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Correlation of Melting Temperature and Cesium Chloride Buoyant Density of Bacterial Deoxyribonucleic Acid

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Samples of bacterial deoxyribonucleic acid from bacteria having guanine plus cytosine (GC) contents in the range of 27 to 72 moles per cent GC were analyzed by optical melting (T_m) and equilibrium buoyant density methods. The relation between these properties is shown to be linear. The relative value of 1.99 moles per cent GC per degree C change in T_m is calculated, and a reference method for the calculation of GC contents relative to a standard is derived.

Considerable use of the knowledge of the mean guanine plus cytosine (GC) content of the deoxyribonucleic acid (DNA) of bacteria has been made for taxonomic purposes (8). The two methods most frequently employed for this determination have been the estimation of the midpoint (T_m) of the optical melting curve (11) and the reference method for CsCl buoyant density equilibrium (14). Both methods were calibrated against reported values of GC content gleaned from the literature, with the uncertainty attendant on the identities and equivalence of the biological materials. Gasser and Mandel (4) verified the linearity of the relation between the chromatographic analyses and the CsCl buoyant densities from a series of 45 pairwise analyses. A series of 12 samples in the range of 58 to 65% GC were analyzed for T_m and by paper chromatographic resolution of the hydrolyzed bases (2); although a general agreement of these data with the formula of Marmur and Doty (11) was found, there still is not sufficient data to establish the overall form of the relation. In this communication, we compare T_m and mean ρ_{CsCl} values of samples of DNA to show the linearity of this relation and to define the limits beyond which a disagreement in the values obtained for a particular sample might be indicative of an unusual component or structure of the DNA (14).

MATERIALS AND METHODS

DNA preparation. DNA samples were isolated from bacteria harvested in the late logarithmic phase of growth. Growth conditions and media were suited to the particular organism. The isolation procedure was modified from that of Marmur (10). In this modification the cell lysate was immediately deproteinized with phenol saturated with saline ethylenediaminetetraacetate (EDTA) (0.15 M NaCl, 0.1 M Na_2EDTA, *p*H 8.0). After treatment with ribonuclease, Pronase which had been previously incubated in SSC (0.15 M NaCl plus 0.015 M sodium citrate) at 37 C for 60 min was added to a final concentration of 100 μg/ml, and the mixture was incubated for 30 min at 37 C. The mixture was then chilled and extracted with phenol as above. These treatments were performed in addition to the manipulations of the original purification procedure (10). *Bacillus subtilis* phage 2C was the gift of J. Marmur; it was propagated in aerated cultures of *B. subtilis* Marburg at 37 C in Penassay broth (Difco). N_4 bacteriophage was obtained from G. C. Schito; it was propagated in *Escherichia coli* K-12S (15). Bacteriophage DNA was isolated by the phenol method (9). All DNA samples were dissolved and stored in 0.1 × standard saline citrate (SSC/10; SSC is 0.15 M sodium citrate, *p*H 7.0) at 5 C over $CHCl_3$. Samples were stored at concentrations of about 0.2 to 0.7 mg/ml and adjusted to final concentrations by appropriate additions of 10-fold concentrated SSC or dilution with distilled water and the final buffer. DNA concentrations were estimated from the absorbance at 260 nm assuming $E^{1\%}_{1\,cm} = 200$ in a Zeiss PMQ II spectrophotometer. All samples displayed A_{260}/A_{280} ratios of 1.94 to 1.96 and A_{260}/A_{230} ratios of 2.2 to 2.5. Sedimentation velocities by the boundary method at 25 C, in SSC at 50 μg/ml, were in excess of 19*S* for all samples of bacterial DNA.

T_m and ρ_{CsCl} determinations. The midpoint of the ultraviolet (UV) absorbance-temperature profile was determined in a Beckman DU spectrophotometer at 260 nm (11). Corrections were applied for absorbance measured at 320 nm and for the thermal expansion at

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each temperature interval. Temperatures were read to the nearest 0.1 C in the cuvette chamber by using a thermometer previously calibrated against a National Bureau of Standards reference.

