (i.e., C2′-endo) ring pucker and realize that extreme variability in furanose ring pucker is very likely in all native DNA. Such a variability in furanose ring conformations would have the effect of making the DNA molecule very flexible and possibly capable of undergoing considerable packing or condensation without changing the average values of the torsional angles of its backbone. This conclusion has also been reached by Wartell and Harrell (1986), who showed by careful band-shape analysis of the backbone vibrational region of the Raman spectra that native DNAs contain marker bands characteristic of both the C2′-endo and C3′-endo vibrations.

In this Raman spectroscopic study the dodecamer I in solution is used as a model for a B-form helix that is not rigid and exhibits a great deal of local conformational variation. This model is then used to analyze the conformation of the DNA found in the intact sperm of the salmon. The results of this study show that, even in the highly condensed DNA of the intact salmon sperm head, any distortion away from the B form as exhibited by the conformation of I is small, but it does make slight but measurable changes in the Raman spectra.

Materials and Methods

Raman spectra were obtained with right-angle scattering geometry by using an argon ion laser at 514.5 nm with an output power of approximately 200 mW at the source and a Spex 1301 double monochromator. The sample was in a glass capillary held in a thermostated copper block. The monochromator was interfaced to a Hewlett-Packard 200 series computer enabling computer-controlled data aquisition and processing. The resolution of the monochromator was 2.5-cm^{-1} half-width at half-height. Raman spectra of single sperm heads were taken with an intracavity laser Raman microscope spectrometer to be described elsewhere. Some of the Raman spectra were fit to a sum of Lorentzians by a nonlinear least-squares procedure. Correction to the band shapes arising from the experimentally determined slit function was included in the analysis by the use of a Voigt function. The Raman spectra were prepared for analysis by a simple base-line adjustment procedure that assumes straight but sloped base lines. For the small regions of interest studied here, this technique was found to be quite adequate.

Salmon semen was obtained from fresh silver salmon at the Dexter Fish Hatchery, Dexter, OR. The salmon were milked by standard hatchery procedures. The semen was kept on ice and rushed to the laboratory where the spectra were obtained. Approximately 2 h elapsed from the time the semen was obtained to the time when the Raman spectra were initiated. At the beginning of the scans, the salmon sperm were still motile as evidenced by standard light microscopic techniques. Salmon semen spectra were obtained at a temperature of 15 °C. Purified salmon sperm DNA was obtained from the salmon sperm by standard methods. The Raman spectra of the purified DNA obtained from our preparation of salmon sperm was compared with the Raman spectra of salmon sperm DNA purchased from Sigma Chemical Co., and the two spectra were essentially identical. DNA from *M. lysodeikticus* was purchased from Sigma and used without further purification.

The dodecamer $d(CGCGAATTCGCG)_2$ was prepared by the standard methods described elsewhere (Schoenwaelder & van de Sande, 1985). The hexamer $d(GCATGC)_2$ was the gift of Dr. Werner Leupin of the Institute for Molecular Biology and Biophysik in Zurich.

Results and Discussion

Figure 1 shows the Raman spectra of the dodecamer $d(CGCGAATTCGCG)_2$ in aqueous solution at pH 7, 50 mM salt (bottom spectrum) and 6 M salt (top spectrum). Figure 2 shows the spectra of the DNA from *M. lysodeikticus* taken under similar conditions. It is apparent that these spectra are very similar to that of the canonical B-type DNA fiber. In particular, the Raman band at 835 cm^{-1} characteristic of the C2′-endo ring pucker (Brown & Peticolas, 1975; Erfurth et al., 1972, 1975) is evident in all four spectra. Since the GC content of the oligonucleotide and the native DNA is very similar, it is reasonable to expect that the relative intensity of all of the bands assigned to the base vibrations would be the same. A study of Figures 1 and 2 shows that this is approximately the case. However, as we will show below,

