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NUCLEASE ACTIVITY IN TISSUES OF ANGIOSPERM PARASITES*

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Abstract—The tissues of angiosperm parasites possess powerful ribonuclease and deoxyribonuclease activities. The general properties of the enzymes, as studied in homogenates, resembled those of plant tissues in general. However, RNase of *Cuscuta* species differed from that of *Orobanche cernua* in that the latter was inhibited by Ca^{2+} and Mg^{2+} and stimulated by EDTA while the former was mostly unaffected. There were significant changes in enzymic activity in different regions of the filament of *Cuscuta reflexa*; the changes were suggestive of an essentially degradative role for the enzymes.

INTRODUCTION

ANGIOSPERM parasites such as *Cuscuta* and *Orobanche* may be dependent on host for the supply of nucleotides, or simpler intermediates, for the elaboration of their requirements of nucleic acids, or various cofactors. At the same time, the parasite tissues may possess mechanisms for degradation of nucleic acids. The presence of nucleases in parasite tissues will permit the endogenous mobilization of RNA and DNA for the formation of mono- and oligo-nucleotides, to be reutilized for specific metabolic needs.

The present investigation was aimed at (a) demonstration of nuclease activity in *Cuscuta* and *Orobanche* (b) a comparison of the properties of the enzymes in the parasites and two typical hosts as studied in homogenates and (c) changes in enzyme activity from region to region in the filament of *C. reflexa*.

RESULTS AND DISCUSSION

Effect of Cysteine

Addition of cysteine to the dispersion medium resulted in homogenates with higher enzymic activity than when water was the medium. The optimal cysteine concentration for RNase and DNase activity in every homogenate was determined in advance and employed in the subsequent experiments.

(a) *Parasite*

Compared to a homogenate in water, RNase and DNase activities in *Cuscuta reflexa* were more than doubled in a homogenate in 0·0125 M cysteine. In *C. campestris*, a 20–25 per cent increase in RNase and DNase activity was observed when ground in 0·025 M cysteine. Optimum cysteine concentration for RNase and DNase activity in *Orobanche* was 0·0167 M. Whereas the increase in DNase was marginal, RNase was increased to over 5-fold in comparison with homogenates in water.

* Abbreviations used: RNase, Ribonuclease; DNase, Deoxyribonuclease.

(b) *Host*

In *Medicago sativa* shoots, RNase and DNase activities were stimulated 17 per cent each, when the dispersion medium contained 0·01 M cysteine. In roots, DNase was only marginally increased, but RNase activity was more than doubled when ground in 0·01 M cysteine. The DNase activity of shoots of *Petunia hybrida* was doubled whereas the RNase activity was increased only 25 per cent when ground in 0·01 M cysteine. Extracts of the roots of *P. hybrida* were characterized by the fact that different cysteine concentrations were required for optimum RNase and DNase activities. RNase activity was optimal at 0·0167 M cysteine (3-fold higher activity than in water); for DNase, 0·01 M cysteine led to 10 per cent activation.

pH-*Activity Relationship*

RNase activity was determined at pH 4·0, 4·5, 5·0 and 5·5, using 0·1 M acetate buffer. DNase activity was tested at pH 5·5, 6·5, 7·5 and 8·5 using tris universal buffer.

(a) *Parasite*

Cuscuta campestris, C. reflexa and *Orobanche cernua* had optimum pH of 5·0 for RNase activity. DNase in these tissues was optimum at pH 5·5, except in *C. reflexa* with optimum pH at 6·5.

(b) *Host*

RNase activity was optimum at pH 4–5 and DNase activity at pH 5·5 in the shoots and roots of *Medicago sativa.* In *Petunia hybrida,* RNase was optimum at pH 5·0 and DNase at 5·5, both in shoots and roots.

Enzymic Activities in Parasites and Hosts Compared

The specific nuclease activities of *Cuscuta* filaments harvested from *M. sativa* and *O. cernua* grown on *P. hybrida* and of control host tissues are recorded in Table 1. It is necessary to emphasize that the activities obtained in the presence of cysteine in the dispersion medium constitute the true basis for comparison, since in its absence phenolics inactivate the enzymes.

