The Relationship between the Base Composition of Bacterial DNA and Its Intracellular Melting Temperature As Determined by Differential Scanning Calorimetry

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(Received 25 June 1987; revised 11 January 1988)

The correlation between the melting temperature of intracellular DNA, determined by differential scanning calorimetry (DSC) of whole bacteria, and its guanine + cytosine (G + C) content, was examined for 58 species of bacteria. Samples of vegetative cells were heated in a Perkin-Elmer DSC-2C at 10 °C min⁻¹ from 5 to 130 °C, cooled to 5 °C and then re-heated as before. Literature values for the mole fraction of G + C, X_{GC} , were linearly related to the temperature, T_{max} , at which the reversible peak, p_r , observed on the second heating run was at a maximum, via the equation $X_{GC} = (T_{max} - 73.8)/41.0$. This equation accounted for 91.9% of the variance in X_{GC} with 95% confidence limits of $\pm 7.3\%$, approximately 1.6 times the corresponding uncertainty ($\pm 4.5\%$) quoted by De Ley (*Journal of Bacteriology* **101**, 738–754, 1970) for estimates based on the spectroscopically determined melting temperature of purified DNA. Random errors of measurement of T_{max} did not greatly limit the precision of the prediction and it was concluded that factors additional to base composition affected the temperature of DNA melting within the bacterial cell. Displacement of T_{max} values from the fitted line was particularly noticeable in *Campylobacter*, *Corynebacterium* and *Bacterionema* species and part of the residual variation appeared to be species specific, possibly caused by differences in intracellular solute concentration.

INTRODUCTION

The guanine + cytosine (G + C) content of bacterial DNA varies between about 25 and 75 mol%. The value is constant for a given organism and is therefore considered an essential item in the description of any species. Base composition can be determined directly by hydrolysis of a DNA sample followed by chromatographic separation of the bases, or indirectly from buoyant density or melting point data (De Ley, 1970; Mandel *et al.*, 1968; Ko *et al.*, 1977).

Although these methods are widely used they require pure DNA as the starting point and are therefore time-consuming to do. Technical problems sometimes arise because of the difficulty of lysing certain types of bacteria to extract DNA, or because of contamination of DNA samples with polysaccharides or other substances. Simpler and quicker methods of determining the base composition of bacterial DNA would therefore have wide application in bacterial classification and identification.

The thermal denaturation of the components of bacterial cells can be observed and recorded by differential scanning calorimetry (DSC) (Verrips & Kwast, 1977; Miles *et al.*, 1986). Thus when whole vegetative bacterial cells were heated in a calorimeter, a characteristic series of peaks was obtained, one of which (p_r) was reversible and was identified as the melting

Abbreviation: DSC, differential scanning calorimetry.

endotherm of DNA (Miles *et al.*, 1986). In a limited investigation of eight species of bacteria, the temperature ($T_{\max}$) at which peak p_r was maximal, was highly correlated with the G + C content of the DNA. This observation suggested that DSC of whole bacteria might provide a simple means of determining G + C values without first having to purify DNA. The purpose of this paper was to examine the relationship between the melting temperature of intracellular DNA and its G + C content, in a wide selection of bacteria having G + C values in the range 25–70%.

METHODS

Organisms. A list of the 59 organisms examined is given in Table 1 (58 species were used in the correlation exercise; *Escherichia coli* was used for additional experiments).

E. coli was maintained on stabs made from nutrient broth no. 2 (Oxoid CM67) solidified with 0.6% agar. Stab cultures were stored at 1 °C and subcultured every 6 months. *Bacillus* spp. were maintained in the sporulated state on nutrient agar slopes at 1 °C. Other organisms were maintained in Robertson's cooked meat medium containing double the usual quantity of meat (Southern Group Laboratories). Each organism was grown at its optimum temperature, then stored at 4 °C. Subcultures were made every 3–6 months depending on species.

Preparation of samples for calorimetry. Most organisms were grown on solid media and samples for DSC were obtained by gently scraping growth from an agar plate with a spatula. Where organisms were grown in liquid medium, samples were taken from a centrifuged pellet of cells. Media and incubation conditions are detailed in Table 1.

Calorimetry. Samples of 10–20 mg (usually 15–20 mg) were weighed to ± 0.1 mg, sealed in aluminium volatile pans and heated in a Perkin-Elmer DSC-2C at 10 °C min⁻¹ from 5 to 130 °C, using an empty pan as reference. This heating rate was chosen arbitrarily, after preliminary tests showed that it yielded well-separated and clearly defined thermogram peaks. Samples were then cooled at 360 °C min⁻¹ to the lower limit then reheated as before. Data were collected and the calorimeter was controlled with a Perkin-Elmer data station but thermogram scans were transferred to a VAX-11/750 computer for high-resolution plotting and determinations of peak temperatures. Temperature and power scales were calibrated according to the manufacturer's instructions using the melting of indium and ice as standards. The sample dry mass was determined by piercing the pan and drying it overnight in an oven at 105 $\pm$ 1 °C.

