The pentose nucleic acid of *Azotobacter vinelandii**

In connection with studies on the existence of regularities in the composition of pentose nucleic acids (PNA)[1,2] it appeared of interest to examine the nucleotide distribution in the PNA of a nitrogen-fixing organism. Our previous work[2] had suggested one wide-spread regularity, namely, the occurrence in PNA of nearly equal amounts of bases carrying 6-amino groups (adenine + cytosine) and of bases having 6-keto groups (guanine + uracil). Some interest was added to the study of the PNA of *Azotobacter vinelandii* by the recent demonstration that this organism possesses an enzyme capable, *in vitro*, of producing, or attacking, polynucleotides with the consumption, or the release, of 5'-nucleoside diphosphates or inorganic phosphate, respectively[3]. If this enzyme were the principal, or sole, agent in the synthesis of PNA by the living cell, considerable fluctuations in the composition of PNA and the absence of regularity would be expected, since the fortuitous availability of individual precursors or the extent of the reverse reaction, *viz.*, phosphorolysis, would make their influence felt. That this is not the case is shown in the accompanying table.

The strain serving for cultures I to V (Table I) was *Azotobacter vinelandii* ATCC No. 9104, grown for 48 hours at 30° in Burk's nitrogen-free medium with aeration (I to III) or shaking (IV, V). We are very grateful to Dr. R. H. BURRIS of the University of Wisconsin for culture VI and to Drs. B. SIEGEL and M. TUNIS for a few preliminary observations. The washed cells were suspended in distilled water and broken either by being shaken with ballotini (90 minutes) or, in the majority of cases, by treatment in a sonic oscillator (Raytheon, 10 kc, 10 minutes). For the rest, previously published procedures and precautions[2,4] were followed**.

TABLE I

COMPOSITION OF PNA OF *Azotobacter vinelandii**

Bacterial culture No.	*Number of hydrolyses*	*Moles per 100 moles nucleotide*				*Molar ratio 6-Am/6-K*
		A	*G*	*C*	*U*	
I	1	24.8	30.3	24.8	20.1	0.99
II	6	23.7	30.6	25.6	20.1	0.97
III	3	24.0	31.2	25.2	19.7	0.97
IV	1	23.5	32.6	25.1	18.8	0.95
V	2	25.0	30.4	26.0	18.6	1.04
VI	4	24.7	29.8	26.5	19.1	1.04
All preparations	17	24.2	30.6	25.7	19.5	1.00
Coefficient of variation		3.1	4.3	3.4	4.6	4.4

* Abbreviations — A, G, C, U: adenylic, guanylic, cytidylic, uridylic acids, respectively; 6-Am: 6-amino compounds (adenylic, cytidylic acids); 6-K: 6-keto compounds (guanylic, uridylic acids).

As will be gathered from Table I, the nucleotide proportions did not show much variation in the several cultures. Whether this minor fluctuation is merely due to technical reasons or whether it reflects an actual, though insignificant, play in composition cannot yet be decided owing to the lack of similar information on other microbial species. What should, however, be emphasized is that in the PNA of all cultures the sum of adenylic and cytidylic acids (6-Am) and that of guanylic and uridylic acids (6-K) were near equality: a result unlikely to be achieved

* Aided by research grants from the National Institutes of Health, U.S. Public Health Service the American Cancer Society (on recommendation of the Committee on Growth, National Research Council), and the Rockefeller Foundation. One of us (A.L.) holds a Quincy Ward Boese Postdoctoral Fellowship of Columbia University.

** The sugar component of the PNA was identified, but only tentatively, as ribose by paper chromatography after the acid hydrolysis of the crude pentose mononucleotides that had been isolated by the alkaline degradation of PNA and precipitation as the lanthanum salts. Two minor sugar zones were also seen on the chromatograms, but may have been contaminants.

by the haphazard alignment of precursors. It may be assumed that the agent concerned with the selection and the arrangement of the nucleotides in the living cell has so far escaped identification. Whether it operates in a manner similar to that suggested before[2], remains to be seen.

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Received March 7th, 1956

Sodium sulfide inhibition of liver lactic dehydrogenase*

Inhibition of carbonic anhydrase[1], trypsin and chymotrypsin[2], carboxypeptidase[3,4], desoxyribonuclease[5], and glutamic dehydrogenase[6] by Na_2S has been reported in recent years. The first interpretation of sulfide inhibition might be that metal ions were removed as insoluble sulfides or that reductive cleavage of important disulfide bonds might be involved. The latter process usually leads to irreversible inactivation. However, in the case of the experiments to be reported here, another view of the inhibition is necessary.

It seems likely that the principal ionic species of sulfide at pH 8.5 will be SH^-. Under these conditions Na_2S showed a reversible, sensitive inhibition of highly purified rat liver lactic dehydrogenase (LDH)[7]. At concentrations of Na_2S as low as 10^{-3} to $10^{-5}\,M$ marked inhibition, depending on exact conditions, was observed. LDH (2 ml) and 0.01 M sulfide (1 ml) were usually mixed with 2 ml M glycine buffer, pH 8.5, 0°, and allowed to stand for a few minutes, following which aliquots were diluted about 50 times with 0.4 M glycine. For analysis 0.6 ml were added to a Beckman cuvette along with Veronal buffer, sodium D,L-lactate and DPN^+. The final cuvette volume was 3.6 ml. The reaction was initiated by adding DPN^+. In a typical experiment the final cuvette concentrations were:

[LDH], $2\cdot10^{-10}\,M$; [Na_2S], $1.0\cdot10^{-5}\,M$; [Glycine], 0.06 M; [Veronal], 0.03 M; [Sodium D,L-lactate], 0.11 M; [DPN^+], $1.5\cdot10^{-4}\,M$; pH 8.6.

As can be seen in Table I, reversal of sulfide inhibition of LDH could be accomplished by a variety of procedures, the common denominator of which turned out to be the removal or displacement of SH^-. Thus such apparently diverse operations as (1) dilution; (2) dialysis; (3) LDH precipitation and re-solution; (4) increase in ionic strength; (5) addition of such chelating reagents as Versene, *o*-phenanthroline, α,α'-dipyridyl, and pyrophosphate; (6) addition of Zn^{++}, Fe^{++}, or Mn^{++}; (7) oxidation of SH^- by ferricyanide or *o*-iodosobenzoate all served to reverse sulfide inhibition.

TABLE I

SULFIDE INHIBITION OF LDH AND ITS REVERSAL

System	[Na_2S] *in cuvette* $M \times 10^{-5}$	[*Reagent*] *in cuvette* *M*	*Inhibition* %
Na_2S	1.18	—	92
Na_2S	0.58	—	78
Na_2S	0.14	—	10
Na_2S + NaCl	1.66	$6.6\cdot10^{-2}$	68
Na_2S + versene	1.66	$6.6\cdot10^{-5}$	10
Na_2S + Zn^{++}	1.18	$2.0\cdot10^{-4}$	0
Na_2S + *o*-phenanthroline	1.18	$1.3\cdot10^{-3}$	25
Na_2S + $K_3Fe(CN)_6$	3.80	$8.8\cdot10^{-4}$	0

* This investigation was supported by a research grant (C-1856-2 C) from the National Cancer Institute of the National Institutes of Health, Public Health Service.