Buoyant densities in CsCl of each DNA sample were determined in at least two of three different Spinco model E analytical ultracentrifuges. UV absorbance in the cells was determined at 265.4 nm and recorded by the photomultiplier scanner and on film. Centers of the peaks of the UV-absorbing bands were measured from the scanner tracings or from densitometer tracings of the UV photographs prepared with the aid of a Joyce-Loebl Mark III recording densitometer. The densities were calculated with respect to an internal standard of *B. subtilis* phage 2C DNA of a density of 1.7420 ± 0.0004 g/cm³ which was measured with respect to DNA of *E. coli* K-12 (sample no. 1, in Table 1 and Fig. 1) assumed to be 1.7100 g/cm³ (14).

Statistical treatment of data. Data were analyzed by using routine programs for regression and correlation analyses with the aid of an IBM 7094 computer.

The consistent fit analysis (6) used the OMNITAB program modified in Fortran IV by L. Jordan-Filho. This program is available in the Department of Biomathematics of this institution.

RESULTS

DNA species from each of the 33 bacteria listed in Table 1 were analyzed by CsCl buoyant density determinations at least three and as many as five times. Duplicate to quintuplicate

TABLE 1. *Buoyant densities in CsCl and midpoints of thermal transitions in SSC/10 of bacterial DNA species*

Species	Strain	Source[a]	Density[b]	T_m[c]
			g/cm³	C
1. *Mycoplasma hominis*	4330	W. H. Kelton	1.6860 ± 0.0008	62.65
2. *M. gallinarum*	PG16	W. H. Kelton	1.6860 ± 0.0011	63.45 ± 0.8
3. *M. arthritidis*	39	W. H. Kelton	1.6915 ± 0.0006	65.9 ± 0.6
4. *M. arthritidis*	07	W. H. Kelton	1.6910 ± 0.0008	66.3 ± 0.6
5. *Lactobacillus acidophilus*	65K	F. Gasser	1.6962 ± 0.0005	67.9 ± 0.17
6. *Leptospira* sp.	B16	C. D. Cox	1.6965 ± 0.0005	68.4 ± 0.4
7. *Lactobacillus jugurti*	10812	ATCC	1.6985 ± 0.0010	68.95 ± 0.8
8. *L. jugurti*	99	NCDO	1.6980 ± 0.0008	69.1 ± 0.7
9. *Cytophaga* sp.	9D	F. D. Cook	1.6990 ± 0.0008	70.15
10. *Cytophaga* sp.	3D	F. D. Cook	1.6995 ± 0.0006	70.2
11. *Lactobacillus fermenti*	A23272	F. Gasser	1.6997 ± 0.0006	70.9
12. *Pseudomonas atlantica*	301	NCMB	1.7023 ± 0.0006	71.2 ± 0.16
13. *Leptospira* sp.	H10	C. D. Cox	1.7050 ± 0.0008	72.4 ± 0.26
14. *Lactobacillus coprophilus*	106	F. Gasser	1.7040 ± 0.0014	73.0 ± 0.6
15. *L. bulgaricus*	11842	F. Gasser	1.7093 ± 0.0010	74.95 ± 0.25
16. *Escherichia coli*	0111_{a1}	C. Schnaitman	1.7097 ± 0.0013	75.1 ± 0.5
17. *E. coli*	0111_{a1} *ilva*	B. Altenburg	1.7095 ± 0.0013	75.4 ± 0.5
18. *E. coli*	K12 *his*323	T. S. Matney	1.7100 ± 0.0000	75.7 ± 0.22
19. *Lactobacillus fermenti*	0C663	F. Gasser	1.7122 ± 0.0007	75.5 ± 0.17
20. *L. fermenti*	236	CNRZ	1.7110 ± 0.0008	75.9 ± 0.3
21. *Enterobacter cloacae*	13047	ATCC	1.7130 ± 0.0008	77.6 ± 0.9
22. *Bifidobacterium bifidum*	12V	M. Sebald	1.7170 ± 0.0008	79.2 ± 0.4
23. *Spirochaeta stenostrepta*	Z1	E. Canale-Parola	1.7195 ± 0.0010	79.7 ± 0.8
24. *Pseudomonas schuylkiliensis*	B1104	NRRL	1.7198 ± 0.0005	80.1 ± 0.5
25. *Spirochaeta aurantia*	J1	E. Canale-Parola	1.7252 ± 0.0005	80.8 ± 0.1
26. *Nitrobacter agilis*	1	D. Hoare	1.7195 ± 0.0013	81.0
27. *Pseudomonas reptilovora*	B-963	P. V. Liu	1.7230 ± 0.0008	82.2
28. *Comamonas terrigena*	8461	ATCC	1.7235 ± 0.0008	82.4
29. *Thiobacillus novellus*	A1	D. Hoare	1.7265 ± 0.0005	82.8 ± 0.5
30. *Pseudomonas maltophilia*	301	N. Palleroni	1.7253 ± 0.0005	83.1 ± 0.5
31. *Thiobacillus novellus*	A2	D. Hoare	1.7260 ± 0.0010	83.6 ± 0.3
32. *Pseudomonas pseudomallei*	113	M. S. Redfearn	1.7278 ± 0.0005	84.3 ± 0.2
33. *Micrococcus luteus*	381	ATCC	1.7313 ± 0.0010	84.9

