Is DNA Really a Double Helix?

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Do the two chains of the DNA molecule coil round one another plectonemically? If so, what is the approximate value of Lk (the linking number) for any closed, circular DNA molecule? Experiments using gel electrophoresis have shown that supercoiled DNA molecules usually migrate in a series of discrete bands. The only tenable explanation for this quantized behavior is that the molecules in one band all have the same value of Lk and that this value differs by unity from that of the adjacent bands. Various experiments in which circular DNA is unwound by known amounts show that (given this assumption) Lk for relaxed DNA is very roughly equal to $N/10$ (where N is the number of base-pairs), as expected from the classical double helix.

The original model for the double helix was right-handed. The experimental evidence for this feature is suggestive but not yet completely compelling.

1. Introduction

A number of recent papers (Rodley *et al.*, 1976; Sasisekharan & Pattabiraman, 1976,1978; Sasisekharan *et al.*, 1977,1978; Cyriax & Gäth, 1978; Pohl & Roberts, 1978) suggest that the two strands of DNA do not coil round one another but lie side-by-side. In this short review we outline the experimental evidence already existing which shows that the two strands of double-stranded DNA do indeed coil plectonemically round one another. Some new experimental data are given in the Appendix.

Since the double helix was first proposed 25 years ago, most of the general features of the structure have stood up very well to experimental tests. DNA is usually double-stranded, with the sugar-phosphate backbones running anti-parallel rather than parallel. The bases, one from each chain, are paired in the classical way; that is,

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adenine with thymine or guanine with cytosine. The above generalizations are supported by innumerable experiments, not least by the flood of exact sequences (of defined stretches of DNA) which are now appearing at an ever-increasing rate. The precise manner with which guanine pairs with cytosine using three hydrogen bonds is firmly established. It has not always been clear exactly how adenine pairs with thymine, but it now seems likely that, at least in most cases, this pair has a configuration not unlike that originally suggested by Watson and Crick.

On the other hand, there are other features of the structure which, although highly plausible, are not supported by experiment to anything like the same extent. We will leave on one side questions involving the precise co-ordinates of the structure (for example, how much the bases are tilted, how exactly the sugars are puckered, the regularity of the structure with different base sequences) and mention just two general features. Is the structure really a proper double helix, with the two chains wound plectonemically round a common axis? Is the helix predominantly right-handed (as originally claimed) or left-handed?

2. The SBS Structure

Two polymer chemists, Cyriax & Gäth (1978) have suggested a structure for DNA which they call a cis-ladder conformation and two mathematicians, Pohl & Roberts (1978) have rejected the classical double helix because of topological difficulties in the replication of DNA. However, the main reason for reconsidering these questions was the suggestion made by a group of New Zealand workers (Rodley et al., 1976) that the two chains of DNA have a configuration they call a side-by-side (SBS) structure. A similar suggestion was made at about the same time by the crystallographer Sasisekheran and his colleagues (Sasisekheran & Pattabiraman, 1976,1978; Sasisekheran et al., 1977,1978). The reader is referred to these papers for details but, in outline, one can describe the proposed structure by saying that it consists of a sequence of five base-pairs having a right-handed twist followed by five with a left-handed twist, and so on, indefinitely. The New Zealand authors have built a model and given co-ordinates but these are only preliminary, so that it is not easy to judge exactly how good the various bond-lengths, angles, hydrogen bonds and van der Waals' contacts can be made. They also claim, rather surprisingly, that their calculations show that such a model will fit the observed X-ray pattern of fibers of the B form of DNA just as well as does the standard structure, if not better. Apparently their motivation for reinvestigating the structure was due to the difficulty of separating the two chains of DNA during replication. That the two chains, in general, do separate has been clear since the classical experiment of Meselson & Stahl (1958), and this separation can now be considered to be very firmly established.