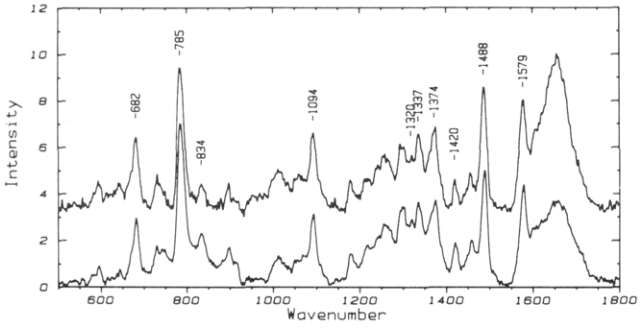

FIGURE 1: Raman spectra of the dodacamer d(CGCGAATTCGCG)$_2$ in aqueous solution at pH 7, 50 mM salt (bottom spectrum) and 6 M salt (top spectrum). These spectra were taken with 514.5-nm light and are the result of several accumulated scans.

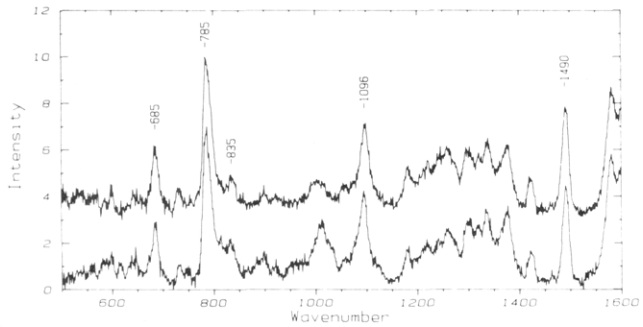

FIGURE 2: Raman spectra of DNA of *M. lysodeikticus* in aqueous solution, pH 7 and 50 mM salt (bottom spectrum) and 6 M salt (top spectrum).

computer-aided band-shape analysis of the conformationally sensitive region 700–880 cm^{-1} shows interesting and significant differences.

It is important to examine the high-salt Raman spectra of the native DNA and the dodecamer in view of the fact that both contain a high percentage of C and G bases. The dodecamer contains tetramers of alternating CG sequences so that there is a possibility that the ends of the dodecamer might go into the Z form at high salt. It has been recently shown that the tetrameric duplexes of CG will go into the Z form at high salt (Thomas & Peticolas, 1984). Examination of the Raman spectra show that indeed there are two new but rather weak bands that do appear in the high-salt Raman spectrum of the dodecamer at 622 and 645 cm^{-1}, which are typical of guanine bands for the Z and the A conformation, respectively (Nishimura et al., 1983; Benevides & Thomas, 1983). Thus, it appears that there may be some slight tendency of the dodecamer to go into a noncanonical B conformation at high salt in which some of the guanine residues are in an environment similar to that found in Z-DNA or A-DNA. It should be noticed however that there is no evidence for these bands in the high-salt spectrum of the native DNA from *M. lysodeikticus*. Hence, we must conclude that even with the high CG content of the bacterial DNA there is no measurable Raman evidence for it to go into the Z form at high salt.

In order to analyze the conformation of these DNAs, we examine in more detail the bands in the region 700–875 cm^{-1}, which include some important conformationally dependent bands due to vibrations of the sugar–phosphate chain. Figure 3 shows this region of the Raman spectrum of the DNA from *M. lysodeikticus* in aqueous solution at low (50 mM) salt. It is plotted on an expanded scale with the Raman spectrum resolved into a sum of Lorentzian bands by the nonlinear least-squares program described under Materials and Methods. In this figure, we see six bands between 700 and 875 cm^{-1}.