The filaments of *Cuscuta* species and the tissues of *Orobanche* possess RNase and DNase with activities significantly higher than in *M. sativa* and *P. hybrida.* Also, the values obtained are within the range reported for plant tissues in the literature.[1–5] The degradative activities known to be mediated in plant tissues through nucleases or the synthetic activities postulated[6–10] can, therefore, occur also in the tissues of angiosperm parasites.

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TABLE 1. RNASE AND DNASE ACTIVITIES IN THE TISSUES OF ANGIOSPERM PARASITES AND THEIR HOSTS

	RNase units/mg protein	DNase units/mg protein
Parasites		
Cuscuta campestris	119	52
C. indecora	144	80
C. reflexa	153	53
Orobanche cernua	248	53
Hosts		
Medicago sativa		
Shoot	7·4	6·4
Root	36·9	32·6
Petunia hybrida		
Shoot	33·9	20·1
Root	43·2	17·6

The whole filaments of *Cuscuta* species and whole growth of *Orobanche* were used in the studies. The homogenates were prepared in the optimal concentrations of cysteine and the assays were at predetermined optimum pH values, at 37°.

Effect of Activators and Inhibitors

A number of supplements were tested, singly, in final concentration of 1×10^{-2} or 1×10^{-3} M. The results obtained are reported in Tables 2–5.

TABLE 2. ACTIVATION AND INHIBITION OF RIBONUCLEASE OF SHOOT AND ROOT PARASITES

	Activation (+) and inhibition (−) of RNase, %		
	Shoot parasites		Root parasite
	C. campestris	*C. reflexa*	*O. cernua*
$CaCl_2$	+8	−5	−50
$MgSO_4$	±	−5	−50
EDTA	+8	±	+50
Sodium citrate	±	+17	−66
NaCl	±	±	±
KCl	−14	+9	+67
NaF	−33	−50	−50
$FeSO_4$	−50	−83	−50
$FeCl_3$	−83	−67	−50
$ZnSO_4$	−58	−83	−50

The various supplements were tested in a final concentration of 1×10^{-2} *M*, except in the case of heavy metals, when the concentration was 1×10^{-3} *M*. The symbol ± denotes that there was neither activation nor inhibition. The homogenates were prepared in water and the enzyme activity determined at optimum pH, at 37°.

TABLE 3. ACTIVATION AND INHIBITION OF DEOXYRIBONUCLEASE OF SHOOT AND ROOT PARASITES

	Activation (+) and inhibition (−) of DNase, %		
	Shoot parasites		Root parasite
	C. campestris	*C. reflexa*	*O. cernua*
$CaCl_2$	+50	+122	+50
$MgSO_4$	+57	+233	+38
EDTA	−71	−50	−50
Sodium citrate	±	±	−50
NaCl	−29	−44	−60
KCl	−36	±	−50
NaF	−50	−33	−50
$FeSO_4$	−50	−25	−60
$FeCl_3$	−100	−50	−60
$ZnSO_4$	−53	−75	−67

The various supplements were tested in a final concentration of 1×10^{-2} M, except in the case of heavy metals, when the concentration was 1×10^{-3} M. The symbol ± denotes that there was neither activation nor inhibition. The homogenates were prepared in water and the enzyme activity determined at optimum pH, at 37°.

TABLE 4. ACTIVATION AND INHIBITION OF RIBONUCLEASE IN HOST TISSUES

	Activation (+) and inhibition (−) of RNase, %			
	M. sativa		*P. hybrida*	
	Shoot	Root	Shoot	Root
$CaCl_2$	−20	−40	−30	−25
$MgSO_4$	−30	−33	−30	−25
EDTA	+10	−13	+10	+
Sodium citrate	+18	−92	−20	−50
NaCl	+4	−92	−40	−50
KCl	−73	−42	−53	−50
NaF	−36	−50	−57	−75
$FeSO_4$	−70	−89	−60	−50
$FeCl_3$	−80	−94	−70	−66
$ZnSO_4$	−50	−59	−70	−50

The various supplements were tested in a final concentration of 1×10^{-2} M, except in the case of heavy metals, when the concentration was 1×10^{-3} M. The symbol ± denotes that there was neither activation nor inhibition. The homogenates were prepared in water and the enzyme activity determined at optimum pH, at 37°.