Now there are many things which could be said against this proposal. It can hardly be denied that the new structure is inelegant. The reversal of the hand of the screw at every fifth residue seems arbitrary The crystal structures of transfer RNA (Kim et al., 1973; Suddath et al., 1974; Robertus et al., 1974) show beyond doubt that, in that case, the helices are consistently right-handed, and so on. Moreover, the proposed structure is not strictly side-by-side. Due to the fact that the right-handed screw is not exactly equal to the left-handed one, the two chains of the proposed structure do, in fact, coil around one another, though much more slowly than in the

classical double helix, one turn taking about 100 base-pairs, rather than 10. Thus, the chains would still have to be unwound to get them apart, though not as much as for the original double helix. One could also argue that since the discovery of the nicking closing enzyme (also called topoisomerase or relaxase) and of the enzyme "gyrase", to say nothing of the various DNA binding proteins and "helicases", the problem of unwinding DNA for replication is by no means as difficult as it appeared 25 years ago.

All the above arguments are suggestive and have some force but they are not compelling. In science ten weak arguments do not add up to one strong one. We are not concerned with the question whether the double helix or the SBS structure is the more plausible. We wish to know whether there is any very hard evidence which decisively favors one structure over the other. For this reason we consider it unwarranted to rely solely on the details of exact model building, our knowledge of stereochemistry, though now fairly good, may not be adequate to provide firm answers, nor is it advisable to put one's faith completely on the fine details of X-ray diffraction patterns. That of the B form has always been rather poor and may not yield a clear, unambiguous decision between the two alternative types of structure. One must turn to evidence of quite a different type.

3. The Linking Number

Fortunately, that evidence now exists and moreover goes to the root of the matter. The essential difference between the two structures emerges rather clearly if we focus our attention on circular DNA molecules. Consider such a circular molecule with about 5000 base-pairs, for example the DNA of the oncogenic simian virus 40 (SV40). What we wish to know, to distinguish between the two structures, is the net number of times each chain is coiled round the other. This is known as the linking number, Lk, of the two chains (White, 1969; Fuller, 1971). Lk is equivalent to the symbol α, called the topological winding number (see the review by Bauer & Vinograd, 1974). For a popular exposition of the meaning of Lk see Crick (1976), where it is written L. For a classical double helix, with one turn every ten base-pairs, we would have $Lk = +500$. (The definition of Lk is such that if the double helix had been left-handed then Lk would have been -500.) For a true SBS structure, $Lk = 0$. For the proposed New Zealand structure, $Lk = +50$, approximately. Which is it?

Notice that for a true SBS model ($Lk = 0$) a very easy experimental proof is possible. One need only take such a piece of circular DNA and raise the temperature until the structure denatures. The two chains should come apart into two distinct intact single-stranded circles. These could be characterized with the electron microscope. With luck the two sorts of circles might even be separated, and then reannealed in various ways to put their identity beyond doubt. We think it is fair to say that the evidence in favor of the classical double helix is sufficiently strong that a proposal for a true SBS structure is unlikely to be widely accepted unless such a dramatic experimental demonstration is provided. Note that one essential control has already been done. We already know that if *one* of the two DNA backbones is nicked, then on denaturation the structure falls apart into a single-stranded circle plus a single-stranded linear molecule (see the review by Bauer & Vinograd, 1974).

This hypothetical experiment is a good test for a true SBS model ($Lk = 0$). Experiments have already shown that a circular DNA molecule, when denatured, does *not* separate into two parts but sediments as a single component whose

sedimentation velocity is approximately three times that of a single-stranded closed circle of the appropriate length (Vinograd & Lebowitz, 1966). This proves that $Lk \neq 0$ (at least for the great majority of those molecules). This does not distinguish between the proposed SBS model ($Lk = +50$) and the double helix ($Lk = +500$).

Fortunately, there already exist experiments in the literature which, properly interpreted, leave one with hardly any doubt that the SBS model is wrong. To these we must now turn.

It is well-known that circular molecules of DNA, such as that of SV40, are "super-coiled", either when extracted from the intact virus or from the virus-infected cell. There is much evidence to suggest that this supercoiling is negative. That is, the linking number of the supercoiled DNA, Lk, is less than that of the unsupercoiled (= relaxed) circular molecule, Lk_0. For the native virion SV40 DNA the linking number deficiency $\Delta Lk = Lk - Lk_0$ is typically about -25. A lot of experimental evidence now supports this interpretation and one might be forgiven for waving one's hand in that direction and leaving the matter at that. (See the comprehensive review by Bauer (1978) for details.) However, it could always be objected that the interpretation of this evidence *assumes* DNA to be the classical double helix. Can we obtain at least an approximate value for Lk without making this assumption?

