Buoyant Density Studies on Natural and Synthetic Deoxyribonucleic Acids in Neutral and Alkaline Solutions*

(Received for publication, January 13, 1972)

ROBERT D. WELLS AND JACQUELYNN E. LARSON

From the Department of Biochemistry, College of Agricultural and Life Sciences, University of Wisconsin, Madison, Wisconsin 53706

SUMMARY

Equilibrium buoyant density centrifugation studies in CsCl and Cs₂SO₄ solutions (both neutral and alkaline) were performed on 11 DNA polymers and 12 naturally occurring DNA's. For the polymers, no apparent relationship exists between DNA nucleotide composition and the extent of density change on alkaline titration. Thus it is not possible to predict the nucleotide composition of a natural single-stranded DNA from its shift in buoyant density.

Large decreases in buoyant density were found on alkaline titration of some DNA polymers in Cs₂SO₄ solution. Also, natural DNA's show a net density decrement due to alkaline titration. Contrastingly, in CsCl solutions, only buoyant density increases were observed.

To determine if these assumptions are valid, we have performed analytical density gradient analyses in CsCl and Cs₂SO₄ solutions, at both neutral and alkaline pH values, on a variety of natural and synthetic DNA's. The results do not show a simple correlation between density change, from neutral to alkaline solution, and base composition.

The density values reported herein serve as useful references for characterization of new satellite DNA's with repeating nucleotide sequences.

EXPERIMENTAL PROCEDURE

DNA Preparations—The single-stranded DNA polymers were prepared by preparative cesium chloride density gradient centrifugation of the parent duplexes in alkaline solution (3). The parent duplexes were prepared by described procedures and were: poly(dA)-poly(dT) (4), poly(dI)-poly(dC) and poly(dG)-poly(dC) (13), and poly(dT-dG)-poly(dC-dA) (14). The single-stranded DNA's were characterized by spectral analyses and were shown to be free (less than 5%) of any contaminating DNA by analytical density gradient centrifugation. Because of the unexpected results observed with poly(dG), this polymer was prepared by strand separation of both poly(dG)-poly(dC) and poly(dT)-poly(dC). Identical results were uniformly observed for all preparations of this DNA. Poly(dT-dC) was prepared by treating poly(dT-dC)-poly(dG-dA) (15) with formic acid-diphenylamine mixture according to Burton and Petersen as previously described (16). After extraction of the reaction mixture, the polymer was exhaustively dialyzed to remove degradation products. Spectral analysis served to characterize the polymer.

Poly(dA-dT)-poly(dA-dT), poly(dI-dC)-poly(dI-dC), and poly(dG-dC)-poly(dG-dC) were prepared as described (2, 4).

Bacteriophage M13 DNA (gift of R. W. Sweet) was prepared by extracting the virus (gift of D. Prati, University of Wisconsin) with phenol and subsequent dialysis (2). Sedimentation velocity studies in 0.9 M NaCl-0.1 M NaOH solution were performed both on the native DNA and on the DNA after mild acid treatment (17); the results indicated that the native DNA was completely (within experimental error) in the circular form. The single-stranded DNA's from H-1 virus and the minute virus of mice (gifts of L. Crawford, Imperial Cancer Research Fund, London) were isolated from the viruses by the sodium dodecyl sulfate procedure of Crawford (18).
DNA (gift of J. F. Burd) was isolated from the virus (gift of William Salivar, Marquette University) by the method used for M13 DNA. *Cancer productus* (dA-dT) was prepared as described (19). DNA's from *Micrococcus luteus*, *Rhederichia coli*, *Bacillus subtilis*, *Bacillus cereus*, *Cytophaga johnsonii*, and T4 phage were prepared as previously described (19) (gifts of L. K. Miller, R. M. Bock, P. F. Schendel, W. Szybalski, and H. Echols). Salmon sperm DNA was purchased from Worthington.

Typical molecular weight values for the DNA polymers have been reported (3) and range from 0.5 to 25 × 10^6. Most of the DNA polymers used herein were the actual preparations characterized by other determinations including ultraviolet spectra (2), circular dichroism spectra (2), absorbance-temperature profiles (2, 14, 20), actinomycin D-binding studies (19, 21), some analytical buoyant density studies (2, 3), and some x-ray diffraction studies (22, 23).

