Decapping Scavenger Enzyme Activity toward N2-Substituted 5′ End mRNA Cap Analogues

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ABSTRACT: mRNA degradation is a key mechanism of gene expression regulation. In the 5′ → 3′ decay pathway, mRNA is degraded by the exosome complex and the resulting cap dinucleotide or short-capped oligonucleotide is hydrolyzed mainly by a decapping scavenger enzyme (DcpS)—a member of the histidine triad family. The decapping mechanism is similar for DcpS from different species; however, their respective substrate specificities differ. In this paper, we describe experiments exploring DcpS activity from human (hDcpS), Caenorhabditis elegans (CeDcpS), and Ascaris suum (AsDcpS) toward dinucleotide cap analogues modified at the N2 position of 7-methylguanosine. Various alkyl substituents were tested, and cap analogues with a longer than three-carbon chain were nonhydrolyzable by hDcpS and CeDcpS. Resistance of the modified cap analogues to hDcpS and CeDcpS may be associated with their weaker binding with enzymes.

INTRODUCTION

The 5′ ends of most RNAs are chemically modified by cap structures which play an important role in many cellular processes. RNA caps facilitate pre-mRNA splicing and export to the cytoplasm, promoting translation initiation and protecting the RNA from degradation by nucleases.1,2 Several chemical modifications have been described at RNA 5′ ends and all possess at least one phosphorothioate bond.3 Eukaryotic mRNAs have 5′-5′-linked N7-methyl guanosine (mG) caps (mG cap, MMG cap), and short nuclear RNAs (snRNAs) and nucleolar RNAs (snorRNAs) possess a trimethylated version of the cap (m2,2,7G cap, TMG cap). Hydrolysis of the cap structure is a critical step in mRNA turnover.4,5 mRNA decay is an essential process that allows cells to continuously modify gene expression patterns in response to internal and environmental changes. Decay pathways usually start with the removal of the poly(A) tail from the 3′ end of the mRNA, and further degradation proceeds either 5′ to 3′ or 3′ to 5′ direction.5–8

In 5′ to 3′ decay, a cap pyrophosphate bond is cleaved by decapping enzymes, yielding m7GDP/m3GDP and the monophosphorylated RNA which is hydrolyzed by exonuclease Xrn1.9 In eukaryotes, three enzymes are known to decap mRNAs in vivo: Dcp2, Nudt16, and Nudt3.7,8 These enzymes belong to the class of Nudix phosphohydrolases that utilize a cation to substitute so forth the general structure nucleoside triphosphate linked to another moiety X.10,11 In the 3′ → 5′ decay pathway, mRNA is degraded by a multisubunit exosome complex, and the resulting cap dinucleotide or short-capped oligonucleotide is hydrolyzed by two decapping enzymes: scavenger DcpS (Dcs1 in yeast) and Fhit (Hnt2/Aph1 in yeast).12,13 Both DcpS and Fhit are members of the histidine triad (HIT) family of pyrophosphatases, with a conserved histidine triad (HxHxH) in the active site. The HIT motif is utilized to bind substrates and cleave the pyrophosphate bond within the cap, releasing m7GMP.14 For specific cap recognition by DcpS enzymes and effective hydrolysis, a positive charge is required, introduced by a substituent at the N7 position of the guanine moiety,15 as well as the triphosphate chain.12

The mechanism of the enzymatic cap cleavage by decapping scavengers is very similar among different species (human, nematode, and yeast), but their substrate specificity differs significantly. All DcpS enzymes are highly specific for dinucleotides and less active toward short oligonucleotides; long mRNAs are not targeted.16–18 Human and yeast enzymes accept cap analogues containing up to 10 nucleotides as substrates, whereas nematode DcpS recognizes shorter species, capped di-, tri-, and tetranucleotides. Human and yeast decapping scavengers are responsible for degradation of monomethylated (at N7 position) cap analogues only, while nematode enzymes exhibit hydrolytic activity toward mono- and trimethylated cap structures.