(a) *Parasite*

RNase activity in homogenates of *Cuscuta* was not affected by $CaCl_2$ and $MgSO_4$ (1×10^{-2} M) and by EDTA. *Orobanche* differed from the shoot parasite in being inhibited by Ca^{2+} and Mg^{2+} and in being activated by EDTA. KCl (1×10^{-2} M) had a marked activating effect on the enzyme from *Orobanche*, without any significant effect on *Cuscuta*. DNase activity in the two types of parasites was activated by Ca^{2+} and Mg^{2+} and inhibited by EDTA. Both RNase and DNase were inhibited powerfully by Fe^{2+}, Fe^{3+} and Zn^{2+} (1×10^{-3} M); NaF (1×10^{-2} M) was also an inhibitor of the two enzymes.

TABLE 5. ACTIVATION AND INHIBITION OF DEOXYRIBONUCLEASE OF HOST TISSUES

	Activation (+) and inhibition (−) of DNase, %			
	M. sativa		*P. hybrida*	
	Shoot	Root	Shoot	Root
$CaCl_2$	+100	+40	+30	+50
$MgSO_4$	+134	+33	+50	+50
EDTA	−34	−45	−50	−38
Sodium citrate	+44	±	−50	−25
NaCl	−6	+43	−13	+50
KCl	−44	−42	−67	+25
NaF	−36	−7	−50	−75
$FeSO_4$	−50	−50	−50	−50
$FeCl_3$	−50	−50	−75	−75
$ZnSO_4$	−25	−50	−50	−50

The various supplements were tested in a final concentration of 1×10^{-2} *M*, except in the case of heavy metals, when the concentration was 1×10^{-3} *M*. The symbol ± denotes that there was neither activation nor inhibition. The homogenates were prepared in water and the enzyme activity determined at optimum pH, at 37°.

A distinctive feature of nuclease activity of plant tissues is the sensitivity to cations. The rate of nucleic acid degradation by the nucleases, or the synthesis as the case may be, can be a critical factor in cell metabolism. Since some of the metal ions occur in sufficiently high concentration in the intracellular regions with nuclease activity, the cations may modulate the degradation or synthesis of RNA and DNA *in vivo*.

(b) *Host*

RNase activity was inhibited by 1×10^{-2} M Ca^{2+} and Mg^{2+} in both shoots and roots of the hosts. Citrate was without effect on the activity in the shoot, but inhibited the root enzyme. DNase activity was activated by Ca^{+2} and Mg^{2+} and inhibited by EDTA in both shoots and roots of *Medicago sativa* and *Petunia hybrida*. NaCl activated DNase in roots of both hosts but was without effect in shoots. Both RNase and DNase were inhibited by Fe^{2+} Fe^{3+} and Zn^{2+} (1×10^{-3} M) and NaF (1×10^{-2} M) in both shoots and roots of *M. sativa* and *P. hybrida*.

The concentration of the various added supplements, 10^{-2} M, may, normally, be considered unphysiological. However, several authors have found that the response by nuclease to added KCl and NaCl occurred only at high concentrations. As already pointed out, the effective concentration of an ion to be taken into account is that at the site of enzyme action and not the total intracellular concentration. It is also necessary to emphasize that for metals to exert their action *in vivo* they must be present in an 'available form' such as in the ionic state.

Alteration in Nuclease Activity Along the Filament of C. reflexa

The enzyme activity was determined in different regions of the filament harvested from *L. camara*. The results obtained in two different experiments are reported in Table 6.