[a] Abbreviations for culture collections: ATCC, American Type Culture Collection; NCDO, National Collection of Dairy Organisms; CNRZ, Centre National de la Recherche Zootechnique; NRRL, Northern Regional Research Laboratory; NCMB, National Collection of Marine Bacteria.

[b] Mean density in CsCl ± σ calculated from reference DNA of *Bacillus subtilis* phage 2C (1.7420 g/cm³) with *E. coli* K-12 DNA (sample 18) assumed as 1.7100 g/cm³.

[c] Mean T_m ± σ measured in 0.015 M NaCl plus 0.0015 M Na_3 citrate, pH 7.0 (SSC/10).

determinations of the T_m in SSC/10 were made on the same samples. These data are recorded in Table 1 and plotted in Fig. 1; the standard deviations for samples on which the determinations were too few for individual calculation were plotted as the square roots of the pooled estimates of the variances in the measurements of $\bar{\rho}$ and $\bar{T}_m$ (i.e., $\sigma_\rho = 0.0008$ g/cm^3, $\sigma_{T_m} = 0.46$ C). Regression analysis (T_m, dependent; $\bar{\rho}$, independent) yields a best fit for the first order polynomial with a correlation coefficient of 0.98. This linear relation for our data is

$$T_m \text{ (SSC/10)} = 489.4\ (\rho - 1.556) \quad (1)$$

Because the least squares fit assumes no error in the independent variable, which is obviously not the case, the consistent fit (6), taking into account the variance in both dimensions, was calculated. This too is linear and is described by

$$T_m \text{ (SSC/10)} = 512.2\ (\rho - 1.563) \quad (2)$$

At present, there is no mathematical theory which will permit the calculation of the confidence bands for the consistent fit. As an approximation, we may assume that the confidence limits for the least squares fit will describe those about the consistent linear fit. At the 95% confidence level, the analyses of samples 25 and 26 fall outside

