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A Cytochemical Study of Nucleic Acids in Plant Cells

VI. Purine and Pyrimidine Composition of DNA from *Oscillatoria princeps* and *Vicia faba*[1)]

By

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While investigations on the nitrogenous composition of nucleic acids from animal and bacterial sources were carried out by several authors such as WYATT (1951), SMITH and WYATT (1951), CHARGAFF and LIPSHITZ (1953) and CHARGAFF *et al.* (1953), those from plant sources were very meagre.

In the cells of *Oscillatoria princeps*, it was reported that the nuclei were Feulgen negative, while these nuclei contained certain amount of DNA as revealed by a biochemical method (SHINKE, ISHIDA and UEDA, 1957; ISHIDA, 1961). It may be assumed that, therefore, DNA in the cells of this plant is different from that of other Feulgen positive plants in respect to its base composition.

It is intended in the present study, therefore, to compare the purine and pyrimidine composition of DNA from *Oscillatoria princeps*, with that of *Vicia faba* which is a Feulgen positive plant.

It was reported by CHARGAFF (1955), on the other hand, that there was no significant difference among base compositions of DNA from different animal organs of the same species. It is a second aim of the present study to see whether such regularities in base composition as stated above are to be found in plant or not.

Materials and Methods

Oscillatoria princeps, a Feulgen negative alga, used in the present study, was collected from freshwater pools. Material collected was washed showering with tap water and finally with distilled water to avoid contamination by other microorganisms. The whole algal body was used for analysis. For the study of Feulgen positive plants, seedlings of *Vicia faba* (7–8 cm in length) were used.

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Dividing zone of the roots or 5 mm segments from apex of roots, elongating zone or 10 mm segments avoiding the 10 mm segments next to the dividing zone, and whole shoot tips, 5 mm in length, were removed from seedlings and used for chemical analysis.

Isolation of DNA: Isolation of DNA was carried out by method of SCHMIDT and THANNHAUSER (1945) with a slight modification (SUGIYAMA, SHINKE and ISHIDA, 1954; ISHIDA, 1961). 'DNA fraction' obtained by this method contained a fairly large amount of proteins and a small amount of RNA as contaminants. For the purification of DNA, following procedures were employed.

Purification of DNA: Dilute KOH solution was added to the 'DNA fraction', and pH of this mixture was adjusted at 7.8. This mixture was incubated with ribonuclease[2] for 15 hours at 38°C. After the incubation, DNA was precipitated by addition of N-HCl and 5% trichloroacetic acid (TCA). Precipitate obtained was re-dissolved with dilute KOH solution. This procedure was repeated twice. Precipitate was washed repeatedly with a small amount of 70% methanol and finally with 97% ethanol, then it was dried.

Powder obtained by above procedure was dissolved in alkaline M-NaCl solution, and deproteinized by repeated shaking with chloroform-amylalcohol (35 : 10) mixture until no gel was formed on emulsification (SEVAG, 1934). After this treatment, DNA solution was condensed on warm water bath. DNA was precipitated by an addition of N-HCl and 5% TCA solutions to this condensed solution. DNA-precipitate was then washed with a small amount of 70% methanol, absolute ethanol and finally with ether, followed by drying at room temperature.

Quantitative hydrolysis of DNA with formic acid: DNA obtained by the above procedure was hydrolysed at 175°C for 1 hour with 98% formic acid in sealed pyrex-glass bombs. After hydrolysis, formic acid was evaporated, and then the hydrolysate was dissolved in a small amount of 0.1 N-HCl and spotted on a filter paper.

Analysis by paperchromatography: Isopropanol-HCl mixture[3] was used as a solvent for chromatography. Chromatograms were obtained after the bases were run on filter papers[4] with this solvent system for 22 hours at 25°C. Position of the bases on the filter papers was detected by ultraviolet ray absorption method and by mercury salt method (VISCHER and CHARGAFF, 1948).