Ribonuclease activity was doubled when proceeding from the haustoria-bearing to the after-haustorial and near-apical regions. The apical region registered a fall, the activity being

TABLE 6. NUCLEASE ACTIVITY IN DIFFERENT REGIONS OF THE FILAMENT OF *C. reflexa*

Region of filament	RNase, units/mg protein		DNase, units/mg protein	
	(1)	(2)	(1)	(2)
Haustoria-bearing	41·8	36·0	12·5	9·6
After-haustorial	80·0	69·1	28·8	22·3
Near-apical	97·2	73·2	33·7	25·6
Apical	48·0	40·9	7·5	5·8

Values under columns (1) and (2) represent data obtained in two different experiments, carried out with samples harvested at wide interval. Ten per cent homogenates of the tissues were prepared in optimal cysteine concentration and used in RNase and DNase assay at the optimum pH and at 37°. The values for enzymic activity reported in this Table are not comparable with those in Table 1 since the hosts were different.

almost of the same order as that of the haustoria-bearing region. The DNase activity was doubled when passing from the haustoria-bearing to the after-haustorial region. A further increase, though of a small order, occurred in the near-apical region, but this was followed by an abrupt drop of the enzymic activity in the apical region. The DNase activity in the near-apicalregion, the maximum, was over 4-fold that in the apical region, the minimum. The variations in enzymic activity obtained in the present experiment are analogous to the variations observed earlier[11] in the activities of a number of enzymes associated with carbohydrate metabolism.

Results reported earlier[12] showed that the contents of RNA and DNA, calculated in terms of tissue protein, were higher in the haustoria-bearing and the apical regions of the vine and lower in the intervening regions. The reciprocal relationship between enzymic activity and the content of substrate suggested a degradative role for nucleases throughout the filament.

EXPERIMENTAL

Parasites and Hosts

In the present investigation, *Cuscuta campestris* and *C. reflexa*, (both shoot parasites) and *Orobanche cernua* (root parasite) were selected for the study along with the two typical hosts, *Medicago sativa* (host for *Cuscuta*) and *Petunia hybrida* (host for both *Cuscuta* and *Orobanche*).

Seedlings of *M. sativa* were transplanted in pots filled with well-mixed garden soil. When the plants were 40–50 cm tall (after 1 month), they were separated into 2 batches, one batch to serve as control and the other as hosts for the parasite. The technique of infection by *Cuscuta* was by twining cut lengths of the vines round the host shoots. Heavy growth of dodder occurred in 15–20 days, when the filaments were harvested for the enzyme assay. Simultaneously, the control hosts were also harvested for enzyme assay. Well mixed garden soil was filled into a number of pots. Half the pots were heavily inoculated with the seed of *Orobanche cernua*, the other half serving as control. Seedlings of *P. hybrida* were transplanted singly in all the pots. Scapes of *O. cernua* commenced to emerge from infected soil surface after two months. After waiting for 10–15 days, the parasite was harvested and used in the experiments. Simultaneously, tissue was collected from the control plants.

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Preparation of Homogenates

Homogenates were prepared in freshly neutralized cysteine hydrochloride of predetermined optimal strength, filtered through two folds of muslin and made to 10% (w/v). For study of the properties of the enzymes in homogenates, pooled samples of entire vine of *Cuscuta* inclusive of curls and the whole growth of *Orobanche* were used as source material of parasite. When the effect of cations, anions and chelating agents on enzymes was to be tested, the tissue dispersions were in water. Simultaneous analyses were carried out on *M. sativa* and *P. hybrida* separately on shoot and root systems. For determination of activity in different regions of the vine of *C. reflexa*, the technique of demarcation of regions was as reported earlier.[12]

Nuclease Assay

RNase. The assay system was based on the method of Reddi[13] and employed yeast RNA (Schwarz) as the substrate.

DNase. The assay system was based on the method of Chang and Bandurski[14] and made use of thymus DNA (BDH) as the substrate.

Units of enzyme activity. One unit of RNase or DNase was defined as the amount leading to an increase of 1·0 in optical density at 260 μm under the respective conditions of assay. Specific activity was expressed as units/mg protein.

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