**Density Gradient Centrifugation** Density gradient centrifugation studies were performed in a model E ultracentrifuge modified for four cell operation as previously described (2). The CsCl runs were at 52,640 rpm and the CsSO₄ runs were at 44,770 rpm; all were performed at 25°C. All neutral CsCl values are relative to an E. coli DNA density of 1.703 g per ml. The actual density marker was usually poly(dA-dT) (1.672 g per ml) (3). In alkaline CsCl solution, poly(dA-dT)-poly(dA-dT) was the marker at 1.722 (3). This DNA also served as the density marker in neutral and alkaline CsSO₄ solutions; the determination of the absolute value in neutral solution was reported (19). The alkaline density value of 1.416 g per ml for this DNA was established by two methods. First, three separate density determinations by the isoconcentration distance method of Hill, Voet and Vinograd (24) each gave a value of 1.416 g per ml; second, identical neutral solutions containing poly(dA-dT)-poly(dA-dT) and poly(dA) were banded at the same time in two cells. Both bands in both cells were found to perfectly align and the density of poly(dA) was found to be 1.379 g per ml, in good agreement with a previously reported value (25). Next, 0.005 ml of 4 M NaOH was added directly into one cell with a Hamilton syringe and 0.005 ml of water was added to the second cell. Upon attainment of equilibrium, the poly(dA) bands were still in perfect alignment in the two cells, indicating that the density of poly(dA) in CsSO₄ solution is not changed in alkaline solution, as reported previously (25). However, the poly(dA-dT) poly(dA-dT) clearly exhibited the less dense value of 1.416 g per ml in alkaline solution.

The density values for the polymers were obtained on at least two different authentic preparations for each polymer. All density values are reproducible within ±1 mg per ml unless stated otherwise. Neutral density gradient studies were performed in 2 × 10⁻³ M sodium phosphate-1 × 10⁻⁴ M EDTA (pH 7.3). Alkaline density gradient studies were performed in 0.062 M NaOH solution; the pH of the complete solution just prior to (or just after) centrifugation was measured as 12.5 to 13.3 with a small combination electrode (20) which had been standardized with a calcium hydroxide standard solution (pH 12.5 at 25°C). At these quite alkaline pH values, it is realized that these values simply represent instrument readings. However, they are reproducible from run to run. Vinograd et al. (11) have inferred that the high concentration of salt which is present does not affect this reading.

**RESULTS**

Guanine and thymine bases are ionized in alkaline solution; thus, it is expected that a DNA containing these bases would become more dense in alkaline solution due to the subsequent acquisition of cation ions. In addition, if a DNA contains any secondary structure in concentrated salt solution, this ordered configuration will be virtually eliminated in alkaline solution, thus providing for an additional density increment. However, the latter increment is probably relatively small compared to the former increment (11).

**CsCl Density Gradients**—The buoyant densities of eight single-stranded DNA polymers in neutral and alkaline cesium chloride solution are shown in Table I. Three of the single-stranded DNA's studied contain only bases which are normally ionized at pH 7.3 and are poly(dA), poly(dC), and poly(dC-dA). Poly(dA) and poly(dC-dA) do not increase in density as the pH of the solution is raised but, in fact, show a slight decrement of 5 and 4 mg per ml, respectively. That these observed decrements are genuine was proved by the following additional experiment. Two identical solutions of polymer in salt solution at pH 7.3 were centrifuged to equilibrium when the bands were found to perfectly align in the two cells. Then, 0.010 ml of water was added to one cell and 0.010 ml of 4 M NaOH was added to the other cell. On recentrifuging the DNA solutions to equilibrium, the density decrease elicited by the alkali was confirmed. The reason for the density decrement is unknown at present; however, other density decrements are observed (see below).

Poly(dC) contains only a single base which is not customarily titrated in alkali (the enol pK of cytidylic acid is 13.2 (26)). Contrary to expectations, Table I shows that this DNA increases in density 37 mg per ml when the pH is raised from 7.3 to 12.5 to 13.3. This is due to poly(dC) existing in a highly ordered state at pH 7.3 since it has an unusually high pK (27). Evidence for this interpretation is presented below with a complete titration curve in CsSO₄ solution. However, the density increment for poly(dC) is greater than that for any other polymer (Table I), even those containing only titratable bases. This increment (37 mg per ml) is appreciably greater than expected solely on the basis of secondary structure (11).