Effect of growth medium and incubation temperature on $T_{\max}$ of peak p_r . A loopful of surface growth of *E. coli* KL16 was spread over the surface of several plates of TSA or M9 minimal agar (Miller, 1972). Plates were placed in polythene bags and incubated as follows before sampling for DSC in the usual way: 8 d at 15 °C; 6 d at 20 °C; 3 d at 25 °C; 1.5 d at 30 °C and 1 d at 37 or 42 °C.

Effect of phase of growth on $T_{\max}$ of peak p_r . TSB (1 litre) contained in a 2 l flask was inoculated with 0.5 ml of an overnight culture of *E. coli* KL16 and incubated at 37 °C in a shaking water-bath. Growth was monitored turbidimetrically at 680 nm in a Pye-Unicam SP600 spectrometer, and samples were removed when the OD₆₈₀

Table 1. *Identification, source and growth conditions used for each strain*

Strain	Source*	Growth† medium	Growth temperature (°C)	Incubation time (h)
<i>Actinobacillus lignieresii</i> NCTC 4189		TSA	37	24
<i>Alcaligenes piechaudi</i> NCTC 11970		TSAP	35	72
<i>Alteromonas putrefaciens</i> NCIB 10471	B. G. Shaw	TSA	25	24
<i>Alteromonas putrefaciens</i> NCTC 10735		TSA	30	18
<i>Alteromonas putrefaciens</i> NCTC 10737		TSA	30	18
<i>Alteromonas putrefaciens</i> NCTC 10738		TSA	30	18
<i>Alteromonas putrefaciens</i> NCTC 10763		TSA	30	18
<i>Bacillus amyloliquefaciens</i> DSM 7	} L. Shute	TSA	30	24
<i>Bacillus cereus</i> ATCC 14579		TSAP	30	18
<i>Bacillus circulans</i> ATCC 4513		TSA	30	24
<i>Bacillus laterosporus</i> ATCC 64		TSAP	30	18
<i>Bacillus macerans</i> ATCC 8244		TSA	30	24
<i>Bacterionema matruchotii</i> NCTC 10254		BHI	37	48
<i>Bordetella bronchiseptica</i> NCTC 8344		TSA	35	24
<i>Campylobacter coli</i> NCTC 11366		BA	43	48–72
		(microaerophilic)		
<i>Campylobacter fetus</i> subsp. <i>fetus</i> NCTC 5850		TRS	37	48–72
		(microaerophilic)		

Table 1. (continued)

