Determination of Microbial DNA Base Pair Ratios by Agar Gel Electrophoresis* (33080)

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Determination of DNA base pair ratios is an important procedure in biological studies (1, 2). The usual methods for base pair calculations such as thermal denaturation (3) and buoyant density measurements (4) are limited to laboratories possessing the required specialized equipment. A previous publication described a method for rapid agar-gel electrophoresis of RNA bases with subsequent quantitation by ultraviolet spectrophotometry (5). An adaptation of this technique permitting DNA base separation and quantitation is presented along with a comparison of DNA base pair ratios determined by buoyant density, thermal denaturation and agar-gel electrophoresis.

Materials and Methods. Preparation of DNA. Escherichia coli B *and Bacterium anitratum* 5W71 (ATCC No. 15150) were grown overnight in 50 ml of tryptose phosphate broth on a rotary shaker. Starter cultures were added to liter flasks containing 400 ml of fresh medium prewarmed at 37°C. After 4–6 hours incubation of the cultures on the shaker, cells were harvested by centrifugation and washed once with saline–EDTA. The washed cells were used immediately or frozen and stored at −10°C. The DNA was extracted according to the method of Marmur (6) or by a minor modification substituting saline–EDTA saturated phenol for sodium perchlorate and chloroform–isoamyl alcohol in the initial deproteinization step. Comparable DNA from *Streptomyces venezuelae* S13 and Actinophage MSP 19 were also prepared.

Determination of mol % G + C by thermal denaturation. The midpoint of the hyperchromic rise at 260 mμ associated with elevated temperature was determined. The temperature at this midpoint, *Tm*, is correlated with mol % G + C. Determinations for *E. coli* and *B. anitratum* were made in a Beckman DU spectrophotometer equipped with thermospacers adjacent to the cell compartment as described by Marmur and Doty (3). For *S. venezuelae* and MSP 19 it was necessary to use a weaker buffer (0.015 *M* NaCl + 0.003 *M* Na citrate, pH 7) to lower the melting temperature. A new curve, based on the known mol % G + C and the *Tm* value for *E. coli* in this buffer, was drawn parallel to Marmur and Doty's curve. This new curve had the formula: $Tm = 55.0 + 0.41$ (mol % G + C). The mol % G + C was also determined from an empirical curve established by Mandel.

Determination of mol % G + C by buoyant density. Buoyant density was determined in the analytical ultracentrifuge, using a field formed CsCl density gradient and ultraviolet optics according to the method of Schildkraut *et al.* (4).

Determination of mol % G + C by agar-gel electrophoresis. Hydrolysis of DNA. The DNA in aliquots containing 1.0 mg was precipitated with two volumes of 95% ethanol in the hydrolysis tube, washed twice with 95% ethanol and dried in a stream of nitrogen. The precipitate was then hydrolyzed with formic acid as described by Bendich (7). The dry hydrolyzed residue was dissolved in 0.25 ml of 0.5% HCl (v/v), mixed well and subjected to electrophoresis.

Electrophoresis. The electrophoretic procedure was essentially the same as that described for RNA bases (5). Stock 4 × buffer solution was prepared by dissolving 13.77 gm of ammonium acetate in distilled water, titrating to pH 3.1 with glacial acetic acid and diluting to 1 liter. Agar–buffer solution was prepared by melting 300 mg of Oxoid Ionagar no. 2 in 88 ml of distilled water, then adding 12 ml of 4 × stock

* Supported in part by PHS Grant CA 08217 from the National Cancer Institute, The Michigan Heart Association, and the Detroit General Hospital Research Corporation.

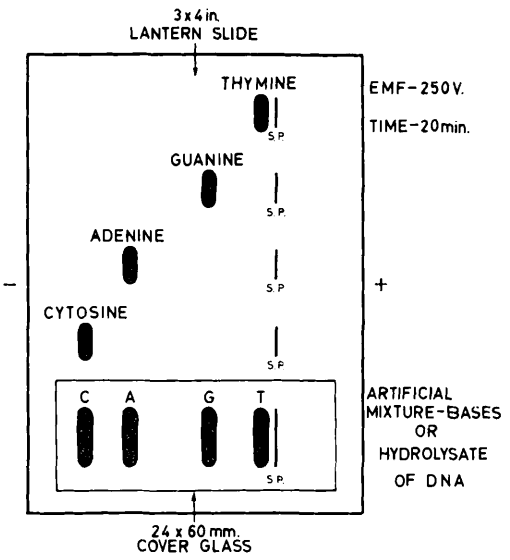

FIG. 1. Schematic diagrams of the electrophoretic separation of DNA bases.