Poly(dT), poly(dG), and poly(dT-dG) contain only bases which are normally ionized at pH 12.8. Table I shows that all

| DNA | CsCl density at | Density change |
|-----|----------------|----------------|
| pH 7.3 | pH >12.5 | g/ml | mg/ml |
| Poly(dA) | 1.628 | 1.623 | -5 |
| Poly(dC) | 1.685 | 1.722 | +37 |
| Poly(dC-dA) | 1.689 | 1.685 | -4 |
| Poly(dG) | 1.739 | 1.774 | +35 |
| Poly(dG) | 1.754 | 1.780 | +39 |
| Poly(dT-dG) | 1.794 | 1.826 | +32 |
| Poly(dT-dC) | 1.734 | 1.741 | +7 |
| Poly(dI) | 1.810 | 1.834 | +24 |

* Broad bands were found for all preparations; this value is ±3 mg per ml.

* The value previously reported (3) is incorrect.
three of these single-stranded DNA's undergo a marked density increase when the cesium chloride solution is made alkaline. However, the density increase was not approximately the same for all three cases. Poly(dT-dG) showed a density increase of 32 mg per ml, whereas poly(dT) and poly(dG) showed density increases of 35 and 20 mg per ml, respectively. Poly(dT-dG) has no ordered structure in neutral solution as judged by absorbance-temperature studies. It has been reported that poly(dT) has no secondary structure in neutral solution (25), whereas a highly ordered structure might be expected for poly(dG) under these neutral conditions. Unexpectedly, poly(dG) shows the smallest buoyant density increase which might be interpreted as indicating that this DNA undergoes the least loss of ordered structure as the solution is made alkaline and hence possesses little (or no) order in neutral solution. These results suggest that each of these polymers has unique and characteristic properties, which are as yet poorly understood. In addition, it should be noted that the density increase observed for these three single-stranded DNA's, which contain 100% alkali-titratable nucleotides, is approximately the same as observed for naturally occurring single-stranded DNA's which contain only 55 to 60% guanine plus thymine (see below).

Poly(dT-dC) shows a density increment of only 7 mg per ml on titration which is much less than found for either poly(dC) or poly(dT). Poly(di) shows a density increment of 24 mg per ml on titration.

For comparison, density studies were performed on four naturally occurring single-stranded DNA's (Table I). φX174, M13, minute virus of mice, and H-1 DNA all contain 54 to 57% guanine plus thymine. Accordingly, they have similar buoyant density values in both neutral and alkaline solution. The satellite (dA-dT) from C. productus, which contains a small amount of guanine + cytosine (28), behaves similarly to biosynthetic poly(dA-dT)-poly(dA-dT) (see below).

The three double-stranded DNA polymers, poly(dA-dT)-poly(dA-dT), poly(dG-dC)-poly(dG-dC), and poly(dI-dC), are also amenable to this investigation due to their repeating, self-complementary nucleotide sequence. Because of the identical nature of the complementary strands of each of the double helices, each DNA exhibits a single band in both neutral and alkaline gradients. Density increments at least as large as those found for natural single-stranded DNA's were expected since all three DNA's possess highly ordered structures in neutral solution (2, 20). In addition, exactly one

### Table II

| DNA                     | pH 7.3 | pH > 12.5 |
|-------------------------|--------|-----------|
| φX174                   | 1.719  | 1.760     |
| M13                     | 1.719  | 1.755     |
| Minute virus of mice    | 1.719  | 1.759     |
| H-1                     | 1.722  | 1.760     |
| Cancer productus (dA-dT)* | 1.679  | 1.726     |

* This DNA is not single-stranded at neutral pH but is renaturable.

### Table III

| DNA | Density at | Density change |
|-----|------------|----------------|
|     | pH 7.3     | pH > 12.5      |
| a. CsCl solution | Poly(dA-dT) | Poly(dA-dT) | +50 |
| Poly(dG-dC) | Poly(dG-dC) | Poly(dG-dC) | +52 |
| Poly(dC-dA) | Poly(dC-dA) | Poly(dC-dA) | +31 |
| b. CsSO4 solution | Poly(dA-dT) | Poly(dA-dT) | -9  |
| Poly(dG-dC) | Poly(dG-dC) | Poly(dG-dC) | +17 |
| Poly(di) | Poly(di) | Aggregate* | -17 |

* Five different authentic preparations of poly(dT-dG) were studied. All showed a single sharp peak in alkaline CsSO4 solution at 1.492 g per ml. However, in neutral CsSO4 solution, four bands (in varying amounts) were observed for all five preparations (1.548, 1.537, 1.524, and 1.494). These densities are ±2 mg per ml.