Strain	Source*	Growth† medium	Growth temperature (°C)	Incubation time (h)
<i>Campylobacter jejuni</i> NCTC 11351		BA (microaerophilic)	43	48–72
<i>Campylobacter jejuni</i> NCTC 11847		BA (microaerophilic)	37	48–72
<i>Chromobacterium violaceum</i> NCTC 9757		TSA	30	24
<i>Corynebacterium minutissimum</i> NCTC 10288		TSA	35	24
<i>Corynebacterium segmentosum</i> NCTC 934		BHIT	37	48
<i>Corynebacterium xerosis</i> NCTC 9755		TSA	30	18
<i>Enterobacter agglomerans</i> NCTC 9381		TSA	30	24
<i>Escherichia coli</i> K12 strain KL16	B. Weiss	TSA	37	18
<i>Flavobacterium</i> P9/2	B. G. Shaw	TSA	25	24
<i>Flavobacterium</i> C20/1		TSA	25	48
<i>Flavobacterium balustinum</i> NCTC 11212		TSA	30	24
<i>Flavobacterium breve</i> NCTC 11099		TSA	30	24
<i>Flavobacterium multivorum</i> NCTC 11343		TSA	30	24
<i>Flavobacterium odoratum</i> NCTC 11036		TSA	30	24
<i>Lactobacillus bulgaricus</i> NCDO 1489		TSB	25	72
<i>Lactobacillus carnis</i> LV61		TSA (anaerobic)	25	54
<i>Lactobacillus divergens</i> DSM 20623		TSB	25	72
<i>Lactobacillus helveticus</i> NCDO 30		TSAP (anaerobic)	25	72
<i>Lactobacillus leichmannii</i> NCDO 299	B. G. Shaw	TSB	25	72
<i>Lactobacillus mali</i> J12		TSB	25	72
<i>Leuconostoc cremoris</i> NCDO 543		TSA (anaerobic)	25	72
<i>Leuconostoc dextranicum</i> NCDO 529		TSB	25	72
<i>Leuconostoc lactis</i> NCDO 533		TSB	25	72
<i>Leuconostoc mesenteroides</i> NCDO 523		TSAP (anaerobic)	25	72
<i>Leuconostoc paramesenteroides</i> NCDO 803		TSAP (anaerobic)	25	72
<i>Micrococcus luteus</i> NCTC 2665	B. G. Shaw	TSA	30	18
<i>Moraxella nonliquefaciens</i> ATCC 19975		TSA	25	48
<i>Moraxella osloensis</i> NCTC 10465		TSAP	25	48
<i>Neisseria elongata</i> subsp. <i>glycolytica</i> NCTC 11050		TSA	35	24
<i>Oerskovia turbata</i> NCTC 11973		TSA	37	24
<i>Proteus morganii</i> NCTC 2815		TSA	30	24
<i>Proteus vulgaris</i> NCTC 4175		TSA	30	24
<i>Pseudomonas acidovorans</i> NCTC 10683		TSA	35	24
<i>Pseudomonas aeruginosa</i> NCTC 10332		TSA	35	24
<i>Pseudomonas maltophilia</i> NCTC 10257		TSA	35	24
<i>Pseudomonas paucimobilis</i> NCTC 11030		TSA	30	18
<i>Pseudomonas paucimobilis</i> NCTC 11031		TSA	30	18
<i>Pseudomonas stutzeri</i> NCTC 10475		TSA	30	24
<i>Pseudomonas testosteroni</i> NCTC 10698		TSA	30	24
<i>Rhodococcus bronchialis</i> NCTC 10667		TSA	30	48
<i>Rhodococcus equi</i> NCTC 1621		TSA	30	48
<i>Streptococcus faecalis</i> NCDO 581	B. G. Shaw	TSA	37	24
<i>Streptococcus faecium</i> NCDO 942		TSA	37	30

* Unless otherwise indicated, strains were obtained from the National Collection of Type Cultures (NCTC), London, UK. Other strains were obtained from Dr B. G. Shaw, AFRC Institute of Food Research, Bristol, UK; Dr L. Shute, Department of Microbiology, University of Bristol, UK; and Dr B. Weiss, John Hopkins University, Maryland, USA.

† TSA, tryptone soya agar + 0.3% yeast extract; TSAP, tryptone soya agar + 0.1% pyruvate; TSB, tryptone soya broth + 0.3% yeast extract; BA, nutrient agar + 5% (v/v) laked horse blood; BAC, as above + 0.15% activated charcoal; TRS, as for TSA but with 0.05% ferrous sulphate, 0.02% sodium metabisulphite and 0.02% sodium pyruvate; BHI, brain heart infusion agar; BHIT, brain heart infusion agar + 0.2% Tween 80. Strains grown microaerophilically were incubated under 85% N₂/10% CO₂/5% O₂ (by vol.); strains grown anaerobically were incubated under H₂ in an anaerobe jar.

readings were 0.28 (400 ml), 1.0 (200 ml) and 2.2 (200 ml). Samples were centrifuged at 8000 *g* for 15 min and the resulting pellet of cells was examined by DSC. Cells from a 24 h culture grown under identical conditions were examined at the same time.

Effect of washing procedures on $T_{\max}$ of peak p_r . Four portions (100 ml each) of an overnight culture of *E. coli* KL16 grown in TSB were centrifuged at 8000 *g* then resuspended in 100 ml of one of the following: distilled water; 0.25 M-sodium/potassium phosphate buffer, pH 7.4 containing 0.01 M-MgCl₂; 0.01 M-Tris/HCl buffer, pH 7.4, containing 0.005 M-magnesium acetate; or TSB. Cells were then recentrifuged and the pellets kept on ice before examination by DSC.

RESULTS

DSC of whole bacteria

Representative thermograms of three species of bacteria having low, medium or high G + C contents are shown in Fig. 1. The pattern of thermogram peaks observed on the first heating run was somewhat variable depending on the species, but it was usually possible to identify peaks corresponding to those labelled *m*, *n*, *p* and *q* in *E. coli* (see Miles *et al.*, 1986). Peaks *m* and/or *n* are possibly due to denaturation of the ribosome whilst *p* is believed to represent melting of DNA (Miles *et al.*, 1986). However, it was difficult to assign labels to peaks on some thermograms and it has not yet been established that peaks occurring in the same relative position necessarily represent denaturation of the same cell component in different bacteria.

