DISCUSSION

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DR. MANDEL: Prof. O'Konski, does the average relaxation time, which you measured for the superhelical structure, really correspond to the rotational relaxation time?

DR. O'KONSKI: In terms of a flexible model, yes. That is, the times are within the range that I would expect. If you would like a segmental model, one way of looking at it is to consider that there are segments of various lengths and inertial effects are negligible, so you can calculate the frictional coefficient of rodlike segments and from that you can get a distribution of relaxation time. If you look at the shortest relaxation time, about ten microseconds, that would correspond to a length of about 2,000 Å for the shortest persistence length.

Now that corresponds quite nicely with the expectation of a persistence length on the order of a couple of thousand angstrom for DNA. That also raises a point with respect to Prof. Houssier's calculation using your model where the discrepancy between the persistence lengths from this measurement and from others and B values he calculates is quite appreciable.

DR. MANDEL: One has to be very careful if one uses persistence length, because I think the wormlike chain is only well defined at the dilution.

DR. O'KONSKI: We are at a very low concentration of the DNA, down to the order of micrograms per mil, and in that region even with the longer calf thymus DNA, the relaxation times go asymptotically to a constant value.

DR. MANDEL: If the molecules are very large, one has to be very careful to define what infinite dilution is. One parameter we can use is the overlap volume, supposing every molecule must turn more or less freely without touching each other at infinite dilution. That should define the region of infinite dilution, and I have the feeling that with very large molecules, this low concentration where no overlap occurs must be really low.

DR. O'KONSKI: I have done these calculations years ago, and I am confident that we are at the essentially infinite dilution value for the PM-2 DNA.

DR. HOUSSIER: We should not compare the values that were presented by Professor Mandel from the dielectric dispersion and the one I presented from electric birefringence, for two reasons.

First of all, the ionic strength was not the same in the two measurements. Second we are discussing average values and the type of averages that we are obtaining from relaxation processes, and from electric polarizability measure-

ments we do not get the same type of average, so we cannot strictly make the comparison this way.

DR. O'KONSKI: Dr. Houssier, you gave for DNA a length of 32,000 Å and an L zero value of 4,000–5,000 Å, which you did not explain. Could you tell me what that value of L-zero corresponds to?

DR. HOUSSIER: I think all the L zero values correspond to the equivalent length calculated from a rigid model.

DR. O'KONSKI: That seems to suggest a very long persistence length, about ten times longer than the B value you calculated from Mandel's theory, which seems to me to be a very serious discrepancy.

DR. HOUSSIER: You don't obtain the persistence length if you use a rigid-rod model. If you analyze the data in terms of a wormlike chain model, you always obtain much lower values of L.

DR. MANDEL: The persistence length does not measure the length of equivalent chains which can represent the same end-to-end distance, for instance. It is just a measure of the rigidity of the chain. The persistence is therefore defined clearly, mathematically or physically, so that there is not direct relation between the persistence length and the length of any model one uses to present the wormlike chain.

DR. M. NADAUTA (*Yale University, New Haven, Conn.*): Dr. Houssier, did you ever try to study the birefringence with *H*-1-depleted chromatin?

DR. HOUSSIER: Yes, I mentioned the results on this. The general observation is that the birefringence is not very much affected by the removal of the *H*-1 fraction. But the reversal of the sign that we observed in the presence of manganese is not observed if *H*-1 is removed.

DR. L. E. CHURNEY (*NIH, Bethesda, Md.*): It seems to me that the only way in which a relaxation time corresponding to persistence length will show up in one of these measurements is if it happens to coincide with one of the normal modes of the flexible molecule. Otherwise, you just get any relaxation times corresponding to the overall motion of the molecule as well as possibly to some of the normal modes in the more flexible ones. I could just add that we made measurements on very small fragments of DNA and on polyadenylic acid fragments. Within the accuracy of a measurement, we see only a single relaxation time of the order of 3 or even 4 log units in the decay time.

DR. O'KONSKI: Similar measurements were made by Dr. Stellwagen on sonicated DNA years ago, and they behave like rigid rods. I want to make a general remark now. Certainly, one needs to use a distribution of relaxation time to represent the data. The three discreet values we use are only a mathematical modeling of the actual relaxation process for a flexible macromolecule. You can get good agreement even though there may be many modes involved. One has to do a transformation of the relaxation data to calculate the distribution function to get at those modes.

The point I am making with respect to the discrepancy is this. The rigid rod has a relaxation time proportionate to the cube of the length, so that relaxation time is a sensitive measure of the length. If you have a discrepancy on the order of ten in length, that corresponds to a discrepancy on the order of a thousand relaxation time. I am inclined to think that the parameter of a few hundred angstroms

calculated from dielectric relaxation measurement might correspond to the mean distance a counterion has to travel in a diffuse layer.

DR. GOLDBERG (*University of Pittsburgh, Pittsburgh, Pa.*): I wanted to ask Dr. Schwarz if the 15 kilovolts per centimeter he spoke of was an internal electric field or an external electric field, or whether there is no significant difference between them in this experiment.

DR. SCHWARZ: The fifteen kilovolt per centimeter is an externally applied electric field, but in this case, because we have organized structures (dipole moments along the helical axis), there is no difference between internal and external field strengths.

DR. STOCKMAYER: I just wanted to say a word about the theoretical situation with respect to the wormlike chain. Actually, if it were frozen into a rigid wormlike conformation with no change of shape, the calculation of the rotatory diffusion would be quite feasible, with appreciable hydrodynamic details. However, the real DNA-type chain probably is also flexing itself, and it is not a bead and spring model, but not a rigid rod. It is something in between. There are many modes of motion, but they are not independent normal modes, so I think the theoretical problem in that case is really formidable. I think the experimenters are going to be forced for a while to deal with effective L-zero values such as Professor Houssier displayed, having a precise theoretical label to put on them.