How this is best done is perhaps a personal matter of how much weight one puts on different types of evidence (since, fortunately, they all lead to the same conclusion). We ourselves find the following line of argument the most compelling.

4. The Bands Found on Electrophoresis

It was established a few years ago by Keller & Wendel (1974) that the electrophoresis of circular DNA on agarose gels containing appropriate amounts of ethidium bromide leads to a pattern having a *discrete series of bands*. It is this observation, and the interpretation of it, which forms the basis of the argument. The obvious interpretation (which was made at the time) is that adjacent bands on the gel differ because their DNA has different amounts of supercoiling. In particular, it was assumed that *Lk for adjacent bands normally differs by unity*. It is our thesis that no other interpretation of the bands is even remotely plausible.

To reach this point we must go over a few well-established experimental facts. First, it should be made clear that only over a limited range of supercoiling are the bands resolved. On a standard polyacrylamide gel, molecules with a different but very low degree of supercoiling tend to run fairly close together, presumably because their configurations are all rather similar, all being fairly open circles. Molecules with a high degree of supercoiling also tend to bunch together on the gel, because they all approximate to rather tight structures. In between there is a range where the bands are fairly well-separated and roughly equally spaced. It is this range that we shall be considering.

The lack of resolution at both low and high levels of supercoiling might seem an insuperable handicap to the argument, but fortunately by various devices it is possible to shift bands from regions of the gel where they overlap to regions where they are well-separated. By these means the total number of "bands" in a population, and their mean position, can be obtained fairly reliably (Keller, 1975; Shure & Vinograd, 1976).

It is also well-established that a population of molecules in contact with the

nicking-closing enzyme (which requires no external source of energy) will end up in the "relaxed" state and that such a population will not show a single band, but a set of adjacent bands, the envelope of which follows approximately a Gaussian distribution (Keller, 1975; Depew & Wang, 1975; Pulleyblank et al., 1975). Moreover, the width of this distribution can be shown to be that expected from independent estimates of the energy of supercoiling. In popular terms, the constant thermal vibration buffets the molecules so that at any one moment some of them are either slightly overcoiled or slightly undercoiled, and the nicking-closing enzyme can catch them in this state and thus produce values of Lk a little different from that for the exactly relaxed state.

(a) Some controls

Bands are only seen upon covalent closure of DNA. A circular DNA with a single-chain scission runs as a sharp, single band. If a band is cut out of the gel and rerun without the nicking-closing enzyme, it runs as a single band in exactly the same place as before. If, on the other hand, it is cut out and left in contact with the nicking-closing enzyme (the DNA being circular and not nicked), it will generate a set of adjacent bands and, if left long enough, will give the set characteristic of the relaxed set.

In addition, if a sample of intact circular DNA, comprising a single band, is heated to just below its melting point and then cooled, the band found on further electrophoresis is exactly the same as if the sample had not been heated. The experimental evidence for this is set out in the Appendix to this paper.

(b) The Cause of the Bands

Now for the interpretation. It is clear that adjacent bands must differ in a property which is discrete (since we obtain bands and not a broad smear) and which survives heating and cooling but not breaking and rejoining of DNA bonds. There is really only one possible interpretation. The bands must differ in Lk. The Linking number, being necessarily an integer for closed circular DNA, has to take one of a series of discrete values. No other independent property, which might have discrete values (e.g., the number of kinks) could possibly survive heating and cooling. Only Lk (which is a topological invariant, provided the two backbones remain intact, but which is otherwise indifferent to the precise arrangement of the molecule in space) can have the desired properties.

Of course, during electrophoresis a given molecule will be constantly taking up different configurations (each with its own Tw and Wr; for an explanation of these terms see Crick (1976)), but over a time which is short compared to the electrophoresis time, the molecule will have run through a sufficient number of these configurations (all having the same value of Lk) that its electrophoretic behavior will be very close to the "average" configuration taken over infinite time. Moreover, when the bands are resolved, these average values for molecules in different bands will differ sufficiently, so that when Lk differs by unity the bands formed by such molecules will not overlap.