When samples were cooled to 5 °C and then reheated, a single, clearly defined endothermic peak, p_r , was observed close to the temperature of *p* seen in the first run. Peak p_r was reversible but tended to become smaller in subsequent cycles of cooling and reheating. For reasons discussed previously (Miles *et al.*, 1986), this peak is believed to represent the intracellular melting of DNA.

In some bacteria p_r was not clearly defined, appearing as a rather flat peak scarcely distinguishable above the baseline. In other cases the re-run yielded more than one peak. Where there was uncertainty, the identity and position of peak p_r were decided by applying one or more of the following criteria: (i) the peak should occur within the temperature range 80–110 °C; (ii) its position should be close to *p* observed on the first run; (iii) it should survive a third cycle of heating and cooling with little change in position.

Relationship between $T_{\max}$ of p_r and the G + C content of DNA

The temperatures at which peak p_r was maximal ($T_{\max}$) were determined by DSC and are listed in Table 2. The relationship between the $T_{\max}$ of peak p_r and the mole fraction of G + C base pairs in the DNA (X_{GC}) is shown in Fig. 2. $T_{\max}$ was related to X_{GC} by the following equation

$$T_{\max} = 75.5 + 37.7X_{GC} \quad (1)$$

[$r = 0.959$, residual standard deviation (RSD) = 1.43 °C]

Although the intracellular melting temperature of DNA was highly correlated with X_{GC} , there was considerable scatter in the data. Displacement of $T_{\max}$ values from the fitted line was particularly noticeable in the coryneform bacteria (*Corynebacterium* and *Bacterionema* spp.) and the *Campylobacter* spp. (see Fig. 2); the former had higher than expected $T_{\max}$ values, the latter had lower than expected values. If these two groups are omitted from the analysis, the equation becomes

$$T_{\max} = 76.8 + 34.9X_{GC} \quad (2)$$

($r = 0.973$, RSD = 1.03)

Melting behaviour of intracellular DNA compared with that of pure DNA in solution

The melting temperature of pure DNA in a solution of NaCl is related to X_{GC} and NaCl concentration by the equation of Frank-Kamenetskii (1971)

$$T_m(^{\circ}\text{C}) = 176 - (2.6 - X_{GC})(36 - 3.06 \ln m_3) \quad (3)$$

Fig. 1. Representative thermograms of bacteria having DNA of low, medium or high G + C content. (a) *C. jejuni* (NCTC 11847) 27.6 mol% G + C, $T_{\max}(p_r) = 85.4^\circ\text{C}$; (b) *E. coli* 51.6 mol% G + C, $T_{\max}(p_r) = 94.3^\circ\text{C}$; (c) *M. luteus* 72.1 mol% G + C, $T_{\max}(p_r) = 103^\circ\text{C}$. Samples were heated at $10^\circ\text{C min}^{-1}$ from 5 to 130°C (—); the re-run thermogram was obtained after cooling by re-running over the same temperature range (---). *m*, *n*, *p*, *q*, *r*, see Results.

Fig. 2. Relationship between the $T_{\max}$ of peak p_r and the mole fraction of G + C base pairs in the DNA. The two groups having lower and higher than expected $T_{\max}$ values (*Campylobacter* spp. and coryneform organisms) are indicated by ■ and ● respectively. The line represents equation (2), a least squares regression of the data omitting the *Campylobacter* spp. and coryneform organisms.

where T_m is the spectroscopically determined mid-point melting temperature of DNA and m_3 is the molar concentration of NaCl.

We may therefore compare the melting behaviour of pure DNA in solution with that of intracellular DNA by substituting $T_{\max}$ for T_m in equation (3) and inserting an appropriate value for m_3 .

Table 2. Details of the G + C content of each strain and the temperature recorded for peak p, (mean of three values)