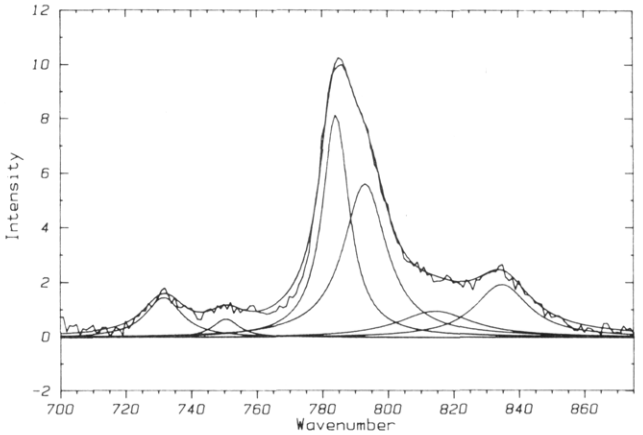

FIGURE 3: Raman spectrum of the DNA of *M. lysodeikticus* in aqueous solution, pH 7, expanded to show the region of 700–875 cm^{-1}. The use of computer-aided band-shape analysis permits the fit of the Raman spectrum in this region into a minimum number of Lorentzian bands.

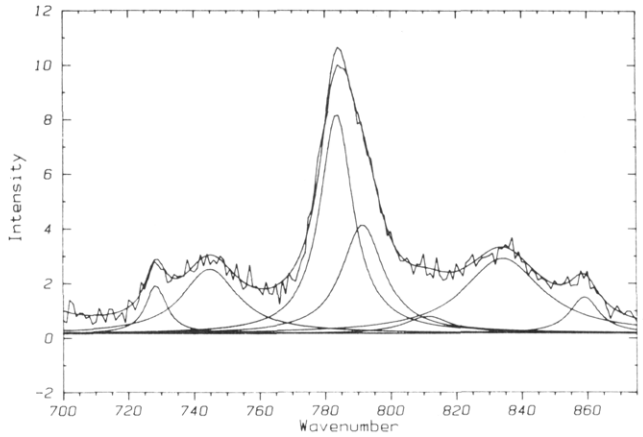

FIGURE 4: Raman spectrum of d(CGCGAATTCGCG)$_2$ in low (50 mM) salt expanded to show the region of 700–875 cm^{-1}, which is fit to the minimum number of Lorentzian bands.

These computer-resolved bands have almost exactly the same form and frequency as those obtained by Wartell and Harrell (1986) for a number of DNAs of various base composition. In particular, we confirm their result that for native DNAs there exists a band at 810 cm^{-1}, which can only been obtained by band-shape analysis. It should be remarked that the computer program we have used here, although using a similar theoretical approach to that of Wartell and Harrell (1986), was developed completely independently in this laboratory. The very close agreement in the analysis of this region of the DNA Raman spectrum between the two laboratories shows that this method of curve analysis is reproducible and independent of the exact program.

Figure 4 shows the spectrum of the dodecamer d-(CGCGAATTCGCG)$_2$ in aqueous solution at low (50 mM) salt expanded in the region 700–875 cm^{-1}. Again, the spectrum is resolved into a sum of Lorentzian line shapes. Here we see a very strong but broad peak at 835 cm^{-1}. This is the canonical marker band for the C2′-endo ring pucker of DNA (Brown & Peticolas, 1975; Erfurth et al., 1972) assigned to the sugar–phosphate backbone. But, interestingly, we see only a very weak, broad band at 810 cm^{-1}. Although weak, this band is required for adequate error reduction in fitting the observed Raman line shapes. From this we conclude that the dodecamer does not have exactly the same distribution of ring puckers as most native DNAs. These latter appear to invariably show a more pronounced band at 810 cm^{-1} as shown