For quantitative estimation of purines and pyrimidines, spots corresponding to each base on the filter papers were cut out and eluded overnight with 0.1 N-HCl at 38°C. Pieces of another filter paper were used for blank test. Optical density of the elute containing each base was determined against the blank with BECKMAN's spectrophotometer.

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2) Obtained from Washington Biochemical Laboratory, U. S. A.

3) Isopropanol 170 ml, concd. HCl (sp. gr. 1.19) 41 ml, water to make 250 ml (WYATT, 1951).

4) Toyoroshi No. 50 and Whatman No. 1.

Result

In *Oscillatoria princeps*, four bases were found. Wavelengths of absorption maximum of these bases locate at 262 mμ, 249 mμ, 275 mμ, and 265 mμ similar to those of adenine (A), guanine (G), cytosine (C) and thymine (T) respectively. They were identified as adenine, guanine, cytosine and thymine because their Rf-values as well as characteristic UV-absorption curves were similar to those of each corresponding standard base. No 5-methylcytosine (5-MC) was able to be detected by the paperchromatographic method.

In *Vicia faba*, however, another base was found in DNA extracted from the dividing and elongating zones of the roots as well as the shoot tips, in addition to four bases found in *Oscillatoria* DNA. This fifth base was identified as 5-methylcytosine by its Rf-value and absorption maximum located at 282 mμ. UV-absorption curves of five bases stated above in 0.1 N-HCl are shown in Figs. 1-5.

In Fig. 1, it is seen that there are no significant differences among the absorption curves of adenine isolated from DNA from three parts of seedlings. Similar results are shown in Figs. 2, 3, 4 and 5 which represent absorption curves of guanine, cytosine, thymine and 5-methylcytosine, respectively. Purine and pyrimidine contents and their molar ratios in DNA, isolated from *Oscillatoria princeps* and three parts of *Vicia* seedlings are summarized in Table 1 and Table 2 respectively.

In Table 2, it is shown that in DNA from *Oscillatoria*, the ratio of purines and pyrimidines is 0.87, and A/T and G/C are much lower than 1, while A+T/G+C is nearly 1.

In *Vicia faba*, no significant difference has been found among the base compositions of DNA isolated from three different parts of the seedlings (Table 1). In Table 2, it is seen that the ratios of purines to pyrimidines, A/T and G/C+5MC in DNA from these three parts, are approximately 1, while A+T/G+C+5MC is 1.39 in DNA from both dividing zone and shoot tips, and is 1.44 in elongating zone. These values are far higher than those found in *Oscillatoria* DNA and are nearly equal to those widely found among higher organisms (*cf.* CHARGAFF, 1955).

Table 1. Purine and pyrimidine composition of DNAs from the cells of *Oscillatoria princeps* and the three parts of *Vicia faba*.

Bases	*Oscillatoria princeps*	*Vicia faba*		
		Dividing zone	Elongating zone	Shoot tip
Adenine	22.5	28.8	30.1	29.6
Guanine	24.1	20.5	19.9	19.5
Cytosine	27.0	17.5	16.8	18.0
Thymine	26.4	29.3	29.0	28.5
5-methylcytosine	—	3.9	4.2	4.4