Strain	G + C content (mol%)	T _{max} (°C)	Reference
<i>Actinobacillus lignieresii</i> NCTC 4189	44.2	89.9	Pohl <i>et al.</i> (1983)
<i>Alcaligenes piechaudi</i> NCTC 11970	65.0	100.4	Kiredjian <i>et al.</i> (1986)
<i>Alteromonas putrefaciens</i> NCIB 10471	43.8	91.8	Owen <i>et al.</i> (1978)
<i>Alteromonas putrefaciens</i> NCTC 10735	46.3	93.1	
<i>Alteromonas putrefaciens</i> NCTC 10737	46.5	93.5	
<i>Alteromonas putrefaciens</i> NCTC 10738	51.9	95.0	
<i>Alteromonas putrefaciens</i> NCTC 10763	53.1	95.2	
<i>Bacillus amyloliquefaciens</i> DSM 7	46.2	95.2	Fahmy <i>et al.</i> (1985)
<i>Bacillus cereus</i> ATCC 14579	35.7	88.6	
<i>Bacillus circulans</i> ATCC 4513	35.5	90.5	
<i>Bacillus laterosporus</i> ATCC 64	40.2	89.9	
<i>Bacillus macerans</i> ATCC 8244	52.2	95.8	
<i>Bacterionema matruchotii</i> NCTC 10254	56.0	100.4	Pitcher (1983)
<i>Bordetella bronchiseptica</i> NCTC 8344	69.0	100.8	De Ley <i>et al.</i> (1986)
<i>Campylobacter coli</i> NCTC 11366	32.3	86.2	Owen & Leaper (1981)
<i>Campylobacter fetus</i> subsp. <i>fetus</i> NCTC 5850	36.2	86.3	
<i>Campylobacter jejuni</i> NCTC 11351	31.6	83.8	
<i>Campylobacter jejuni</i> NCTC 11847	27.6	85.4	Owen & Dawson (1986)
<i>Chromobacterium violaceum</i> NCTC 9757	67.2	99.5	De Vos & De Ley (1983)
<i>Corynebacterium minutissimum</i> NCTC 10288	58.9	100.5	Pitcher (1983)
<i>Corynebacterium segmentosum</i> NCTC 934	59.5	101.1	
<i>Corynebacterium xerosis</i> NCTC 9755	58.7	99.2	
<i>Enterobacter agglomerans</i> NCTC 9381	56.0	96.0	De Vos & De Ley (1983)
<i>Escherichia coli</i> K12, KL16	51.6	94.3	Tamaoka & Komagata (1984)
<i>Flavobacterium</i> P9/2	36.0	89.7	Hayes <i>et al.</i> (1977)
<i>Flavobacterium</i> C20/1	35.5	90.0	
<i>Flavobacterium balustinum</i> NCTC 11212	33.1	89.0	Holmes <i>et al.</i> (1984)
<i>Flavobacterium breve</i> NCTC 11099	32.4	88.8	Owen & Holmes (1980)
<i>Flavobacterium multivorum</i> NCTC 11343	39.3	91.5	Holmes <i>et al.</i> (1981)
<i>Flavobacterium odoratum</i> NCTC 11036	36.1	90.3	Owen & Holmes (1978)
<i>Lactobacillus bulgaricus</i> NCDO 1489	50.0	91.7	Gasser & Mandel (1968)
<i>Lactobacillus carnis</i> LV61	35.2	88.5	Shaw & Harding (1984)
<i>Lactobacillus divergens</i> DSM 20623	34.8	89.0	Holzappel & Gerber (1983)
<i>Lactobacillus helveticus</i> NCDO 30	36.3	91.3	Gasser & Mandel (1968)
<i>Lactobacillus leichmannii</i> NCDO 299	50.5	92.2	
<i>Lactobacillus mali</i> J12	32.5	88.3	Carr <i>et al.</i> (1977)
<i>Leuconostoc cremoris</i> NCDO 543	42.0	91.0	Garvie <i>et al.</i> (1974)
<i>Leuconostoc dextranicum</i> NCDO 529	40.8	90.2	
<i>Leuconostoc lactis</i> NCDO 533	45.3	90.7	
<i>Leuconostoc mesenteroides</i> NCDO 523	41.0	91.1	
<i>Leuconostoc paramesenteroides</i> NCDO 803	41.5	91.1	
<i>Micrococcus luteus</i> NCTC 2665	72.1	103.0	Owen <i>et al.</i> (1969)
<i>Moraxella nonliquefaciens</i> ATCC 19975	42.0	92.0	Bovre <i>et al.</i> (1969)
<i>Moraxella osloensis</i> NCTC 10465	43.5	91.6	
<i>Neisseria elongata</i> subsp. <i>glycolytica</i> NCTC 11050	54.8	96.3	Rossau <i>et al.</i> (1986)
<i>Oerskovia turbata</i> NCTC 11973	72.3	102.7	Tamaoka & Komagata (1984)
<i>Proteus morgani</i> NCTC 2815	52.3	95.6	De Vos & De Ley (1984)
<i>Proteus vulgaris</i> NCTC 4175	40.6	90.1	
<i>Pseudomonas acidovorans</i> NCTC 10683	67.1	100.5	Tamaoka & Komagata (1984)
<i>Pseudomonas aeruginosa</i> NCTC 10332	66.4	100.3	
<i>Pseudomonas maltophilia</i> NCTC 10257	66.5	100.6	
<i>Pseudomonas paucimobilis</i> NCTC 11030	65.5	99.1	Owen & Jackman
<i>Pseudomonas paucimobilis</i> NCTC 11031	64.9	99.1	
<i>Pseudomonas stutzeri</i> NCTC 10475	64.5	97.7	De Vos & De Ley
<i>Pseudomonas testosteroni</i> NCTC 10698	62.5	98.1	
<i>Rhodococcus bronchialis</i> NCTC 10667	65.5	100.0	Pitcher (1983)
<i>Rhodococcus equi</i> NCTC 1621	67.2	101.0	
<i>Streptococcus faecalis</i> NCDO 581	38.7	90.4	Farrow <i>et al.</i> (1983)
<i>Streptococcus faecium</i> NCDO 942	38.3	89.1	