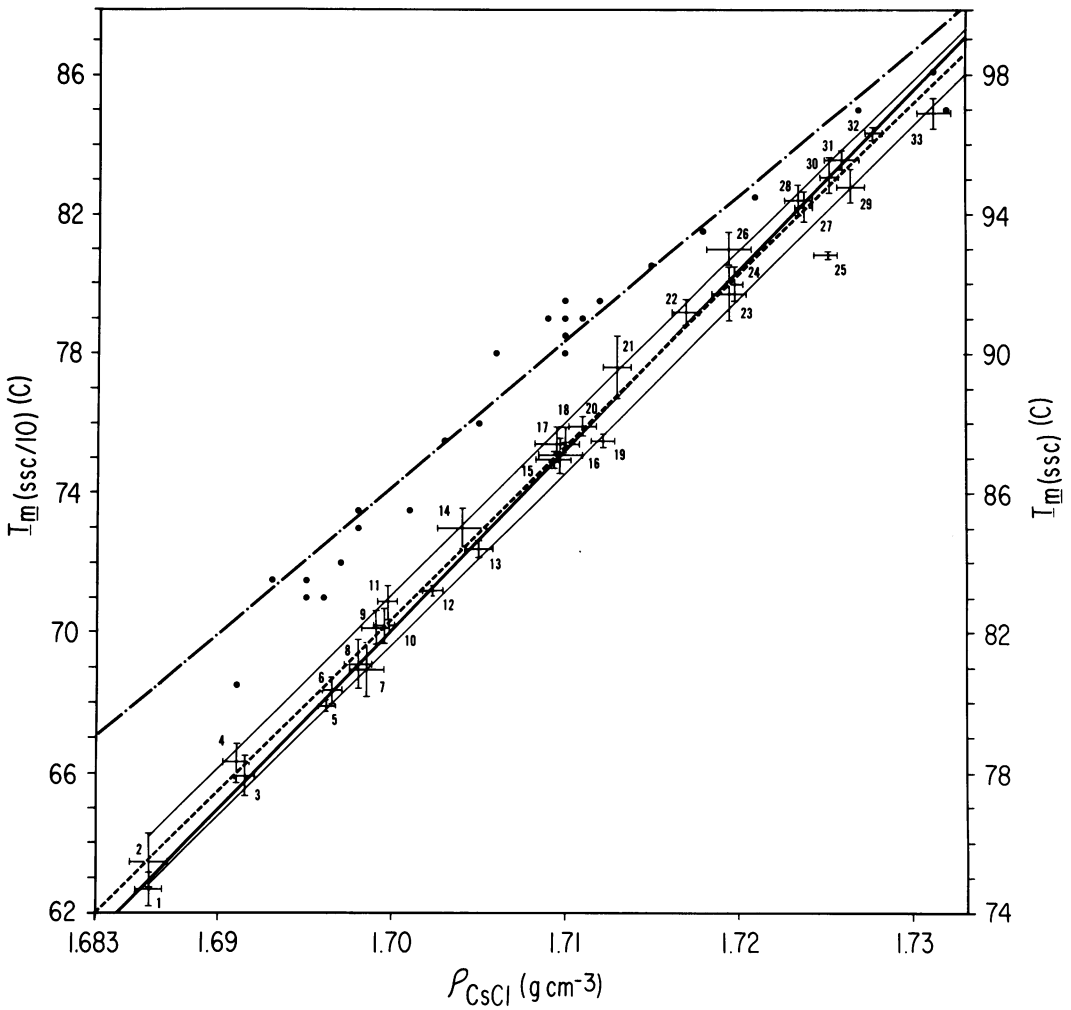

FIG. 1. *Correlation of T_m and buoyant density in CsCl of bacterial DNA. Determinations of ρ_{CsCl} are relative to E. coli K-12 DNA (sample 18) taken as 1.710 g/cm^3. Left ordinate refers to experimental values obtained in this study (samples 1 to 33 as listed in Table 1) and to least squares fit (— —), 95% confidence band (—), consistent linear fit (—) of these data. The right ordinate refers to the data of Table 2 (●) and the combined expression of equation 3 given in the text (— ·).*

this band about either fit. This, of course, may be a statistical probability but it does indicate the necessity of further investigation of new samples of DNA from the same origins, particularly because the melting curves of each sample demonstrated significant departures from the cumulative normal, or sigmoid, shape. That is, the absorbance rise as a function of temperature was much more gradual for the initial 50% of the total hyperchromicity than for all other samples.

