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Further Investigations on the Specificity of an Acid Deoxyribonuclease from *Helix aspersa* (Müll.)

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Linear relationships were found to hold between the percentages of the 3′-*P* terminal and 5′-OH terminal and penultimate nucleotides released by snail acid DNAase from bacterial DNAs and the (dG + dC) contents of the latter. This finding is similar to previous results obtained on spleen acid DNAase; as in the case of this enzyme, the composition of the termini released by the snail DNAase from calf thymus DNA deviate from the linear relationships mentioned above.

The snail enzyme appears to recognize a sequence of at least two nucleotides, namely those which will be released as 3′-P and 5′-OH terminals, respectively.

The investigations to be reported in the present article answer one question which was left open by the previous work [1] on the specificity of an acid DNAase from *Helix aspersa* (Müll.). It concerns the composition of the termini[1] released by this enzyme from bacterial DNAs of different base composition and its variation with varying (dG + dC) contents. An answer to such a question, already obtained for spleen acid DNAase [2], is a pre-requisite for the use of the snail enzyme in studies of nucleotide sequences in DNA by the method of the "analysis of termini" [2]. The results obtained in the present work were also useful in providing information on the length of the nucleotide sequence recognized by the snail enzyme.

MATERIALS AND METHODS

These have already been described [2]. The *Helix aspersa* DNAase used in the present work was obtained according to a procedure to be presented elsewhere.

RESULTS

Termini Released from Bacterial DNAs

Table 1 and Fig. 1 show the results obtained in the analysis of termini as released by the snail enzyme from three bacterial DNAs (from *Haemophilus influenzae*, *Escherichia coli* and *Micrococcus luteus*, respectively) and artificial mixtures of them. As in the case of spleen acid DNAase [2] linear relationships were found to hold between the percentages of termini released and the (dG + dC) contents of the DNAs investigated. The previous data obtained with calf thymus DNA [1] are also shown in Fig. 1 for the sake of comparison.

Termini Recognized by the Snail Enzyme

This problem was investigated by comparing the experimental results obtained for the 5′-OH terminal and penultimate nucleotides with calculated compo-

Table 1. *Termini released from different DNAs by* Helix *acid DNAase*

DNA	Nucleotide	3′-Terminal	5′-Terminal	5′-Penultimate
		%		
H. influenzae	dT	15	25	36
	dG	9	32	19
	dA	74	21	27
	dC	2	22	18
E. coli	dT	16	22	29
	dG	15	38	25
	dA	66	13	24
	dC	3	27	23
M. luteus	dT	14	8	15
	dG	26	46	43
	dA	58	5	9
	dC	2	41	33

[1] With this term we designate, conventionally, the 3′-phosphate terminal nucleotide and the 5′-hydroxy terminal and penultimate nucleotides.

Enzymes. Acid DNAase from snail hepatopancreas (EC 3.1.4.—); acid DNAase from porcine spleen (EC 3.1.4.6); DNAase from bovine pancreas (EC 3.1.4.5).