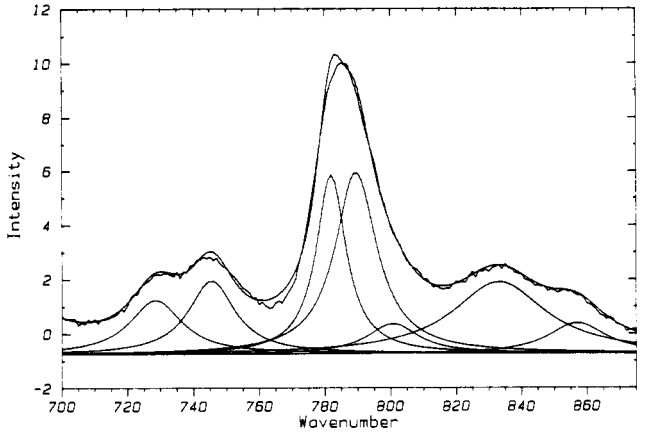

FIGURE 5: Raman spectrum of d(CGCGAATTCGCG)$_2$ in 6 M salt expanded to show the region of 700–875 cm^{-1} with the curve fit to a sum of Lorentzian bands.

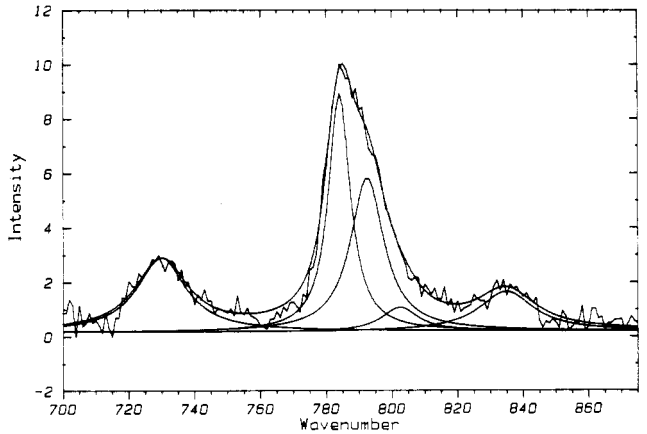

FIGURE 6: Raman spectrum of *M. lysodeikticus* in 6 M salt expanded to show the region of 700–875 cm^{-1} with the curve fit to a sum of Lorentzian bands.

in Figure 3 for DNA from *M. lysodeikticus* and for many other DNAs by Wartell and Harrell (1986). We have also taken the Raman spectrum of the deoxyoligonucleotide d-(GCATGC)$_2$, which has the same relative base concentrations as the dodecamer. It has a Raman spectrum in aqueous solution at low-salt concentration, which is remarkably similar to the dodecamer. Like the dodecamer, this hexamer has a pronounced but broad band at 835 cm^{-1} and the similarly weak band at 810 cm^{-1}, which together with the intensities of the other Raman bands show that in solution the hexamer and the dodecamer deoxyoligomers have very similar conformations. It appears these oligodeoxynucleotides have weaker bands at 810 cm^{-1} than does the native DNA from *M. lysodeikticus* and most other native DNAs, indicating a smaller number of furanose ring conformations with a C3′-endo ring pucker in the oligonucleotides. However, as we will discuss below, the DNA in living salmon sperm also has a weak, broad band at 810 cm^{-1} that appears to be unique among native DNAs.

Figures 5 and 6 show, respectively, the Raman spectra of the dodecamer d(CGCGAATTCGCG)$_2$ and the native DNA from *M. lysodeikticus* at high (6 M) salt expanded to show the region 700–875 cm^{-1}. These spectra should be compared with the low-salt Raman spectra from these DNAs shown in Figures 3 and 4. The conformationally sensitive backbone vibrations in the region 760–860 cm^{-1} show a remarkable insensitivity to this drastic change in salt concentration. But for the DNA from *M. lysodeikticus*, the distinct pyrimidine bands at 730 and 750 cm^{-1} are not present in the high-salt