T_m is linearly related to T_{max} by the empirical equation

$$T_m = 1.26T_{max} - 26.6 \quad (4)$$

This equation was derived by reanalysing the data of Miles *et al.* (1986) for the melting of purified *E. coli* DNA in different concentrations of NaCl, using a value for X_{GC} in the Frank-Kamenetskii equation of 0.516 (Table 2) rather than 0.50, as used originally.

By combining equations (3) and (4) we obtain

$$T_{max} = \frac{202.6 - (2.6 - X_{GC})(36 - 3.06 \ln m_3)}{1.26} \quad (5)$$

By rearranging we obtain

$$m_3 = \exp [-(109 + 36 X_{GC} - 1.26 T_{max})/(7.956 - 3.06 X_{GC})] \quad (6)$$

This equation was used to calculate the apparent internal salt concentration for each test strain. The mean value for all strains excepting *Campylobacter* spp. and the coryneform organisms was 0.283 M.

When this value is inserted in equation (3) and the equation combined with equation (5) we obtain

$$T_{max} = 78.5 + 31.6 X_{GC} \quad (7)$$

This equation, which defines the dependence of melting temperature on the G + C content of pure DNA in a 0.283 M solution of NaCl is very similar to equation (2) which describes the melting behaviour of DNA inside bacterial cells (excluding *Campylobacter* spp. and coryneform organisms).

Uncertainties in the measurement of T_{max}

The uncertainty in the measurement of T_{max} for peak p_r was estimated in two ways. In the first, an analysis of variance was done on data taken from three replicate thermograms for each of 58 species of bacteria. This yielded an RSD value of 0.52 °C. The second estimate was based on analysing the results of 30 separate thermograms of *E. coli*, taken over a period of 15 months. The mean value for T_{max} for *E. coli* was 94.31 °C with an SD of 0.41 °C. As the two results were very similar, the variances were pooled to estimate the overall within organism SD of 0.5 °C. This means that 67% of the individual measurements of a given organism would be expected to lie within 0.5 °C of its mean, and 95% within two SDs, i.e. 1.0 °C. Some analyses were done on the basis of mean T_{max} values: SEM values were 0.35 °C and the 95% confidence limits were ± 0.7 °C. However, the uncertainty in the measurement of T_{max} is probably organism specific since some organisms yield narrow, strong peaks, while others are broad and weak and more difficult to measure.

Effects of growth conditions and method of sample preparation and analysis on T_{max} of peak p_r

Growth temperature and composition of growth medium. In samples of *E. coli* grown at 15, 20, 25, 30, 37 or 42 ± 1 °C on rich medium (TSA) or M9 minimal salts agar, there was no significant effect of incubation temperature on T_{max} (p_r). However, a small but significant effect of medium composition was detected; the mean T_{max} values for cells grown on TSA and M9 agar were 94.4 and 93.1 °C respectively (means of duplicate determination at each of six incubation temperatures). Addition of NaCl to the rich growth medium at concentrations greater than about 2.0% (w/v) caused an increase in T_{max} . This phenomenon has been investigated more fully and will be described elsewhere.

In one of three comparisons we noticed a difference in T_{max} between cells grown in broth and those grown on an agar-solidified version of the same medium (i.e. TSA or TSB). This effect has not yet been examined fully and we are unsure whether it is significant.

Phase of growth. No significant differences in T_{max} were detected in samples of *E. coli* harvested from TSB during mid or late exponential growth or early or late stationary phase (i.e. at an OD₆₈₀ of 0.28, 1.0, 2.2 or after 24 h incubation).

Composition of diluent used to wash cells. Washing *E. coli* cells in water or dilute buffer solutions caused a marked though somewhat variable reduction in $T_{\max}$. For example, $T_{\max}$ values measured after washing cells in water or 0.01 M-Tris/HCl, pH 7.4, plus 0.005 M-magnesium acetate were 3–4 °C lower than in cells washed in growth medium or 0.25 M-sodium/potassium phosphate buffer, pH 7.0, containing 0.01 M-MgCl₂.