### Table IV

| DNA | CsSO4 density at | Density change |
|-----|------------------|----------------|
|     | pH 7.3           | pH > 12.5      |
| Poly(dA) | 1.370  | 1.380  | +1  |
| Poly(dC) | 1.520  | 1.422  | +8  |
| Poly(dC-dA) | 1.416  | 1.416  | -9  |
| Poly(di) | 1.428  | 1.447  | +19 |
| Poly(di) | 1.592  | 1.540  | -52 |
| Poly(dT-dG) | Aggregate* | 1.492  | -17 |
| Poly(di) | 1.500  | 1.470  | -12 |

* CsSO4 Density Gradient—In a further effort to establish a relationship between nucleotide composition of single-stranded DNA and buoyant density, analyses were performed in both neutral and alkaline CsSO4 solutions. Unexpectedly, buoyant density decrements induced by titration were found for a variety of DNA's. Table IIIb shows that both poly(dA-dT)-poly(dA-dT) and poly(dI-dC)-poly(dI-dC) undergo a density decrease, whereas poly(dG-dC)-poly(dG-dC) undergoes a density increase on titration. Two additional determinations

1 R. D. Wells, unpublished work.
are described under “Experimental Procedure” that verify the density decrements.

Table IV shows that neither poly(dA) nor poly(dC-dA) undergo dramatic density shifts on titration as expected from Table I. However, poly(dC) undergoes a density decrement of 98 mg per ml. Fig. 1 shows the titration of poly(dC) from pH 7.1 to 12.6. A sharp drop in density is found at pH 7.9. This is consistent with the high pK of poly(dC) (27). The highly ordered protonated structure has a density of 1.520 and the single-stranded coil has a density of 1.422. Poly(dT) undergoes a density increase of 19 mg per ml but poly(dG) undergoes a density decrease of 52 mg per ml. Poly(dI) shows a very large density decrement. A similar analysis was not possible for poly(dT-dG) due to aggregation in neutral solution; however, it clearly undergoes a density decrement also.

Table V shows that the four single-stranded viral DNA’s also undergo a density decrement of 10 to 13 mg per ml on titration. As in CsCl solutions, C. productus (dA-dT) behaves similarly to biosynthetic poly(dA-dT). studies were performed on seven natural double-stranded DNA’s (Table VI). The density values on native DNA’s are in excellent agreement with reported values (1). On heat denaturation, density increments of 19 to 28 mg per ml were observed. However, in contrast to the results found in CsCl solution (11), greater increments were found for DNA’s with higher guanine + cytosine contents.

Buoyant density increments were also found on alkaline titration of the DNA’s, except for T₄ DNA (Table VI). However, in no case was the increment induced by titration as great as that induced by heat denaturation. A linear relationship was found between base composition and buoyant density increments induced by titration.

Thus, in Cs₂SO₄ solution, titration of a DNA (with ensuing denaturation) gives rise to a smaller density increment than the loss of secondary structure alone (heat-denatured DNA). Hence, a net buoyant density decrement is found due to titration of the bases. The opposite behavior is found in CsCl solution (see “Discussion”). The reason for this difference in behavior between CsCl and Cs₂SO₄ solutions is unclear but may be related to the effective hydration of the nucleic acid under different conditions (30).

**DISCUSSION**

Vinograd et al. (11) previously reported that the magnitude of the change in buoyant density induced by alkaline titration of a DNA was comprised of at least two components. First, denaturation of the DNA helix gave a density increment of 13 to 19 mg per ml (in CsCl solution); larger increases were found for DNA’s with lower guanine + cytosine contents. Second, alkaline titration of the bases gave an additional density increment of 43 to 49 mg per ml (in CsCl solution); larger increases were found for DNA’s with higher guanine + cytosine contents.
In CsCl solutions, alkaline titration produced an over-all density increment (native to denatured titrated DNA) of 62 to 63 mg per ml which was invariant with DNA base composition.

This study was undertaken to attempt to establish a relationship between DNA nucleotide composition and buoyant density change induced by alkaline titration. DNA polymers were the models used because of their simplicity and because unequivocal conclusions can be drawn since they contain completely defined nucleotide sequences and compositions. The results clearly show that a simple relationship does not exist. The results of this more complete study indicate that such extrapolations are not valid.