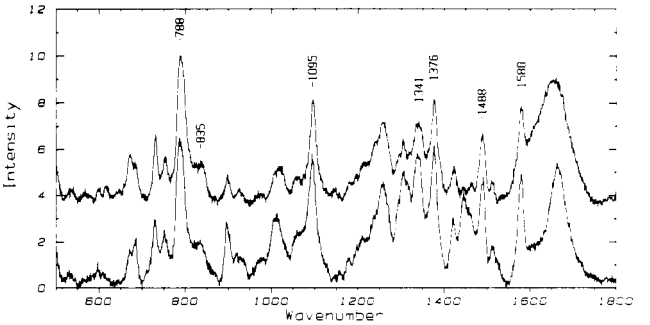

FIGURE 7: Raman spectra of an aqueous suspension of intact salmon sperm (bottom spectrum) and purified salmon sperm from the same sperm sample (top spectrum) in aqueous solution at pH 7 in 50 mM salt.

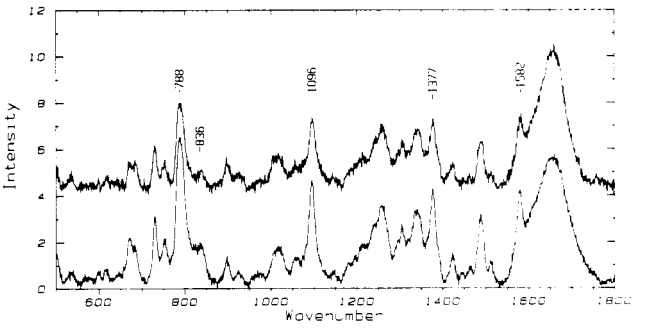

FIGURE 8: Raman spectrum of purified salmon sperm in low (50 mM) (bottom spectrum) and high (top spectrum) (6 M) salt.

form, but a single strong broad band at 728 cm^{-1} appears. Thus, it is apparent that even with its very high percentage of GC base pairs the DNA of *M. lysodeikticus* and the dodecamer remain in the B family even in very high salt concentration.

As was discussed above, it is of interest to examine the Raman spectra of salmon sperm DNA in intact sperm heads and in relatively dilute solution to see if there is an observable difference due to a difference in DNA conformation. It is apparent that, in the packing process or in the interaction of the DNA with the polyamines that occurs in the sperm head, the conformation of the salmon sperm DNA may be changed from that in an ordinary DNA found in dilute (about 0.05 M nucleotide) solution. Figure 7 shows the Raman spectra of a suspension of living intact salmon sperm (bottom spectrum) and that of a dilute aqueous solution (0.05 M nucleotide, 0.05 M salt, pH 7) of the DNA purified from the same salmon sperm preparation used as the sample in the top spectrum. This latter spectrum is quite similar to that reported by Alix et al. (1981). It is apparent that both the purified DNA in dilute low-salt solution and that in the intact salmon sperm are in the B family. Since the bottom spectrum is obtained from a turbid, white suspension of salmon sperm and the top spectrum is obtained from a clear solution of purified salmon sperm DNA, it is remarkable that the spectra are so similar. It is to be expected that the spectrum of the suspension of intact sperm heads would show bands from the membrane components. Indeed, the bands at 896 and 1450 cm^{-1} may very well be due to the membrane components as they are absent in the spectrum of the purified salmon sperm. In addition to these differences, which are plainly due to other components, there is a slight change in the relative intensities of the Raman bands in the 1257–1340-cm^{-1} region, which may be indicative of some slight differences in conformation inside and outside of the sperm head. That the conformation of purified salmon sperm DNA is also independent of salt con-

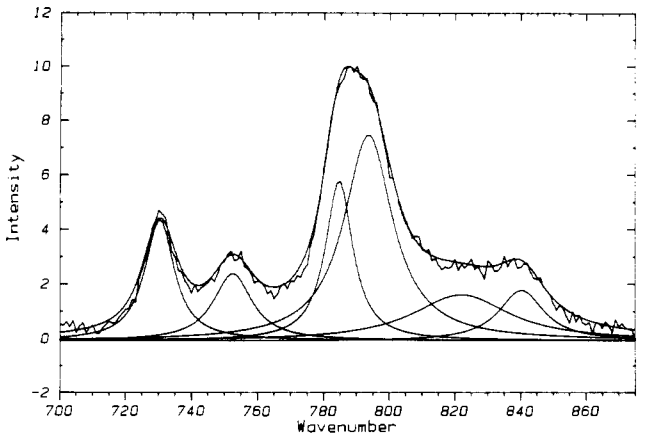

FIGURE 9: Expanded Raman spectrum in the region of 700–875 cm^{-1} of a solution of purified DNA from salmon sperm in 50 mM salt showing a curve fit to a sum of a minimum number of Lorentzian bands.