Strictly the above argument only shows that adjacent bands must differ in Lk. It does not prove that this difference is unity. The total absence of intermediate bands, under any of a whole variety of conditions, makes any other explanation hopelessly forced. Moreover, as we shall see, the assumption that this difference equals

unity fits nicely with many other less direct forms of evidence. Notice that if the difference for adjacent bands was not one but, say, two, this would only *increase* our estimate of the linking number of a circular DNA molecule, and not decrease it as required by the SBS structure.

By counting the bands on the gels one can thus establish the mean value of the supercoiling, $\overline{\Delta Lk}$, for a particular population of circular DNA molecules. One can then study the effects of dyes such as ethidium bromide. This establishes that the binding of about 14 ethidium molecules alters the "supercoiling" by unity (Bauer, 1978). This allows ethidium bromide titration to be used as a subsidiary method for estimating supercoiling so that band counting does not have to be done in every case.

5. Experimental Estimates of *Lk*

We now need to consider experiments of a quite different type, though their interpretation depends on the interpretation of supercoiling outlined above. They fall into two distinct classes. The first class, which has already been published for some time, depend, roughly speaking, on uncoiling part of a negatively supercoiled circular duplex of DNA by one method or another and finding what fraction of the DNA has to be unwound to remove all the supercoiling. One ends up with local regions which either consist of two strands of duplex DNA, approximately side-by-side, as in the method of Liu & Wang (1975) or one single strand of DNA beside a duplex of DNA, as in the D-loop experiment of Vinograd and his colleagues using mitochondrial DNA (Kasamatsu *et al.*, 1971; Schmir *et al.*, 1974) or two single-stranded DNA chains, as in the alkaline titration method (Vinograd *et al.*, 1968; Schmir *et al.*, 1974; Wang, 1974) in which all the guanines and thymines are negatively charged.

The second type of method has been published only recently (Wang, 1978,1979). It depends on a detailed interpretation of the precise positions of gel electrophoresis banding patterns produced by circular DNA duplexes, all about 5000 base-pairs long, which differ from one another by an exactly known number of base-pairs, their difference being either in the range 1 to 58 base-pairs or even higher.

All the different methods lead to the same general conclusion that there are about ten base-pairs per turn for DNA in solution. The more recent work of Wang (1978,1979) has refined this figure. It shows that in solution, under standard conditions of salt and temperature, the number is $10 \cdot 4 \pm 0 \cdot 1$.

Here we shall consider in detail only one of the earlier methods, that of Liu & Wang (1975) on PM2 DNA. For details of the other methods the reader is referred to the papers cited above.

Liu and Wang first prepared PM2 native supercoiled DNA, which is known from ethidium bromide titrations to have $\Delta Lk \simeq -100$. From this material they produced super-supercoiled DNA having $\Delta Lk \simeq -200$. They then annealed this to short single-stranded fragments of PM2 DNA. Because the circular DNA was supercoiled, these fragments combined with it until the entire configuration became relaxed as judged in the electron microscope. That is, in places the original chains of the circular DNA separated and each combined with short, single-stranded complementary stretches. At these regions, therefore, the two *original* chains ran approximately side-by-side, in the sense that they did not twist round each other. Since total Lk

is unaltered, the twisting of the remaining part of the molecules is *increased* and is thus no longer locally supercoiled, so that eventually the entire circular DNA assumes a fairly open (relaxed) configuration rather than a tight (supercoiled) one.

Now PM2 DNA has about 10,000 base-pairs. To relax the super-supercoiled DNA (for which Lk had been shown to be roughly -200), it was found by Liu & Wang (1975) that approximately 20% of its length needed to be covered by the single-stranded complementary fragments. Thus "straightening" 20% of the DNA removed 200 turns. Therefore, the total number of turns must have been about five times this: that is, Lk was roughly 1000 for relaxed PM2 DNA. This is what is expected for a classical DNA double-helix having 10,000 base-pairs, whereas the New Zealand SBS structure would predict that Lk was about 100.

In contrast to the views expressed above, Pohl & Roberts (1978) believe that the left and right handedness of the different segments of the presumed SBS structure could survive denaturation "due to base-stacking interactions." We cannot accept this suggestion as at all plausible, because it is almost impossible to conceive conformations protected by a sufficiently high activation energy to prevent some degree of randomization of the local handedness during the heating period. Nor do we feel it likely that the unwinding angle for ethidium bromide is 5·2°, as they suggest, rather than 26°. It should be noted that Pohl and Roberts also believe that the nicking-closing enzyme does not in fact break and rejoin polynucleotide chains.