We have previously determined the DcpS—cap interactions crucial for the catalytic activity of human and nematode decapping scavengers.12,15,17 We tested the influence of some modifications of the cap structure on the efficient binding and
hydrolysis by DcpS. We found that modifications of the phosphate chain and ribose moiety of m7G affect the binding affinity and hydrolysis rate of cap analogues, whereas the type of the second nucleoside and the size of substituent at N7 are less important. The role of the N2 substitution has not been examined in detail.

Since the development of a procedure to chemically synthesize cap analogues modified within the N2 position of the purine ring, such analogues have become a powerful tool to examine biological properties of cap binding proteins and for potential medical applications. It has been recently shown that N2 substituted analogues of 7-methylguanosine 5′-monophosphates represent a promising class of effective translation inhibitors to counteract elevated eIF4E levels in tumor cells. Such compounds possess an IC50 similar to m7GTP or m7GpppG despite a reduced number of phosphate groups. This feature may support their cell penetration. In addition, when incorporated into mRNA, N2-modified ARCA (anti-reversed cap) analogues improved its translational properties as well as enhancing mRNA stability in HEK293 cells compared to the ARCA-capped transcript. The ARCA cap is considered an excellent substrate to yield capped transcripts. Additionally, 7-methylguanosine cap analogues possessing various substituents at the exocyclic 2-amino group appeared to be a very sensitive and effective tool for the investigation of the molecular mechanism of TMG-capped U snRNA recognition by snurportin 1, an adaptor protein crucial for the active nuclear import of this class of RNA. For these reasons, experiments enhancing our understanding of substrate specificity and hydrolytic activity of decapping enzymes toward differently modified cap structures are of great importance.

In the present work, we investigated the influence of the N2 substitution within the cap structure on the interaction with decapping scavengers (DcpS) from different species (human, Caenorhabditis elegans and Ascaris suum). We compared the hydrolysis rates of dinucleotide cap analogues bearing various alkyl groups within an exocyclic amine group of 7-methylguanosine and the binding affinity of some of hydrolysis products and nonhydrolyzable analogues. Our results suggest that cap analogues possessing a longer than three-carbon chain were nonhydrolyzable by hDcpS and CeDcpS. Resistance of modified cap analogues to hDcpS and CeDcpS may be associated with their weaker binding with enzymes.

## RESULTS AND DISCUSSION

In previous kinetic studies of cap cleavage by DcpS enzymes, we compared the hydrolysis rate between m7GpppG and m7,2,7-GpppG. The considerable difference between the decapping activity of these proteins is the ability of C. elegans and A. suum DcpS to efficiently hydrolyze both MMG and TMG caps, in contrast, the human enzyme is not responsible for TMG cleavage. The presence of two methyl groups at the N2 position of 7-methylguanosine is a key determinant of differential specificities of human and nematode decapping scavengers.

To further investigate the effect of N2 substitution on cap recognition and hydrolysis by DcpS, we examined several dinucleotide cap analogues with different alkyl groups introduced at the N2 position. N2-modified dinucleotide cap analogues synthesized for DcpS activity studies included compounds containing in the N2 position of 7-methylguanosine: methyl (m2m7GpppG), ethyl (etm7GpppG), propyl (prop2m7GpppG), isopropyl (iprop2m7GpppG), butyl (but2m7GpppG), and isobutyl (ibut2m7GpppG) substituents (Figure 1). All compounds were obtained and characterized as described previously.

The hydrolysis process of each compound was studied with human, C. elegans and A. suum DcpS in the same experimental conditions (20 °C, 50 mM phosphate buffer pH 7.2 containing 150 mM NaCl). Reaction progress was monitored by high-performance liquid chromatography (HPLC). The comparison of hydrolysis rate is presented in Figures 2 and Table 1.

