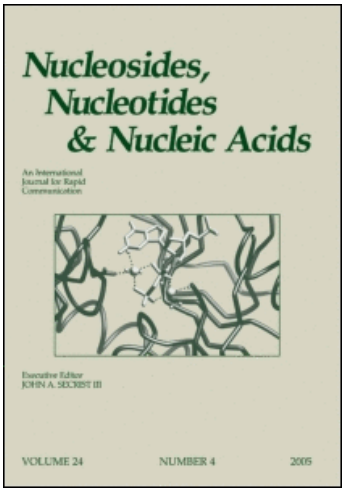

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AFFINITY OF DINUCLEOTIDE CAP ANALOGUES FOR HUMAN DECAPPING SCAVENGER (hDcpS)

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□ *Eukaryotic cells utilize scavenger decapping enzymes to degrade cap structure following 3′-5′ mRNA decay. Human DcpS recently has been described as a highly specific hydrolase (a member of the HIT family) that catalyses the cleavage of m^7GpppG and short capped oligoribonucleotides. We have demonstrated here that cap-1 (m^7GpppGm) is a preferred substrate among several investigated dinucleotide cap analogues m^7Gp_nN ($n = 3$–5, N is a purine or pyrimidine base) and m^7GMP is always one of the reaction product. Cap analogues containing pyrimidine base instead of guanine or diphosphate chain are resistant to hydrolysis catalyzed by human scavenger. Contrary to the other enzymes of HIT family, hDcpS activity is not stimulated by Mg^{2+}.*

Keywords hDcpS; scavenger decapping enzymes; mRNA cap analogues

INTRODUCTION

The human decapping scavenger (hDcpS) is a key enzyme in eukaryotic mRNA degradation process which determines the lifetime of different transcripts in living cells and therefore plays an important role in the post-transcriptional control of gene expression.[1] hDcpS is a highly specific hydrolase catalyzing the cleavage of residual cap structure m7GpppN and/or capped oligonucleotides resulting from the exosom-mediated 3′ → 5′

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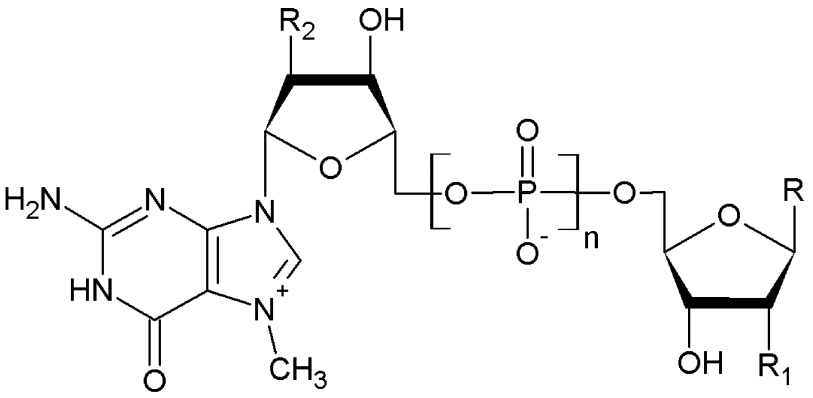
digestion of deadenylated mRNA.[2] Hydrolysis of the cap structure releases m^7GMP and ppN or diphosphate terminated oligo mRNA.[3] Human DcpS is a member of the HIT family of pyrophosphatases containing a conserved histidine triad (His-X-His-X-His) in the active site.[4] The central histidine within the HIT motif plays an important role as a nucleophilic agent in the hydrolysis process.[5] Many aspects of the activity of hDcpS remain unknown. In this article, the substrate specificity of human scavenger with respect to a number of dinucleotide cap analogues has been examined. The aim of the studies was to identify selective substrates for hDcpS and characterize the enzyme activity dependent on the structural modifications introduced into the polyphosphate chain, bases or ribose moieties of investigated cap analogues.