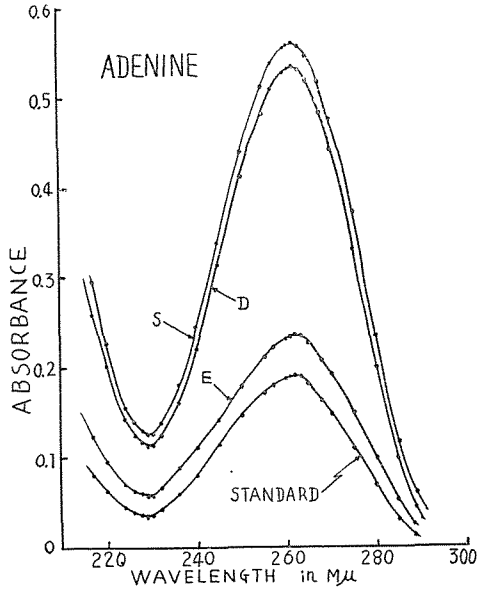

Fig. 1

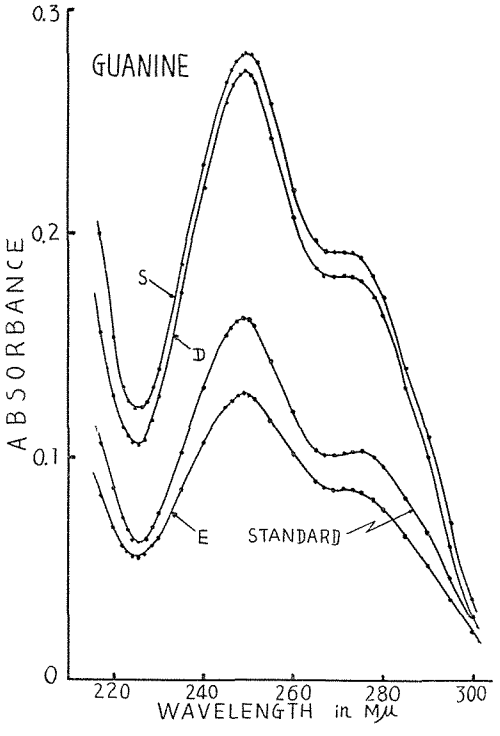

Fig. 2

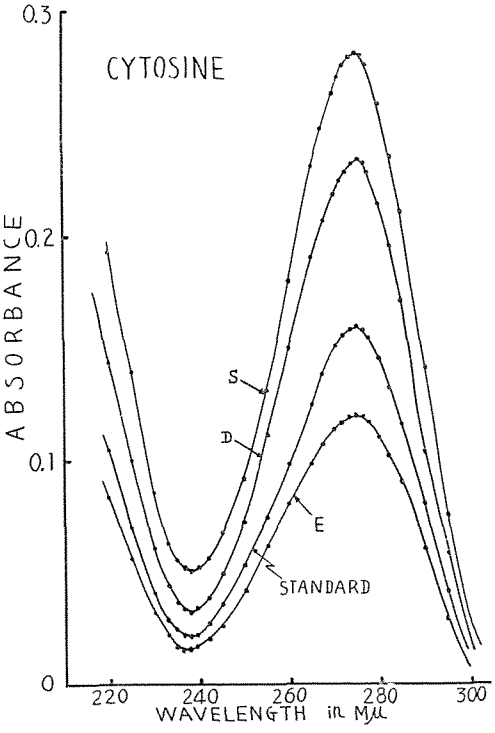

Fig. 3

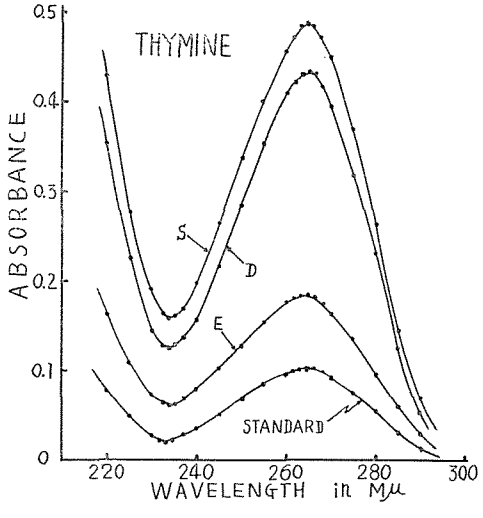

Fig. 4

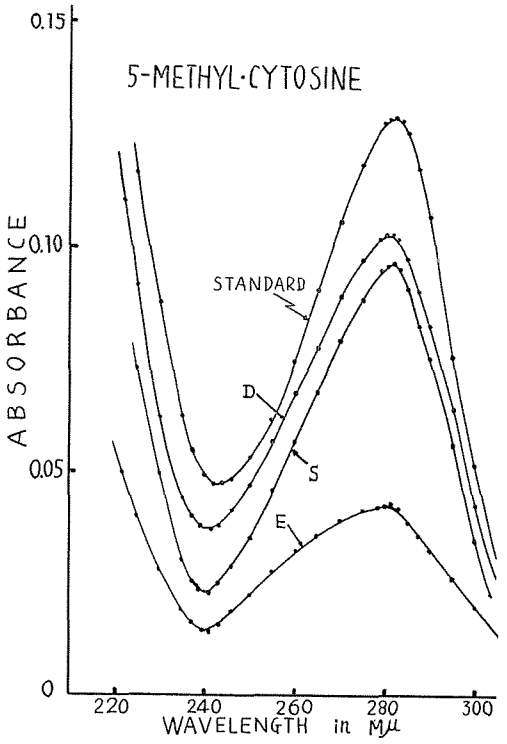

Fig. 5

Figs. 1–5. Showing the UV-absorption curves of five bases of DNA from dividing zone (D), elongating zone (E) and shoot tip (S) of *Vicia faba* seedlings.

Table 2. The molar relationship between purine and pyrimidine bases of DNA from the cells of *Oscillatoria princeps* and the three parts of *Vicia faba*.

	Oscillatoria princeps	*Vicia faba*		
		Dividing zone	Elongating zone	Shoot tip
$\frac{\text{Adenine}}{\text{Thymine}}$	0.85	0.98	1.04	1.04
$\frac{\text{Guanine}}{\text{Cytosine (+5·MC)}}$	0.89	0.96	0.98	0.96
$\frac{\text{Purines}}{\text{Pyrimidines}}$	0.87	0.97	1.00	0.96
$\frac{\text{A + T}}{\text{G+C(+5·MC)}}$	0.96	1.39	1.44	1.39

Discussion and Conclusion

It was reported by several authors that 5-methylcytosine was found little or a little in addition to adenine, guanine, cytosine and thymine in most DNA isolated from animals, while DNA from higher plants contained a fairly large amount of 5-methylcytosine in addition to the four bases (THOMAS and SHEREATT, 1956; *cf.* CHARGAFF, 1955).

It has been shown in the present study that these four bases are found in *Oscillatoria* DNA, but no 5-methylcytosine was detected. Absence of this base has also been reported in *Nostoc muscorum* by BISWAS (1956), and in *Navicula pelliculosa*, *Cylindrotheca gracilis* and *Anagstis nidulans* by LOW (1956), though whether this base is always absent in DNA from lower algae has not been cleared in the present study.

Contrary to the above case, *Vicia* DNA contains a fairly large amount of 5-methylcytosine in addition to these four bases. This result is quite in accord with that obtained by WYATT (1951) in wheat germ DNA and that reported by THOMAS and SHEREATT (1956) in DNA from bracken, clover and rye.

In the study of purine and pyrimidine composition in nucleic acids isolated from many different organisms, WYATT (1951), BRAWERMAN and CHARGAFF (1951) and CHARGAFF and LIFSHITZ (1953) have found that the sum of purine bases is nearly equal to that of pyrimidine bases in DNA from wheat germ and several higher animals. Similar result is obtained in the present study on DNA from *Vicia faba* seedlings (Table 2). Contrary to the above cases, the ratio of purines to pyrimidines in *Oscillatoria* DNA is only 0.87. This is one of the characteristics of *Oscillatoria* DNA.