centration may be seen from Figure 8, where the Raman spectrum of purified salmon sperm DNA in low (50 mM) salt (bottom spectrum) is compared with the same preparation in 6 M salt (top spectrum). Figure 9 shows the Raman spectrum of the purified salmon sperm DNA in 50 mM salt expanded to show the backbone marker bands. Note that in this spectrum there exists a sizable band at 810 cm^{-1} similar to that found in the DNA from *M. lysodeikticus* shown in Figure 3 and by Wartell and Harrell (1986) for many other DNAs. Figure 10 shows the expanded Raman spectra of the intact sperm head taken in a conventional Raman apparatus on an opaque suspension of sperm (top spectrum) and from a single sperm head with the microscope system. In each case, the expanded spectrum in the region 700–870 cm^{-1} is presented. A comparison of the Raman spectra taken on the purified salmon sperm DNA (Figure 9) and the spectra taken on intact sperm heads is remarkably similar. However, using our band-shape analysis program, it may be seen that there are significant differences. The DNA in the intact salmon sperm appears to have a much smaller band at 810 cm^{-1}. Indeed, in the bottom of Figure 10, which shows the Raman spectrum taken with the intracavity laser Raman microscope of a single salmon sperm head, the band at 810 cm^{-1} is absent. This spectrum is also expanded in the region 700–870 cm^{-1} (which are noted by channel number in this spectrum) and analyzed by means of a fit to a sum of Lorentzians. This Raman spectrum of the intact sperm head shows that the 810-cm^{-1} band usually found in DNA in normal aqueous solutions (Wartell & Harrell, 1986) is either very weak or not present in the DNA packed into the sperm head. There is also a slight difference in the relative intensities of the two strong Raman bands in the center of the spectrum at about 780 cm^{-1}. However, considering the fact that these spectra were taken on completely different types of apparatus, the agreement is remarkable. As our development of the microscope Raman technique continues, we anticipate an improved signal to noise and the replacement of the channel numbers by the actual wavenumbers.

Another interesting difference in the Raman spectra of purified salmon DNA and the intact sperm head may be seen in Figure 7. Both of the spectra in Figure 7 show a doublet at 682 cm^{-1} (due to guanine) and 668 cm^{-1} (due to thymine). One sees in the Raman spectrum of the intact salmon sperm (bottom spectrum of Figure 7) that the 682-cm^{-1} peak is higher than the 668-cm^{-1} peak while in the purified salmon sperm DNA just the opposite is true of these intensities. The explanation of this difference may be obtained from Figure 8, where the high-salt form of the purified salmon sperm DNA shows the 668–682-cm^{-1} doublet to have the intensity ratio of the intact sperm head. Thus in this case this inversion of the intensities of this doublet from the values found in the purified salmon sperm DNA in low salt appears to be brought about by high salt concentration in the case of the salmon sperm DNA in solution and the high spermine salt content of the intact sperm head.