RESULTS AND DISCUSSION

Human decapping scavenger was expressed in *E. coli* according to the procedure described previously.[6] Cap analogues were synthesized in the Department of Biophysics (University of Warsaw)[7–10] and subjected to hydrolysis catalyzed by hDcpS at 25°C, in 50 mM Tris buffer pH 7.6 containing 1 mM DTT, 30 mM $(NH_4)_2SO_4$ and different concentrations of $MgCl_2$ (1–10 mM) and KCl (0–500 mM). The substrate concentration in the reaction mixture was 5 μM. The progress of hydrolysis was monitored using fluorimetric method. Reactions were followed by recording the time-dependent increase of fluorescence intensity caused by removal of the intramolecular stacking as a result of enzymatic cleavage of pyrophosphate bond in dinucleotide cap analogues. The rates of hydrolysis were determined on the basis of initial velocity method.[11] Fluorescent experiments were performed on a LS-50B spectrofluorometer (Perkin-Elmer Co., Norwalk, CT, USA).

The structures of investigated compounds are shown in Scheme 1. These dinucleotides were selected for evaluation as potential substrates of human scavenger that hydrolyse the pyrophosphate bond yielding in all cases 7-methylguanosine monophosphate as the reaction product. The relative rates of hydrolysis catalyzed by hDcpS for different dinucleotide cap analogues are shown in Table 1. The preferred substrate is cap-1 (m^7GpppGm) for which maximal activity was observed. Only half of this activity was reached for m^7GpppG and m^7Gppp(iso)G. Cap analogues containing adenine or N^6-methyladenine instead of guanine exhibited about 30% of the activity of cap-1. More significant decrease of the relative velocity was observed when the number of phosphate groups in the phosphate chain increased from 3 to 5. The replacement of guanine by a pyrimidine base caused the loss of substrate properties towards hDcpS. Cap analogue with diphosphate bridge (m^7GppG) is also resistant to enzymatic cleavage by human scavenger. The presence of Mg^{2+} did not stimulate or inhibit hydrolysis



$m^7GpppGm$	R_2=OH	n=3	R_1=OCH_3	R=Guanine
m^7GpppG	R_2=OH	n=3	R_1=OH	R=Guanine
$m^7Gppp(iso)G$	R_2=OH	n=3	R_1=OH	R=Isoguanine
m^7GpppA	R_2=OH	n=3	R_1=OH	R=Adenine
m^7Gpppm^6A	R_2=OH	n=3	R_1=OH	R=N^6-methyladenine
m^7Gp_4G	R_2=OH	n=4	R_1=OH	R=Guanine
m^7Gp_5G	R_2=OH	n=5	R_1=OH	R=Guanine
$m_2^{7,2'O}GpppG$	R_2=OCH_3	n=3	R_1=OH	R=Guanine

SCHEME 1 Structures of dinucleotide cap analogues.

catalyzed by hDcpS. No significant effect on the hydrolytic activity has been either observed when KCl was added to the reaction mixture. However, as it was previously shown the presence of 200 mM KCl stabilizes the enzyme.[11]

The data presented here show the potential usefulness of modified dinucleotide cap analogues for the study of the mechanism of action of human scavenger. The investigated compounds exhibit remarkable selectivity in their behavior as substrates for hDcpS. It is evidence that both purine ring in the first transcribed nucleoside and the triphosphate bridge are crucial for efficient catalysis. The comparison of relative rates of hydrolysis clearly indicates the highest activity for cap1 which is the natural substrate for hDcpS. Contrary to the other members of HIT family of pyrophosphatases which

TABLE 1 Substrate specificity of human DcpS

Cap analogues	V_{max} (relative)
$m^7GpppGm$	1
m^7GpppG	0.52
$m^7Gppp(iso)G$	0.51
m^7GpppA	0.38
m^7Gpppm^6A	0.28
$m^7GppppG$	0.20
$m^7GpppppG$	0.11
$m_2^{7,2'O}GpppG$	0.02

activity is Mn^{2+}/Mg^{2+} dependent,[12,13] hDcpS exhibit the same activity in the presence and absence of Mg^{2+}.

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