buffer. Three 24 × 60 mm glass coverslips placed on a 2.25 × 4-inch glass lantern slide were covered with 10–12 ml of the hot agar–buffer solution. Impregnated strips of Whatman 3 mm filter paper were used to place the DNA hydrolyzate on the solidified agar. A strip was prepared by applying 25 μl, by pipetting on 5-μl aliquotes of hydrolyzate and drying the strip after each application except the last with a hot air blower. Care was taken in the drying step to avoid charring because it produced changes in the separation characteristics of the bases. The buffer boxes were filled to equal heights with 1:4 dilution of stock 4 × buffer. Schleicher and Schuell no. 900 filter paper wicks were connected between the buffer boxes and the agar covered slide. Electrophoresis was carried out at an emf of 250 v applied to the buffer box–gel system for 20 min. Migration was monitored by observing positions of the bases under an ultraviolet light as they migrated toward the cathode. Immediately upon completion of electrophoresis, the electropherogram was dried in an oven at 100°C or under an infrared light (8). An electrophoretic control solution was prepared by dissolving 5.0 mg of each of the DNA bases in 1.0 ml of 5% (v/v) HCl. A filter paper strip impregnated with the acid solution was dried with the hot air blower and the dry strip was applied to the agar gel. This mixture was processed on the same plate simultaneously with a sample.

Ultraviolet spectrophotometry. Individual bases visualized under ultraviolet illumination were recovered by cutting the dried coverslips with a diamond tip pencil and eluting the individual bases from the slips with 0.1 N NaOH. For *E. coli,* MSP 19 and *S. venezuelae,* individual bases were eluted with 1.0 ml and peak absorbances determined using microcuvettes with a 10-mm light path. For *B. anitratum,* individual bases from three simultaneous electrophoretic separations, one slide, were pooled and eluted with 2.0 ml and read in standard cuvettes. All readings were taken from complete UV spectra drawn with a Beckman DB recording spectrophotometer. These scans were compared to those obtained with standards made from commercial bases dissolved in 0.1 N NaOH.

Results. Figure 1 shows that nucleobases produced as a result of formic acid hydrolysis have electrophoretic mobilities identical to commercial compounds electrophoresed either individually or as a mixture. Spectra obtained from eluted spots identified electrophoretically as guanine, cytosine, and adenine were identical to the spectra of the pure compounds. Analysis of the eluate of the spot corresponding to thymine showed a slight hypsochromic shift though the spectral structure was unchanged. Pure thymine subjected to the hydrolytic and electrophoretic steps failed to exhibit the shift. One can only assume that substances produced during the hydrolysis of DNA have an influence on the thymine spectrum thereby causing a spectral shift. However, no other ultraviolet absorbing materials were observed, and the four spots had mobilities identical with known nucleobases, so it was felt that the hydrolytic and electrophoretic processes could be used in determining individual bases and base pair ratios.

The DNA was prepared, hydrolyzed, and subjected to electrophoresis as described. After the electropherogram was dried, the separated nucleobases on the coverslips were cut out and placed in test tubes for elution. Ab-

TABLE I. Comparison of Mol % G + C Values.

Organism	Mol % G + C as determined by:			
	Tm			
	Method[c]	Method[d]	Buoyant density	Electrophoresis[a]
E. coli B	50.2	54.3	—	52.5
	51.3	55.9	—	53.0
	50.8	54.9	—	53.0
	50.0	54.9	—	52.1
	49.8	53.9	—	52.3
	50.8	54.9	—	53.0
B. anitratum	40.5	43.7	40.8[b]	39.7
S. venezuelae	—	73.0	72.0	71.4
Actinophage MSP 19	—	60.3	—	60.2

[a] Each value represents an average of 3 electrophoretic determinations.
[b] Determined by one of us (CDJ) in the laboratories of Dr. Manley Mandel.
[c] Calculated according to methods of Mandel (1964).
[d] Calculated according to methods of Marmur and Doty (1962) (3).

sorbances of the eluted samples were determined and concentrations of individual nucleobases present were calculated by comparison to one of the four standard curves established for the nucleobases. The mol % G + C values were determined and compared with those obtained by the buoyant density and/or *Tm* methods (Table I). For *B. anitratum* and *S. venezuelae* the data obtained by the three methods are quite similar. The data for *E. coli* are interesting. Conversion of *Tm* to mol % G + C according to Marmur and Doty (3) resulted in higher values than those obtained using the curve established by Mandel for such conversions. The mol % G + C values determined by the electrophoretic method usually were intermediate between them. Reasons for these observed differences are not apparent at the present time.

