*SEPARATION OF B. SUBTILIS DNA INTO COMPLEMENTARY STRANDS, III. DIRECT ANALYSIS**

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Since the completion of the two preceding papers of this series[1, 2] we have had the opportunity of examining, by direct base analysis, the composition of two preparations of separated strands isolated, as described before,[1] from the DNA of two strains of *B. subtilis*. The results will be found in Tables 1 and 2.

TABLE 1. *Composition of native DNA and of separated DNA strands of B. subtilis.*

		Strain W23				Strain Mu8u5u16			
		Strand L	Strand H	Computed for L + H	Native	Strand L	Strand H	Computed for L + H	Native
Bases, (mole %)	A	29.4	27.7	28.5	28.2	30.8	27.0	28.9	29.5
	G	23.6	19.2	21.4	21.9	23.8	19.9	21.8	21.7
	C	21.0	23.2	22.1	21.6	19.2	23.3	21.3	20.7
	T	26.0	29.9	28.0	28.3	26.2	29.8	28.0	28.1
Molar ratios	Pu/Py	1.13	0.88	1.00	1.00	1.20	0.88	1.03	1.05
	A/T	1.13	0.93	1.02	1.00	1.18	0.91	1.03	1.05
	G/C	1.12	0.83	0.97	1.01	1.24	0.85	1.02	1.05
	A + T/G + C	1.24	1.36	1.30	1.30	1.33	1.31	1.32	1.36
	6-Am/6-K	1.02	1.04	1.02	0.99	1.00	1.01	1.01	1.01

The composition of the native DNA of strain W23 was taken from a previous paper,[3] that of strain Mu8u5u16 has been newly determined. The slight distortion of the ratios is presumably due to contamination with some RNA. Abbreviations: A, adenine; G, guanine; C, cytosine; T, thymine; Pu, purines, Py, pyrimidines; 6-Am, 6-amino nucleotides (A + C); 6-K, 6-keto nucleotides (G + T).

TABLE 2. *Comparison of data on composition of separated strands of B. subtilis DNA.*

		Composition (mole %)				Molar Ratios		
Preparation	Analytical method	A	G	C	T	Pu/Py	A + T/G + C	6-Am/6-K
Strand L	Direct*	30.1	23.7	20.1	26.1	1.16	1.28	1.01
	Transcript†	30.0	23.2	20.4	26.3	1.14	1.29	1.02
Strand H	Direct*	27.3	19.6	23.2	29.8	0.89	1.33	1.02
	Transcript†	27.2	20.7	23.0	29.0	0.92	1.29	1.01
Computed for L + H	Direct*	28.6	21.7	21.7	28.0	1.01	1.30	1.01
	Transcript†	28.6	22.0	21.7	27.7	1.02	1.29	1.01

* Based on the averages of the figures in Table 1.

† Based on the averages of the figures on the nucleotide composition of the polyribonucleotides synthesized by RNA polymerase with the separated DNA strands as the templates, taken from Table 1 of the preceding paper.[2]

Technical Details.—The DNA preparation from strain W23 corresponds to the one shown in Figure 1*B* of a previous paper.[1] The DNA preparation from strain Mu8u5u16 has also been discussed previously.[1, 2] It was denatured by alkali and subjected to separation by intermittent gradient elution (0.7 *M*–0.14 *M* NaCl, 500 ml) on a methylated albumin-kieselguhr column:[1] recovery, 80%; fraction L, 42%; fraction H, 23%; RNA, 15%.

The eluates corresponding to each peak were pooled, dialyzed in the cold against at least four changes of 2000 ml of dist. H_2O for 48 hr, concentrated in a flash evaporator, and finally taken to dryness in the hydrolysis tubes under a stream of nitrogen. About 1 mg of each fraction was hydrolyzed at 100° for 1 hr in a sealed tube in 0.02–0.04 ml of 7.5 *N* $HClO_4$; the hydrolysate was diluted with H_2O to a volume of 0.1–0.15 ml and centrifuged, and two 0.02-ml portions were subjected to chromatography in butanol-0.6 *N* ammonia (6:1, v/v); the same quantities were also developed in isopropanol-conc. HCl-H_2O (17:4:4, v/v/v). Each figure in Table 1 consequently is the average of four determinations in two solvent systems.

Results.—The findings reported in this communication bear out the conclusions drawn in the preceding paper[2] as to the complementary character of the two fractions isolated by the separation of denatured *B. subtilis* DNA. The comparison in Table 2 of the two sets of data—direct base analysis and indirect analysis of the RNA products synthesized enzymically—shows that the transcripts of these single-stranded DNA fractions are indeed faithful copies. The complementarity ratios calculated from the averages in Table 2, with the first figure always referring to the direct analysis and the second to the analysis of the transcript, are: A_L/T_H, 1.01, 1.03; G_L/C_H, 1.02, 1.01; C_L/G_H, 1.02, 0.99; T_L/A_H, 0.95, 0.97.

The point stressed in the preceding communication,[2] namely, the equality—even in the separated DNA strands—of 6-amino and 6-keto nucleotides, in the absence of all other pairing regularities, is also supported by the present observations (see Tables 1 and 2).

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[1] Rudner, R., J. D. Karkas, and E. Chargaff, these PROCEEDINGS, **60**, 630 (1968).
[2] Karkas, J. D., R. Rudner, and E. Chargaff, these PROCEEDINGS, **60**, 915 (1968).
[3] Lin, H. J., and E. Chargaff, *Biochim. Biophys. Acta*, **145**, 398 (1967).