In Fig. 1, we have also plotted the data for 29 of the 30 samples (Table 2) which were analyzed in the Harvard laboratory to give the original relations between GC content and each of the physical variables (11, 14). The analysis by each method was of the same preparation of DNA (J. Marmur, *personal communication*). Combining the equations for each relation to GC content we obtain

$$T_m \text{ (SSC)} = 418.2 (\rho - 1.494) \quad (3)$$

We note the difference in the slope of this equation from the slopes of equations 1 and 2 above. Displacement of the lines is to be expected because of the difference in ionic strengths of the buffers used in the two studies. There is some discrepancy in the literature as to the difference in T_m to be expected for a 10-fold dilution of the buffer. Schildkraut and Lifson (13) predict a reduction of 16.6 C, Silvestri and Hill (17) predict 15.4 C, Cantoni (1) cites 15.55 C, and Dove and Davidson (3) report 18.5 C. We have plotted the mean T_m values for five DNA samples obtained in various concentrations of SSC in Fig. 2. The slopes of the lines are parallel and all are linear in the range of concentration in which we are interested. The T_m in SSC is reduced by 16.3 ± 0.5 C when the samples are melted in SSC/10; this agrees best with the value of Schildkraut and Lifson (13). Within the limits of our measurements and under these conditions, we do not observe any obvious monotonic relation between the slope of the T_m and the logarithm of ionic strength and base compositions of the samples. Such a relation has been reported (12), but other data do not confirm this relation in the region where the individual slopes are constant (3, 5, 7), as is the case in this study.

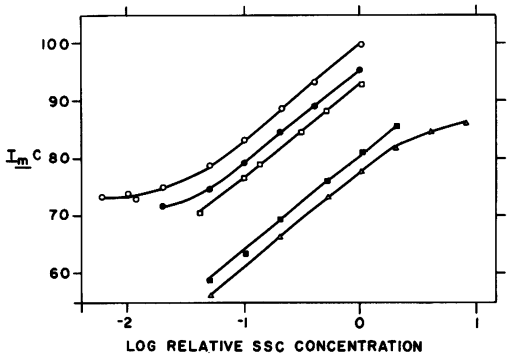

FIG. 2. *Variation of the T_m with the logarithm of the concentration of standard saline citrate (0.15 M NaCl + 0.015 M Na_3 citrate, pH 7.0). DNA from Thiobacillus novellus, ○; Spirochaeta stenostrepta, ●; Escherichia coli, □; Mycoplasma gallinarum, ■; B. subtilis phage 2C, △.*

The DNA of *B. subtilis* phage 2C is representative of a group of bacteriophages in which the T_m and buoyant densities are in gross disagreement with each other because of the replacement of thymine by hydroxymethyluracil. A density of 1.742 g/cm³ and a T_m of 77.7 C in SSC were found for this DNA. Coliphage N_4 DNA has been thoroughly characterized (16) and is a very

TABLE 2. *Buoyant density in CsCl and midpoint of thermal transitions in SSC for paired analyses of DNA from the literature*

Species	Density[a]	T_m[b]
	g/cm³	C
Aerobacter aerogenes	1.716	93.5
Bacillus cereus	1.696	83
B. megaterium	1.697	85
B. stearothermophilus	1.705	88
B. subtilis	1.703	87.5
B. thuringiensis	1.695	83.5
Brucella abortus	1.715	92.5
Clostridium perfringens	1.691	80.5
Diplococcus pneumoniae	1.701	85.5
Erwinia carotovora	1.709	91
Escherichia coli B	1.710	90.5
E. coli K-12	1.710	90.5
Haemophilis influenzae	1.698	85.5
Micrococcus lysodeikticus	1.731	99.5
M. pyogenes var. *aureus*	1.693	83.5
Mycobacterium phlei	1.732	97
Neisseria flavescens	1.706	90
N. meningitidis	1.709 (1.703)[c]	91
N. sicca	1.710	90
Pasteurella tularensis	1.695	83
Proteus morganii	1.710	91
P. vulgaris	1.698	85
Pseudomonas aeruginosa	1.727	97
P. fluorescens	1.721	94.5
Salmonella typhimurium	1.712	91
S. typhosa	1.711	90.5
Sarcina lutea	1.731	98
Serratia marcescens	1.718	93.5
Streptomyces viridochromogenes	1.729	100.5

[a] Taken from Table 1 of Schildkraut, Marmur, and Doty (14).