The only way to attempt to escape our conclusion would be to assume that the chains in these "denatured" regions must always have a certain number of crossovers. One must then postulate that this number, per unit length of DNA, is fairly high and is the same whether both sides are double-stranded (as in the experiment of Liu & Wang described above) or when one side is double-stranded and other single-stranded (as in the D-loop experiment) or when both sides are single-stranded and charged as in the alkaline titration experiment. This interpretation is so forced that we can rule it out. Nor do we see how the SBS model can explain the recent experiments by Wang (1978,1979) on the band patterns formed by DNA molecules which differ slightly in length.

In addition, as stated above, there is much indirect structural evidence, such as model building, single crystal studies of base-pairs (with or without intercalated drugs) and the structure of transfer RNA which all suggest that duplex DNA has about ten base-pairs per turn, rather than 100 or higher, whereas there is no reliable experimental evidence which suggests the contrary.

It might be claimed that while circular viral DNA forms a classical double helix, straight DNA in solution forms a SBS structure. This seems highly unlikely. Nor can DNA in chromatin differ too much from a classical double helix, because the observed ΔLk between DNA in chromatin and in solution is too small, being about unity for every 200 base-pairs or so.

6. The Handedness of DNA

What about the handedness of the double helix? Here the argument is suggestive but not conclusive. The data show without doubt that bound ethidium reduces supercoiling of native DNA molecules. One ethidium molecule alters the local twist by about 26°. From model building and general considerations it seems unlikely that it does this by *increasing* the local twist (i.e. from 36° to 62°). It is more likely to

decrease it (from 36° to 10°), and this is supported by certain X-ray studies (Tsai et al., 1977; Jain et al., 1977) as well as by a comparison between denaturation and ethidium binding (see the review by Bauer, 1978).

If it had been firmly established, using the electron microscope, that the usual supercoiled DNA formed a *right*-handed double-helical supercoil (which rather tiresomely is equivalent to a left-handed solenoidal supercoil; see Fuller, 1971) this would strongly indicate that DNA itself is right-handed, because of the ethidium bromide evidence outlined above. Unfortunately the present experimental evidence on supercoiled DNA (Schmir et al., 1974; Bourguigon & Bourgaux, 1968; Rhoades & Thomas, 1968; Pulleyblank & Morgan, 1975) is suggestive rather than conclusive. There seems to be no good reason why a careful study should not be able to decide the true hand but until this has been done one must reserve judgement. There are of course a number of other suggestive lines of evidence, such as the ease of model building, X-ray studies on crystals of small polynucleotides of one sort or another and also the fact that the short RNA double-helices in transfer RNA are right-handed. There seems rather little doubt that the DNA double-helix is right-handed and that this will be firmly established by experiment before too long but the present evidence might not be strong enough to convert a sufficiently obstinate skeptic.

7. Conclusion

The SBS structure is thus incorrect, but this is not to say that the proposals have not served a useful purpose. They have shown rather clearly that while certain general features of the classical double-helix are established beyond reasonable doubt (special cases aside), other features need more careful scrutiny. The SBS model was ingenious because it incorporated the well-established features while altering the less certain ones. It has undoubtedly made us sharpen the arguments for the double helix. It has raised the question of how far a structure can depart from a double-helix and still give the very striking absences seen in the diffraction pattern. More calculations here would be of value. Above all, it has underlined a need that has been apparent now for some time, but which seemed perhaps less urgent than it does now. This is the solution, to high resolution, of single crystal structures of *short* lengths of the DNA double-helix having a defined base-sequence. This is now technically possible, both from the supply side and from the X-ray side, given a little luck. From these we could obtain more exact parameters than we could ever hope to obtain from fibers. We could also see how the details of the structure vary with base-sequence. The stereochemistry could be compared with the detailed structures now being proposed by computation alone (see Levitt, 1978). In addition the diffraction data could be used to show that the helix is right-handed, as has been done for transfer RNA. Even so, it might be sensible to build and calculate the energy of the best *left*-handed structure and of the best SBS one, since it is by no means certain that, under certain conditions, DNA cannot be forced into such configurations. DNA is such an important molecule that it is almost impossible to learn too much about it.

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