Unexpectedly, alkaline titration of all natural DNA’s and most polymers in CsSO₄ solution elicits a net density decrement, not a density increment as found in CsCl solution. The reason for this behavior is not clear at present. An understanding of this, and related buoyant density phenomena may have to await the elucidation of the physical and chemical parameters which govern the observed density of a DNA sample.

REFERENCES

1. Szybalski, W. (1968) Methods Enzymol. 12, 330-360
2. Wells, R. D., Larson, J. E., Grant, R. C., Shortle, D. E., and Cantor, C. R. (1970) J. Mol. Biol. 54, 465-497
3. Wells, R. D., and Blair, J. E. (1967) J. Mol. Biol. 27, 273-288
4. Burd, J. F., and Wells, R. D. (1970) J. Mol. Biol. 53, 435-459
5. Hogness, D. S., Doerfler, W., Egan, J. B., and Black, L. W. (1966) Cold Spring Harbor Symp. Quant. Biol. 31, 123-138
6. Headson, Z., and Szybalski, W. (1967) Virology 32, 633-643
7. Flamm, W. G., Walker, P. M. B., and McCallum, M. (1969) J. Mol. Biol. 42, 441-455
8. Leffler, A. T., Luborsky, S. W., and Mora, P. T. (1969) Nature 223, 1153-1154
9. Leffler, A. T., Chesskooff, E., Luborsky, S. W., McFarland, V., and Mora, P. T. (1970) J. Mol. Biol. 48, 455-468
10. Hershberger, C., Mickel, S., and Rownd, R. (1971) J. Bacteriol. 106, 238-242
11. Vinograd, J., Morris, J., Davidson, N., and Dove, W. F. (1963) Proc. Nat. Acad. Sci. U. S. A. 49, 12-17
12. Doerfler, W., and Hogness, D. S. (1965) J. Mol. Biol. 14, 257-260
13. Inman, R. B., and Baldwin, R. L. (1964) J. Mol. Biol. 8, 452-469
14. Wells, R. D., Ohtsuka, E., and Khorana, H. G. (1965) J. Mol. Biol. 14, 221-240
15. Byrd, C., Ohtsuka, E., Moon, M. W., and Khorana, H. G. (1965) Proc. Nat. Acad. Sci. U. S. A. 53, 79-86
16. Harwood, S. J., and Wells, R. D. (1970) J. Biol. Chem. 245, 5625-5634
17. Mitra, S., Reichard, P., Inman, R. B., Bertsch, L. L., and Kornberg, A. (1967) J. Mol. Biol. 24, 429-447
18. Crawford, L. V. (1966) Virology 29, 605-612
19. Wells, R. D., and Larson, J. E. (1970) J. Mol. Biol. 49, 319-343
20. Grant, R. C., Kodama, M., and Wells, R. D. (1972) Biochemistry 11, 805-815
21. Wells, R. D. (1969) Science 166, 75-76
22. Langer, R. (1969) J. Cell Physiol. 74, 1-20
23. Mitsui, Y., Langridge, R., Shortle, B. E., Cantor, C. R., Grant, R. C., Kodama, M., and Wells, R. D. (1970) Nature 226, 1166-1169
24. Ifft, J. B., Voet, D. H., and Vinograd, J. (1961) J. Phys. Chem. 65, 1138-1145
25. Riley, M., Maling, B., and Chamberlin, M. J. (1966) J. Mol. Biol. 20, 350-380
26. Levene, P. A., and Simms, H. S. (1925) J. Biol. Chem. 65, 519-534
27. Inman, R. B. (1964) J. Mol. Biol. 8, 629-631
28. Smith, M. (1964) J. Mol. Biol. 9, 17-23
29. Inman, R. B., and Baldwin, R. L. (1962) J. Mol. Biol. 5, 172-184
30. Vinograd, J., and Hearst, J. E. (1962) in L. Zechmeister (Editor), Progress in the chemistry of organic natural products, Vol. 20, pp. 372-422, Springer-Verlag, Berlin
Buoyant Density Studies on Natural and Synthetic Deoxyribonucleic Acids in Neutral and Alkaline Solutions
Robert D. Wells and Jacquelynn E. Larson

J. Biol. Chem. 1972, 247:3405-3409.

Access the most updated version of this article at http://www.jbc.org/content/247/11/3405

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/247/11/3405.full.html#ref-list-1