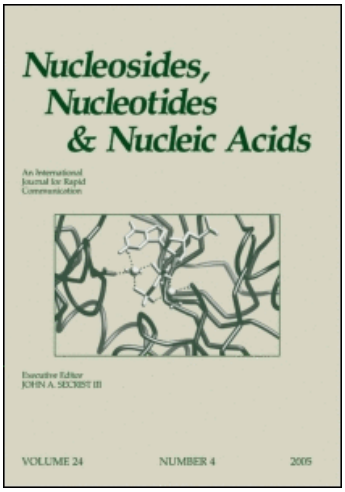

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KINETICS OF *C. ELEGANS* DcpS CAP HYDROLYSIS STUDIED BY FLUORESCENCE SPECTROSCOPY

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□ *DcpS (scavenger decapping enzyme) from nematode C. elegans readily hydrolyzes both monomethyl- and trimethylguanosine cap analogues. The reaction was followed fluorimetrically. The marked increase of fluorescence intensity after the cleavage of pyrophosphate bond in dinucleotides was used to determine K_m and V_{max} values. Kinetic parameters were similar for both classes of substrates and only slightly dependent on pH. The hydrolysis was strongly inhibited by methylene cap analogues ($m^7Gp(CH_2)ppG$ and $m^7Gpp(CH_2)pG$) and less potently by ARCA ($m^{7,3'O}GpppG$).*

Keywords DcpS; nematode cap hydrolysis; fluorescence; enzyme kinetics; inhibitors

INTRODUCTION

The majority of mRNAs in the nematode *C. elegans* have a unique cap structure consisting of a trimethylated guaninosine residue ($m^{2,2,7}G$) linked by a 5′,5′ triphosphate bridge with the mRNA chain. Hydrolysis of the mRNA cap resulting from 3′→ 5′ decay is carried out by a specific hydrolase known as the scavenger decapping enzyme, DcpS.[1] Unlike other eukaryotic DcpS

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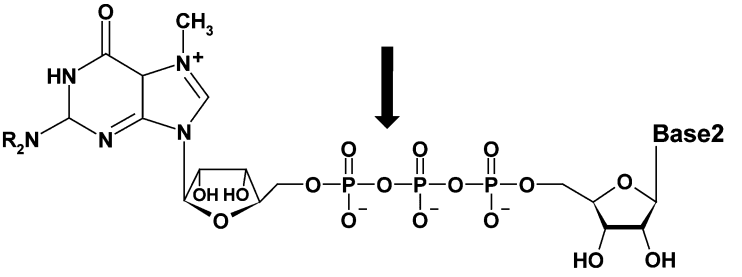
enzymes, the nematode DcpS has the ability to hydrolyze the TMG cap, $m^{2,2,7}$GpppG.[2,3]

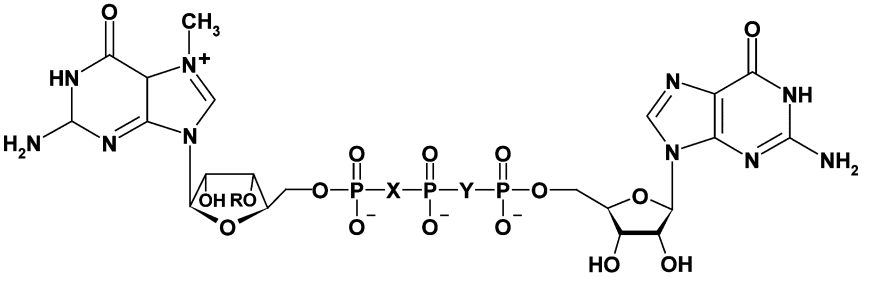
The relatively strong fluorescence of the $m^{2,2,7}$G moiety is enhanced several-fold during hydrolytic cleavage of dinucleotide cap analogues with purine residues at the second nucleotide. This fluorescence increase was used to determine kinetic parameters of hydrolysis catalyzed by nematode DcpS and to characterize the most effective inhibitors of this enzyme.

EXPERIMENTAL

Syntheses and purification of cap analogs (Scheme 1) were performed as described previously.[4–6] The concentrations of investigated compounds were determined spectrophotometrically, using the respective molar extinction coefficients.[7] Other chemicals were commercial products of reagent grade, and water was micro-filtered through a Millipore filtering system.



$m^{7,2,2}$GpppA: R=CH_3, Base2 = Adenine
$m^{7,2,2}$GpppG: R=CH_3, Base2 = Guanine
m^{7}GpppA: R=H, Base2 = Adenine
m^{7}GpppG: R=H, Base2 = Guanine



$m^{7,3'O}$GpppG: R = CH_3; X = Y = O (ARCA)
m^{7}Gp(CH_2)ppG: R = H; X = CH_2, Y = O
m^{7}Gpp(CH_2)pG: R = H; X = O, Y = CH_2

SCHEME 1 Structures of the purine dinucleotide 5′-cap analogs tested as DcpS substrates (left) and inhibitors (right). Site of the enzymatic hydrolysis is indicated by arrow.

The UV absorption spectra of dinucleotides, were recorded with an UV-Vis Cary 300. Fluorescence kinetic measurements were carried out with a Perkin-Elmer LS-50B spectrometer using excitation at 300 or 290 nm and spectral bandwidth of 10–15 nm. The cell-housing block was thermostated at 30 ± 0.2°C.