Sample mass. Sample mass would be expected to affect temperature lag in the sample during heating and hence the position of thermogram peaks. However, this effect was small compared with the random experimental uncertainty, since when multiple samples of *E. coli* of mass between 8 and 22 mg were analysed there was no significant correlation between $T_{\max}$ of peak p_1 and sample mass or sample mass squared.

DISCUSSION

The melting temperature of DNA, as determined by DSC of whole bacteria, was linearly related to the mole fraction of G + C (X_{GC}) in the DNA. Of the variation in X_{GC} , 91.9% was accounted for by the variation in $T_{\max}$, and single determinations of $T_{\max}$ predicted X_{GC} with an RSD of 3.64%, via the equation

$$X_{GC} = (T_{\max} - 73.8)/41.0 \quad (8)$$

This differs from equation (1) because that equation minimized the squares of the errors in $T_{\max}$, whereas this minimized the squares of the errors in X_{GC} . The 95% confidence limits of predictions at the mean of the data were $\pm 7.3\%$, approximately 1.6 times the corresponding uncertainty ($\pm 4.5\%$) quoted by De Ley (1970) for estimates from T_m in purified DNA. The expedient of making predictions on the basis of the mean of three determinations of $T_{\max}$ rather than one reduced the 95% confidence limits only marginally to $\pm 7.1\%$, suggesting that random errors of measurement of $T_{\max}$ were not greatly limiting the precision of the predictions, and that factors additional to base composition affect the temperature of DNA melting within the bacterial cell.

There are several possible explanations for the scatter of points about the fitted regression line. First, the literature G + C values may be subject to systematic error, having been determined in several different laboratories; second, some of the strains may have been mislabelled; third, there may be species-specific differences in the intracellular environment of DNA which affect its melting temperature; fourth, differences in growth conditions or method of sample preparation may have influenced DNA melting for unknown reasons.

As a rough check of the first two possibilities, the data were reanalysed including only $T_{\max}$ values measured with strains obtained from the NCTC shortly before use. There was less chance of these strains having been mislabelled and many of their G + C contents had been determined in the same laboratory. However, this exercise failed to reduce the SE about p_1 (RSD = 1.50 °C for NCTC strains compared with 1.43 °C for all strains), suggesting that species-specific factors may be a more important source of variation. The observation that two particular groups of organisms, the campylobacters and coryneforms, had $T_{\max}$ values which diverged widely from the fitted line, lends support to this idea.

Variation in DNA melting might arise from stabilizing interactions with other molecules within the cell, e.g. polyamines, RNA or protein (Worcel & Burgi, 1972; Flink & Pettijohn, 1975; Sjastad, 1982). It is thus possible that DNA melting may be represented by more than one peak during the first heating run. However, in our experiments, DNA melting temperatures were determined from the re-run thermograms. These usually yielded single sharp peaks suggesting that interactions with other molecules were not an important influence at least on the re-melting of DNA. However, we cannot rule out such interactions as a source of variability because the re-run sometimes yielded a multi-peaked thermogram and because the position of the DNA melting peak shifted slightly to a lower temperature when samples were heated for a third time. These last two observations could suggest that stabilizing interactions were not completely destroyed by the first cycle of heating and cooling but persisted to varying degrees depending on the type of bacterium. Alternatively, the heating and cooling could affect the

thermostability of intracellular DNA for other reasons. The occurrence of mismatched regions of DNA, for example, might lower the melting point of the re-annealed DNA complex. Mismatching could arise as a result of heat-induced depurination or deamination reactions (Ullman & McCarthy, 1973*a, b*) or possibly from the formation of DNA-RNA heteroduplex regions during cooling.

An additional and probably more important source of variation is intracellular solute concentration which is known to vary quite widely between bacterial species. For example, the osmolality of the cytoplasm of *Staphylococcus aureus* or *Streptococcus faecalis* is two to three times greater than that of *E. coli* or *Salmonella typhimurium* (Mitchell & Moyle, 1956; Marquis & Carstensen, 1973; Stock *et al.*, 1977).

By using equation (6) it was possible to calculate the apparent internal ionic strength of each bacterium used in the study. The highest value was 0.65 M, in *Bacterionema matruchotti*; the lowest was 0.12 M, in *Campylobacter jejuni* 11357. These values are expressed as the molarity of an equivalent solution of NaCl; the corresponding osmolalities would be 1.19 and 0.22 Os kg⁻¹ respectively.