Our analysis indicates some differences in substrate specificity of the three enzymes. Human and C. elegans DcpS exhibit higher specificity than the A. suum enzyme, as they only efficiently hydrolyze cap analogues with a small substituent at the N2 position (methyl or ethyl group). The rate of hydrolysis catalyzed by human DcpS is 2–3 times lower in comparison with m7GpppG. In the case of C. elegans DcpS, m7m’GpppG and et’mGpppG are hydrolyzed more efficiently than m7GpppG. Prop’mGpppG is a poor substrate for human and C. elegans DcpS; it is hydrolyzed only by C. elegans DcpS but very slowly. The reaction with human DcpS occurs when
the enzyme concentration increases 1 order of magnitude in comparison with m7GpppG. Dinucleotides containing larger substituents at the N2 position (isopropyl, butyl, and isobutyl) are resistant to enzymatic digestion by human and C. elegans DcpS. In contrast to these two enzymes, A. suum DcpS accepts all investigated dinucleotides as substrates. They are hydrolyzed with a comparable rate except etm7GpppG, which is cleaved very rapidly.

Our kinetic data indicate that the size of the substituent introduced at the N2 position strongly influences the rate of hydrolysis catalyzed by human and C. elegans DcpS. For nonhydrolyzable but2m7GpppG, binding affinity studies with both enzymes were performed (Figure 3). As presented in Table 1, the hydrolysis rate of cap analogues is significantly lower for the butylated dinucleotides compared to the parent cap analogues. The data show that the hydrolysis rate decreases as the size of the substituent at the N2 position increases.

**Table 1. Hydrolysis Rate of Cap Analogues**

| cap analogue | hDcpS (μM/min) | CeDcpS (μM/min) | AsDcpS (μM/min) |
|--------------|----------------|-----------------|-----------------|
| m7GpppG      | 4.33 ± 0.23    | 1.83 ± 0.21     | 1.84 ± 0.19     |
| m2m7GpppG    | 1.91 ± 0.18    | 3.73 ± 0.28     | 1.82 ± 0.15     |
| et2m7GpppG   | 0.92 ± 0.12    | 6.27 ± 0.36     | 5.94 ± 0.22     |
| prop2mGpppG  | nh b           | 0.12 ± 0.02     | 2.14 ± 0.19     |
| irop2mGpppG  | nh nh          | 2.51 ± 0.16     | 2.13 ± 0.18     |
| but2m7GpppG  | nh nh          | 2.64 ± 0.12     | 2.13 ± 0.18     |
| ibut2m7GpppG | nh nh          | 2.13 ± 0.18     | 2.13 ± 0.18     |

“Experiments were performed at 20 °C, in 50 mM phosphate buffer pH 7.2, containing 150 mM NaCl. Initial concentration of cap analogues was 20 μM. Reactions were initiated by the addition of 2 μL of DcpS enzyme to 1 mL of reaction mixture. The V0 values were calculated as averages of three independent experiments. bNot hydrolyzed.

**Figure 3.** Fluorescence titration curves of human DcpS with m2m7GMP (top drawing) and but2m7GpppG (bottom drawing). The increase of fluorescence intensity at cap analogue concentration above 1 μM corresponds to the fluorescence of unbound compounds.

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**Figure 3.** Fluorescence titration curves of human DcpS with m2m7GMP (top drawing) and but2m7GpppG (bottom drawing). The increase of fluorescence intensity at cap analogue concentration above 1 μM corresponds to the fluorescence of unbound compounds.

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**Table 2. Binding Affinity of Human and C. elegans DcpS toward but2m7GpppG and m2m7GMP Determined at 20 °C, in 50 mM Phosphate Buffer pH 7.2, Containing 150 mM NaCl**

| cap analogue | Kd (μM) | ΔG° (kcal/mol) |
|--------------|---------|----------------|
| CeDcpS       |         |                |
| but2m7GpppG  | 5.11 ± 0.71 | −8.99 ± 0.08   |
| m2m7GMP      | 7.36 ± 1.56 | −9.20 ± 0.13   |
| hDcpS        |         |                |
| but2m7GpppG  | 5.78 ± 0.64 | −9.06 ± 0.06   |
| m2m7GMP      | 3.50 ± 0.70 | −8.79 ± 0.11   |
Table 2, the association constants ($K_a$) and Gibbs free energies ($\Delta G^\circ$) of but-m'GpppG binding with human and C. elegans DcpS are very similar. The $K_a$ values ($5.11 \pm 0.71$ and $5.78 \pm 0.64 \mu M^{-1}$) are 1 order of magnitude smaller than $K_a$ determined previously for dinucleotides nonmodified at the N2 position.\(^{12,23}\) Such weak interactions of but-m'GpppG with human and C. elegans DcpS may explain its resistance to hydrolysis catalyzed by both enzymes.