Reactions were carried out in 50 mM TRIS buffer, pH 6.8–7.9, containing 0.15 M KCl and 2 mM DTT. Initial reaction rates were determined fluorimetrically, using final product fluorescence as an internal standard. Corrections for enzyme instability were introduced whenever necessary. Kinetic parameters were calculated using the initial velocity method.

RESULTS AND DISCUSSION

Fluorescence of Trimethylguanosine Cap Analogs

Dimethylamino analogs of 7-methylguanosine ($m^{2,2,7}G$) and respective nucleotides exhibit fairly intense fluorescence (Figure 1), which is attributed to the cationic form of the compounds (pK_a 7–8). This fluorescence is significantly (i.e. by 70–80%) quenched in purine, but not pyrimidine dinucleotides, presumably due to stacking interactions. Hydrolysis of the pyrophosphate bridge abolishes quenching and leads to marked fluorescence increase, providing sensitive assay of hydrolytic activity, especially at pH <7.5.

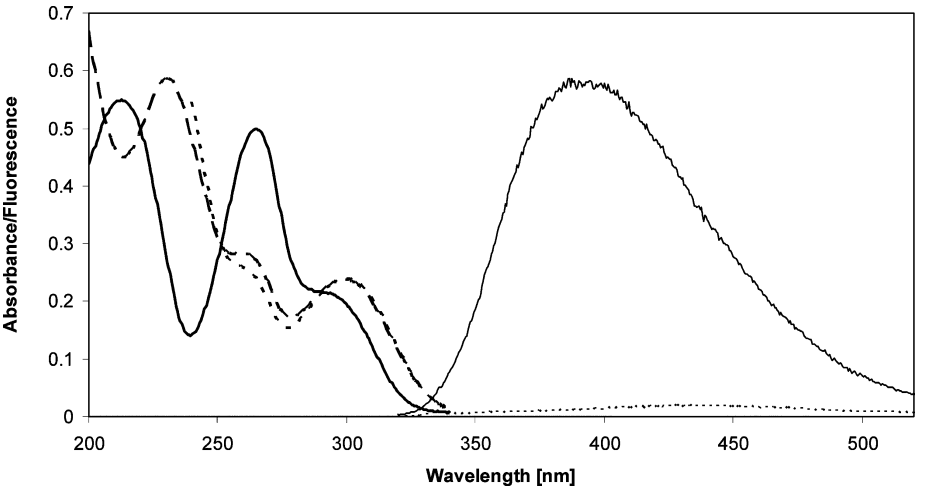


FIGURE 1 Fluorescence (right) and absorption (left) spectra of $m^{7,2,2}GTP$ at various pH: - -, pH 4.5; - - - -, pH 10; dashed line, pH ~9.

TABLE 1 Kinetic parameters for enzymatic hydrolysis of cap analogs by DcpS from *C. elegans*, determined fluorimetrically at 30°C. Accuracy of the K_m determination is ~20%

Substrate	pH	K_m [μM]	V_{max} [relative]	F/F_0
$m^{7,2,2}$GpppA	6.8	2.7	1.0	3.7
	7.9	<2	0.6	2.6
$m^{7,2,2}$GpppG	6.8	2.0	1.3	4.3
	7.9	3.6	1.5	3.5
m^7GpppA	6.8	~0.7	4.5	2.12
	7.9	~0.5	3.5	1.46
m^7GpppG	6.8	~1.2	5.2	2.27

Kinetic Studies

Several purine-containing dinucleotide cap analogs were tested as substrates of the nematode DcpS. The kinetic parameters of the hydrolytic reaction are given in Table 1.

It should be noticed that trimethylated cap analogs generally exhibit similar substrate properties than their monomethylated counterparts, except m^7GpppG and m^7GpppA are hydrolyzed somewhat faster at pH 6.8. Low K_m values reflect high specificity of the DcpS hydrolases, in line with the data for mammalian DcpS.[8] The kinetic parameters are only slightly dependent on pH.

The reaction course indicated definite product inhibition by monophosphates of cap analogs, more pronounced in the case of monomethylated substrates, but low enzyme stability precluded quantitative analysis in this case.

Inhibition of the DcpS Hydrolase by Cap Analogs

Inhibitory properties of non-hydrolyzable or poorly hydrolyzable cap analogs ($(m^7Gp(CH_2)ppG$, $m^7Gpp(CH_2)pG$ and $m^{7,3'O}$GpppG) were tested, using $m^{7,2,2}$GpppA as the most convenient substrate. Reactions were run at pH 6.8, and typical inhibitor concentrations were 0.5–10 μM. The results, showing strong and apparently competitive inhibition of the DcpS, are summarized in Table 2. Two inhibitors, ARCA and m^7GppCH_2pG,

TABLE 2 Inhibition of DcpS from *C. elegans* by cap analogs (inhibition constants are accurate to ~25%)

Inhibitor	Substrate	pH	K_i [μM]	Competition
$m^7Gp(CH_2)ppG$	$m^{7,2,2}$GpppA	6.8	0.07	+
$m^7Gpp(CH_2)pG$	$m^{7,2,2}$GpppA	6.8	<0.05	+
$m^{7,3'O}$GpppG (ARCA)	$m^{7,2,2}$GpppA	6.8	1.0	nd

are also very slowly hydrolyzed by DcpS (rates are <1% of the hydrolysis of $m^{7,2,2}$GpppA).

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