If deviations from the predicted G + C values were due to differences in internal salt concentrations, there should be a correlation between values calculated from DSC data and those derived by other methods. However, although data are available on the composition of cytoplasmic solutes, it is not clear on which basis comparisons should be made: whereas the ionic strength dependence of DNA melting has been determined using solutions of simple salts (Marmur & Doty, 1962; Dove & Davidson, 1962), the free solutes in the cytoplasm are a heterogeneous mixture of K⁺ and Na⁺ cations, Cl⁻ and phosphate anions and amino acids. Additionally, a large proportion of the total counter-ions are fixed negative charges on macromolecules (Damadian, 1971). The effect of such complex mixtures on DNA melting is not known. An approximate comparison can be made on the basis of cation content. In *E. coli* the concentration of Na⁺ plus K⁺ has been measured chemically at around 0.16 to 0.28 equiv. per litre of cell water, depending on the phase of growth (Schultz & Solomon, 1961; Schultz *et al.*, 1962). The mean apparent salt concentration in *E. coli* calculated from DSC data was 0.254 M, which is within this range. The concentrations of Na⁺ plus K⁺ in *Streptococcus faecalis* and *Micrococcus luteus* (previously known as *M. lysodeikticus*) were about 0.6 and 0.35 equiv. per litre of cytoplasmic water respectively (Marquis & Carstensen, 1973). The calculated values from DSC data were 0.26 and 0.40 M respectively.

Comparisons can also be made on the basis of osmotic strength. However, this will reflect the concentrations of all free solutes, not all of which will affect DNA melting. The osmolality of the contents of *E. coli* has been estimated from plasmolysis experiments at 0.5–0.6 Os kg⁻¹ (Mitchell & Moyle, 1956; Schultz & Solomon, 1961). A lower value of 0.3 Os kg⁻¹ for *Salmonella typhimurium* was obtained by vapour pressure equilibration and other methods (Stock *et al.*, 1977). The osmolality corresponding to the apparent salt concentration in *E. coli* was 0.47 Os kg⁻¹ which falls between these estimates. The osmolalities of *S. faecalis* and *M. luteus*, from plasmolysis experiments, were 1.0 and 0.52 Os kg⁻¹; the osmolalities calculated from DSC data were 0.49 and 0.74 Os kg⁻¹ respectively. Thus, on whichever basis comparisons are made, our calculated internal solute concentrations do not always coincide with reported values either absolutely or relatively.

The discrepancy between our data and those reported by others could indicate that estimates of internal solutes based on analysis of cell extracts, or on osmotic measurements, do not represent their effective activities within the cytoplasm. On the other hand, internal salt concentrations derived from DSC measurements of heat-denatured cells may not always represent those in viable intact cells. For example, heat-induced loss of permeability control could result in leakage of cations from the cell. The resulting degree of dilution of the cytoplasmic content would then depend on the volume and composition of any fluid external to the cytoplasmic membrane.

Variations in growth conditions or method of sample preparation could influence DNA melting either by affecting internal salt concentration or for other, unknown reasons. Of the variables we tested, phase of growth or incubation temperature had no effect on $T_{\max}$ of peak p_r .

There was, however, a slight effect of growth medium: cells grown on complex media had higher $T_{\max}$ values than those grown on minimal media. We do not believe that the differences in growth media would account for the rather large deviations of $T_{\max}$ values from the fitted line observed with some bacteria. For example *Corynebacterium minutissimum* was grown on the standard medium, TSA, but still had much higher than expected $T_{\max}$ values.

Washing cells in water or dilute buffer solution before examination by DSC had a large effect on the temperature of DNA melting. Exposure of cells to solutions more dilute than their growth medium is known to cause loss of internal cations (Epstein & Schultz, 1965). The resulting decrease in internal salt concentration would account for the observed depression in $T_{\max}$. In our experiments cells were never washed before analysis so this would not have been a source of variability.

In this paper we have shown that the melting temperature of DNA in a wide range of bacteria can be measured by DSC of whole cells. Melting temperatures were largely dependent on base composition; hence G + C contents of bacteria can be estimated without having to purify DNA. The method is simple and would be particularly useful for organisms whose DNA is difficult to extract or purify. At present the method is less precise than those using pure DNA as the starting material. Part of the residual variation appears to be species specific and is probably due to differences in intracellular solute concentration between species. We are currently investigating ways of overcoming possible variability from this source.

We thank Mrs D. A. Seymour for preparing bacterial samples for analysis, Drs L. Shute and B. Weiss for providing strains and Dr B. G. Shaw for providing strains and helpful advice.

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