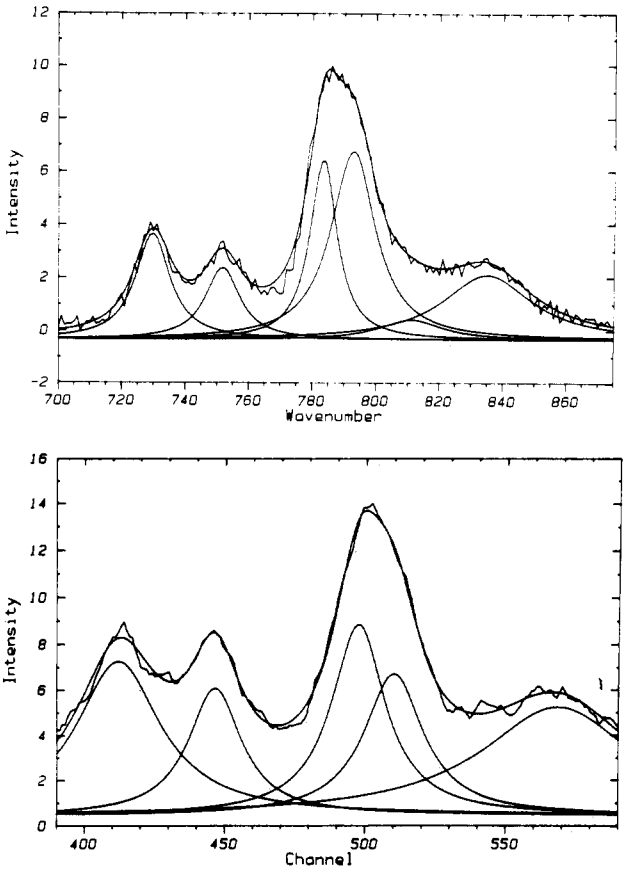

FIGURE 10: (Top) The expanded Raman spectrum of a sample of intact salmon sperm showing a curve fit to a minimum number of Lorentzian bands. (Bottom) The same spectrum taken with the laser Raman microscope system from a single living sperm head.

The fact that the Raman spectra of the deoxydodecamer model B-DNA, the DNA inside the intact salmon sperm head, and the purified salmon sperm DNA have bands with very similar frequencies but with slightly altered intensities indicates that the average conformation of these nucleic acids must be very similar. The presence of numerous bands in the region of the backbone vibrations indicates that there is a heterogeneity of furanose ring conformations indicating great flexibility in the DNA backbone. It should be noted that the technique of Raman spectroscopy appears to be the only physical technique that can compare the average conformation of DNA in a living cell such as a sperm head and the conformation of DNA in solution or in a crystal. However, we cannot comment on the possibility that there exists a very small amount of DNA in the sperm head (1% or less) with an unusual conformation such as, for example, the left-handed Z form. The bands arising from such a small concentration of unusual conformation would be very difficult to pick out. However, it is certain that there are no major changes in conformation between the salmon sperm DNA in aqueous solution and that in the intact sperm head.

Registry No. I, 77889-82-8.

REFERENCES
Alix, A. J. P., Bernard, L., & Manfait, M. (1981) *Inorg. Chim. Acta*, 147-152.
Benevides, J. M., & Thomas, G. J., Jr. (1983) *Nucleic Acids Res. 11*, 5747-5761.
Brown, E. B., & Peticolas, W. L. (1975) *Biopolymers 14*, 1259-1271.
Dickerson, R. E., & Drew, H. R. (1981) *J. Mol. Biol. 149*, 761-786.
Erfurth, S. C., Kiser, E. R., Kiser, J., & Peticolas, w. L. (1972) *Proc. Natl. Acad. Sci. U.S.A. 69*, 938-941.
Erfurth, S. C., Bond, P. J., & Peticolas, W. L. (1975) *Biopolymers 14*, 1245-1257.
Germann, M. W., Schoenwaelder, K. H., & van der Sande, J. H. (1985) *Biochemistry 24*, 5698-5702.
Goodwin, D. C., & Brahms, J. (1978) *Nucleic Acids Res. 5*, 835-850.
Nishimura, Y., Tsuboi, M., Nakano, S., Hiquchi, T., Sato, T., Shido, S., Uesugi, S., Ohtsuka, E., & Ikehara, M. (1983) *Nucleic Acids Res. 11*, 1579-1588.
Thomas, G. A., & Peticolas, W. L. (1983) *J. Am. Chem. Soc. 105*, 986-992.
Thomas, G. A., & Peticolas, W. L. (1984) *Biochemistry 23*, 3202-3207.
Wartell, R. M., & Harrell, J. T. (1986) *Biochemistry 25*, 2664-2671.