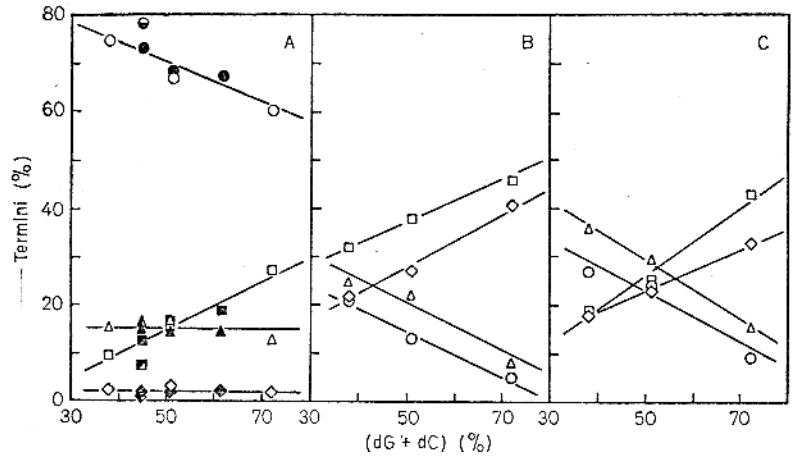

Fig. 1. *Plot of the percentages of the (A) 3′-P terminal (B) 5′-OH-terminal and (C) 5′-OH-penultimate nucleotides released by snail DNAase as function of DNA (dG + dC) contents.* The average sizes of the digests, $\bar{P}_n$, were close to 18. (□) Deoxyguanosine; (○) deoxyadenosine; (△) thymidine, (◇) deoxycytidine. Open symbols show results obtained with bacterial DNAs and filled symbols with their mixtures. *H. influenzae* + *E. coli* DNA, 44% (dG + dC); *H. influenzae* + *M. luteus* DNA, 51% (dG + dC); *E. coli* + *M. luteus* DNA, 61% (dG + dC). Half-filled symbols correspond to calf thymus DNA [1]

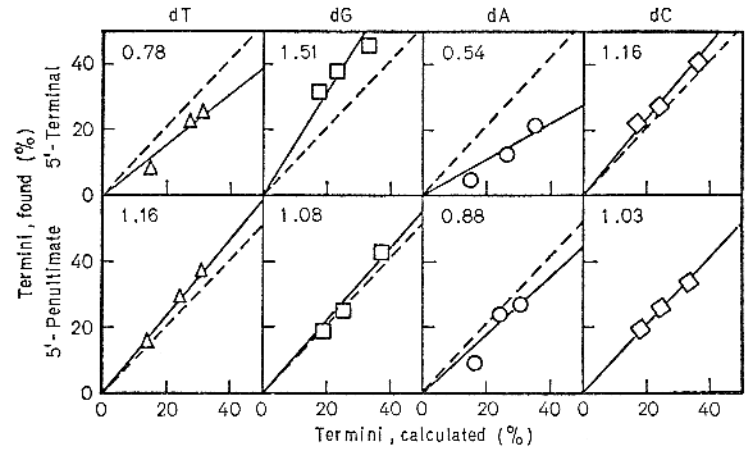

Fig. 2. *A plot of percentages of termini found by analysis of snail DNAase digests ($\bar{P}_n \approx 18$) against the percentages calculated from nearest-neighbor data.* For this type of plot see Fig. 6 of Thiery *et al.* [2]. Broken lines of slope equal to 1 are shown for the sake of comparison

sitions of the nearest neighbors of the 3′-*P* and 5′-OH terminals, respectively [3]. Fig. 2 shows that the experimental data of the 5′-OH terminals are quite different from those expected of the basis of the nearest neighbor analysis; the opposite is true for the 5′-OH penultimate position.

DISCUSSION

The conclusions which may be drawn from the present work are straightforward. (a) Linear relationships hold between the percentages of all termini released and the percentage of (dG + dC) in the bacterial DNAs investigated. The termini released by calf thymus DNA showed a deviation from these linear relationships. Both results were expected for reasons already discussed in the case of spleen DNAase [2]. The comments made for spleen acid DNAase [2] are also valid for the snail enzyme. (b) The snail enzyme appears to recognize a sequence of at least two nucleotides, namely those which will be released as 3′-*P* and 5′-OH terminals, respectively; the composition of the 5′-OH penultimate nucleotide corresponds to that expected for the nearest neighbor of the 5′-OH terminal and appears therefore not to be selected by the enzyme. Nothing is known, so far, concerning the composition of 3′-*P* penultimate position.

It is evident from the results presented in this and the preceding paper [1] that snail acid DNAase has a specificity different from that of the spleen enzyme [2]. More recent results have shown that this

is the case also for pancreatic DNAase [4] and *E. coli* endonuclease I [5]. It is extremely likely, therefore, that specific towards nucleotide sequences, such as those shown by the three enzymes explored so far, is a general property of DNAases. Such specificities can be used in studies of nucleotide sequences, the existence of different specificities in different DNAases increasing the analytical potentialities of this approach.

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