Summarizing the results of studies carried out by himself and other investigators, CHARGAFF (1955) has emphasized that A/T as well as G/C+5MC are approximately 1. Result of the present study of *Vicia* DNA shows that these values are nearly 1, while in *Oscillatoria* DNA, A/T is 0.85 and G/C is 0.89. Peculiarity of DNA from *Oscillatoria* is also found in molar ratio of A/T as well as that of G/C.

The results of the studies on DNA composition reported by several authors (ZAMENHOF and CHARGAFF, 1950; SMITH and WYATT, 1951; WYATT and COHEN, 1952; CHARGAFF *et al.*, 1953; *cf.* CHARGAFF, 1955) have shown that molar ratio of A+T/G+C+5MC is greatly different in different organisms. In most organisms, this value is far higher than 1. CHARGAFF (1955) called DNA of this type 'AT-type'. The results obtained in the present study show that in *Vicia* DNA this ratio is from 1.39 to 1.44 (Table 2). Contrary to the above case, the ratio A+T/G+C is far less than 1 in DNA from *Mb. tuberculosis*. DNA of this type is called 'GC-type' by CHARGAFF (1955). In *Oscillatoria* DNA, however, this ratio is 0.96. It is stated, therefore, that *Oscillatoria* DNA belongs neither to 'AT-type' nor 'GC-type' but belongs to 'intermediate type', which has been found in DNA from *E. coli* (SMITH and WYATT, 1951). Considering from the

results obtained in the present study, it is concluded that *Oscillatoria* DNA is peculiar in respect to its purine and pyrimidine composition.

CHARGAFF (1955) has pointed out that there is little difference among purine and pyrimidine compositions of DNA isolated from different organs of one species. In *Vicia* seedlings, it is confirmed that A/T ratios of DNA from the dividing zone, elongating zone and shoot tip, are nearly 1. Similar values are found in purine and pyrimidine ratio in DNA from three different parts of seedlings. The ratios of A+T/G+C+5MC in all three parts mentioned above are far larger than 1. These results show that there is no significant difference among the base compositions of DNA from three different parts of *Vicia* seedlings.

It is concluded from the present study that regularities on base ratios of DNA from *Vicia* seedlings are quite in accord with those proposed by CHARGAFF (1955), while these regularities are not found in *Oscillatoria* DNA.

Summary

1) Purine and pyrimidine compositions of DNA from the cells of *Oscillatoria princeps* and the three different parts, dividing and elongating zones of young roots and shoot tips of seedlings of *Vicia faba*, were determined.

2) DNA fraction was isolated by a modified SCHMIDT and THANNHAUSER's method and RNA contamination in this fraction was removed by treatment with RNase followed by removal of proteins by SEVAG's method.

3) DNA was hydrolysed with formic acid at 175°C for 1 hour. Each purine and pyrimidine base was isolated by paperchromatography using isopropanol-HCl mixture as a solvent.

4) Purines and pyrimidines separated from *Oscillatoria* DNA were identified as adenine, guanine, cytosine and thymine by their Rf-values and characteristic UV-absorption curves. No 5-methylcytosine was detected in this alga.

5) In *Oscillatoria* DNA, molar ratio of purine and pyrimidine bases was 0.87, ratios of adenine to thymine was 0.85, and of guanine to cytosine, 0.89. Ratio of adenine plus thymine to guanine plus cytosine was 0.96. These values were much lower than those obtained in other organisms, DNA of which belonged to 'AT-type', but higher than DNA of 'GC-type'. DNA of this alga, therefore, belonged to the 'intermediate type'.

6) Five bases contained in DNA from *Vicia faba* were identified as adenine, guanine, cytosine, thymine and 5-methylcytosine. This nucleic acid was characterized by a high content of the last base.

7) Base compositions of DNA from *Vicia faba* closely resembled those found in DNA from several higher plants such as wheat, clover and others. *Vicia* DNA, therefore, belonged to the 'AT-type'.

8) No significant difference was found among the base compositions of DNA from three different parts of *Vicia* seedlings stated in (1).

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