[b] Taken from Table 1 of Marmur and Doty (11).

[c] The value 1.703 appears to be a typographical error in the table cited in reference 14.

convenient source of monodisperse, double-stranded DNA of 4×10^7 daltons. A discrepancy in T_m and buoyant density was noted (ρ = 1.707 g/cm^3 and T_m in SSC of 87.2 C; reference 16). These values yield a point falling well outside the 95% confidence band of Fig. 1 after the correction for buffer strength is applied. We determined the density to be 1.703 g/cm^3 and the T_m in 0.10 SSC to be 71.6 C; these data do not indicate any discrepancy with the relations of equations 1 and 2 and a GC content of 45 moles per cent (16).

DISCUSSION

The relation between the midpoint of the optical thermal denaturation profile and the buoyant density in CsCl for double-stranded bacterial DNA samples is linear. This relation holds for samples with densities as low as 1.686 g/cm^3 and as high as 1.731 g/cm^3. Therefore, it is probable that it also holds for the entire range in which biological samples of DNA containing the regular Watson-Crick structure and the usual bases will be encountered. We conclude that the relation of T_m to GC content in DNA must also be linear because of the demonstration of linearity between buoyant density and chemically determined GC contents (4).

Using equation 2, the consistent linear fit relating T_m and ρ_{CsCl}, together with the equation of Schildkraut et al. (14), we can relate a T_m value obtained in SSC/10 to the mole fraction GC in double-stranded DNA as

$$GC = (Tm_{0.1SSC}/50.2) - 0.990 \quad (4a)$$

If the T_m is determined in any higher concentration of saline citrate buffer up to SSC concentration, then the logarithm of the relative increase in buffer concentration of SSC [log (SSC_x/0.10 SSC)] times 16.3 should be incorporated in the equation

$$GC = \{[Tm_{SSCx} - 16.3 \log \cdot (SSC_x/SSC_{0.1})]/50.2\} - 0.990 \quad (4b)$$

When T_m determinations are made on two DNA samples in a saline citrate buffer at *p*H 7.0 in this range of concentrations (0.0195 to 0.195 M Na^+), a 1.0 C difference in (T_m is equivalent to a difference of 0.0199 moles fraction of GC content. For the establishment of a reference method for determination of GC content by comparison of optical thermal transition midpoints, we use the following equation

$$GC_x = GC_{STD} + 0.0199 (T_{mx} - T_{STD}) \quad (5)$$

The GC contents are expressed in mole fractions and the T_m on the Celsius scale. Hence, if the standard chosen is DNA of *E. coli* K-12 and the GC content were assumed to be 0.51 (51% GC in accordance with assumed density of 1.710 g/cm^3 and the relation of Schildkraut et al. (14), a DNA of unknown base composition with a T_m 4.1 C higher than that found for the *E. coli* DNA would be estimated to contain 59.2 moles per cent GC and is predicted to have a buoyant density of 1.718 g/cm^3 from equation 2. The standard deviation of the determination then is the root mean square of the standard deviations of T_m for the sample and the standard. As a prudent practice, we recommended that individual laboratories establish their own standards of DNA (for T_m and ρ_{CsCl}) relative to *E. coli* K-12 DNA, rather than use equations 4a or 4b at face value. Minor differences in techniques and in chemical supplies used in different laboratories and in a given laboratory over a period of time may result in differences in reported T_m values.

Inasmuch as the major use of determinations of mean GC contents is as an exclusionary criterion in taxonomic studies, the matching of T_m or ρ_{CsCl} values should be sufficient, provided there is nothing unusual about the sample or samples being studied. The significant departure of a pair of values for T_m and ρ_{CsCl} from the consistent linear fit given here should serve as a warning that the analyses may be poor, or the sample degraded, contaminated with extraneous matter, or may contain unusual bases or sugar moieties. The latter circumstances have not been encountered thus far in bacterial DNA samples.

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