Wobble dG·dT Pairing in Right-Handed DNA: Solution Conformation of the d(C-G-T-G-A-A-T-T-C-G-C-G) Duplex Deduced from Distance Geometry Analysis of Nuclear Overhauser Effect Spectra†

Dennis Hare

Infinity Systems, 14810 216th Avenue NE, Woodinville, Washington 98072

Lawrence Shapiro and Dinshaw J. Patel*

Department of Biochemistry and Molecular Biophysics, College of Physicians and Surgeons, Columbia University, New York, New York 10032

Received May 29, 1986; Revised Manuscript Received July 25, 1986

ABSTRACT: We report below on features of the three-dimensional structure of the d(C-G-T-G-A-A-T-T-C-G-C-G) self-complementary duplex (designated 12-mer GT) containing symmetrical G·T mismatches in the interior of the helix. The majority of the base and sugar protons in the 12-mer GT duplex were assigned by two-dimensional nuclear Overhauser effect (NOESY) spectra in H_2O and D_2O solution. A set of 92 short (<4.5-Å) proton–proton distances defined by lower and upper bounds for one symmetrical half of the 12-mer GT duplex were estimated from NOESY data sets recorded as a function of mixing time. These experimental distances combined with nucleotide bond length parameters were embedded into Cartesian space; several trial structures were refined to minimize bond geometry and van der Waals and chirality error. Confidence in this approach is based on the similarity of the refined structures for the solution conformation of the 12-mer GT duplex. The G and T bases pair through two imino–carbonyl hydrogen bonds, and stacking is maintained between the G·T wobble pair and adjacent Watson–Crick G·C pairs. The experimental distance information is restricted to base and sugar protons, and hence structural features such as base pair overlap, glycosidic torsion angles, and sugar pucker are well-defined by this combination of NMR and distance geometry methods. By contrast, we are unable to define the torsion angles about the bonds C3′–O3′–P–O5′–C5′–C4′ in the backbone of the nucleic acid.

The ready availability of synthetic oligonucleotides of defined sequence has permitted a systematic attempt to probe the conformation of DNA in solution by NMR techniques [see reviews by Patel et al. (1982a), Kearns (1983), and Wemmer and Reid (1985)] and in the solid state by X-ray methods [see reviews by Dickerson et al. (1982), Rich et al. (1984), and Kennard (1985)]. These approaches have been extended to studies of errors in DNA such as base pair mismatches in an attempt to delineate the conformational basis for their recognition and repair (Patel et al., 1982b).

Chart I

C1 — G2 — T3 — G4 — A5 — A6 — T7 — T8 — C9 — G10 — C11 — G12

G12 — C11 — G10 — C9 — T8 — T7 — A6 — A5 — G4 — T3 — G2 — C1

The G·U mismatch in RNAs and the G·T mismatch in DNAs drew initial attention on the basis of the proposal of wobble base pair formation by Crick (1976). Support for this model came from identification of G·U pairing in transfer RNA by crystallographic (Quigley & Rich, 1976; Jack et al., 1976) and NMR solution (Schimmel & Redfield, (1980) investigations. An NMR chemical shift study has been reported for poly(dG-dT) in solution (Early et al., 1978).

† The research was supported by National Institutes of Health Grants GM 34504-02 to D.J.P. and GM 35620-02 to D. H. The NMR spectrometers were purchased from funds donated by the Robert Woods Johnson, Jr., Trust and the Matheson Trust toward setting up the NMR Center in the Basic Medical Sciences at Columbia University.

0006-2960/86/0425-7445$01.50/0