Discussion. The *Tm* and buoyant density methods of determining mol % G + C exploit physical properties of intact DNA. In the *Tm* process, where there is a greater bond strength between guanine and cytosine than between adenine and thymine, more heat is required to separate strands of DNA with high mol % G + C. The buoyant density of DNA is also related to base content, the density increasing as the mol % G + C increases. Conversion of *Tm* or buoyant density values to mol % G + C must be made by establishing these values for DNA's of known chemically determined base composition. Such conversions are based on data derived empirically from established DNA base values (3,4).

The data presented in this paper indicate that actual mol % G + C values, as determined on the electrophoretically separated bases, are comparable to base pair ratios obtained on aliquots of the same DNA by the *Tm* and buoyant density methods. The simplicity and availability of the equipment and techniques required in the electrophoresis procedure is an obvious advantage over the specialized nature of that required for *Tm* or buoyant density determinations. There is another advantage in the electrophoretic method which is a temporal one. Isolation and purification of DNA is a step required by all three procedures. Hydrolysis of DNA followed by electrophoretic separation and quantitation can be accomplished in a matter of hours. Another advantage to the method is that multiple samples can be separated simultaneously. Thus, triplicate analysis can be performed on aliquots of any individual DNA hydrolyzate, a practice followed in this procedure. In this way a reliability check is

built into the system. In addition, the electrophoresis process allows for the determination of the individual concentrations of all bases of the DNA molecule; data not available from the more sophisticated approaches. The limitation of *Tm* determinations to the range of 25–75 mol % G + C for which it has been established (3) is another advantage in favor of the electrophoretic method whose range is restricted only by the limits of determination of the individual bases by ultraviolet spectrophotometry.

A disadvantage of the described method is the requirement of a relatively large DNA sample in the analytical process. The mol % G + C values can be determined by thermal denaturation on 50 μg samples and by buoyant density on solutions containing 1–2 μg. The electrophoretic method requires the application of approximately 100 μg of hydrolyzed DNA in a volume of 25 μl. In these experiments, 1.0 mg of dry DNA was hydrolyzed and taken up in a total volume of 0.25 ml, thereby providing the means for replicate determinations. In the final analysis, the advantages of simplicity of instrumentation and technique must be weighed against the disadvantage of the increased DNA requirement.

Summary. The determination of DNA base pair ratios has been described using a rapid agar-gel electrophoresis method for the separation of DNA bases obtained from formic acid hydrolyzates. The actual mol % G + C values calculated from spectrophotometric data on the electrophoretically separated bases compared favorably with values obtained by thermal denaturation or buoyant density determinations on separate aliquots of the same DNA.

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Received Feb. 26, 1968. P.S.E.B.M., 1968, Vol. 128.

Glucagon Clearance by the Isolated Perfused Rat Liver* (33081)

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The liver is often quoted as a major site for the degradation of glucagon (1,2). However, the evidence for this is only indirect, including destruction of glucagon-^{131}I by liver homogenates (3), loss of hyperglycemic properties of insulin after liver perfusion (4), and transhepatic gradients in glucagon levels in the intact animal (5). Since the introduction of precise and sensitive immunoassay methods for glucagon (6,7), the opportunity now exists to study the clearance of the hormone directly in a liver perfusion system. Immunoreactive glucagon (IRG) has been detected not only in the pancreas (PIRG) but also in the gut (GIRG) (8–11). It is not yet known if there are any similarities between PIRG and GIRG, but evidence is accumulating that they might not be identi-

* Supported by USPHS Grants AM 02456-09, AM 05020-13, and a United Health Foundation Grant.

[1] In receipt of Fellowship 4-F2 AM30 11203 from the National Institutes of Health.

[2] In receipt of Fellowship F2 Am-32,466-02 from the National Institutes of Health.