We also compared the binding affinity of m'Gm'GMP for human and C. elegans decapping scavenger. We found that C. elegans DcpS have similar $K_a$ values for m'Gm'GMP ($7.36 \pm 1.56 \mu M^{-1}$) (Table 2) and m'GMP ($8.00 \pm 1.56 \mu M^{-1}$),\(^{23}\) whereas human DcpS exhibits 6-fold lower affinity for m'Gm'GMP ($3.5 \pm 0.7 \mu M^{-1}$) (Table 2) compared to m'GMP ($19 \pm 1 \mu M$).\(^{23}\) The decreasing affinity of human DcpS for cap analogue-containing substituent at the N2 position affects the rate of hydrolysis.

Our results reveal different activity of human and nematode DcpS depending on the type of the alkyl group introduced at the N2 position. For human DcpS, m'GpppG is the best substrate. Dinucleotides bearing the methyl or ethyl group at the N2 position were also hydrolyzed by this enzyme but less efficiently. In contrast, hydrolytic activity of C. elegans DcpS is higher toward m'Gm'GpppG and et'2m7GpppG than for m'GpppG. Prop'm'GpppG is a very poor substrate for human and C. elegans DcpS, and dinucleotides with butyl, isopropyl or isobutyl group at the N2 position are resistant to cleavage by both enzymes. A. suum DcpS accepts as substrates all investigated dinucleotides, hydrolyzing them with a comparable rate. These results indicate that the region within the cap-binding pocket of A. suum DcpS, which is involved in the interaction with N2 position, is more flexible in comparison with human and C. elegans DcpS. This flexibility may explain why A. suum DcpS can accommodate cap dinucleotides with significantly large alkyl groups at the N2 position (such as propyl, isopropyl, butyl, and isobutyl) and hydrolyze them with a similar rate as m'GpppG.

Some other types of cap modifications have been tested previously to determine their influence on binding affinity for DcpS and susceptibility to DcpS-mediated hydrolysis. It was shown that modifications within the triphosphate chain as well as at the 2'-O- or 3'-O-position of m'G ribose affect the binding affinity for decapping scavengers and protect it from enzymatic cleavage.\(^{12}\) A distinct effect was observed when various substituents were introduced at the N7 position. Dinucleotide cap analogues bearing differently sized groups at this position (methyl, ethyl, butyl, and benzyl) were hydrolyzed with a comparable rate by the human DcpS.\(^{15}\) In the case of nematode DcpS, cap analogues substituted by ethyl, butyl, or benzyl were hydrolyzed more efficiently than m'GpppG.\(^{15}\)

In summary, we conclude that the human DcpS enzyme exhibits the highest substrate specificity. Substitution of different positions within m'G generally decreases the hydrolytic susceptibility of dinucleotides. C. elegans DcpS accepts different modifications at the N7 position but only small substituent at N2. A. suum DcpS exhibits the hydrolytic activity toward dinucleotide cap analogues with various modifications at the N7 or N2 position.

■ EXPERIMENTAL SECTION

Cap Analogue Synthesis. N2-substituted cap analogues investigated in this work (x'm'GpppG) were synthesized and characterized as described in detail earlier.\(^{19,22}\) The concentrations of all compounds were determined on the basis of their absorption coefficients.

DcpS Production and Purification. Human or C. elegans DcpS was expressed from the respective pET vectors in the Rosetta 2(DE3) Escherichia coli strain. Protein expression was induced with 0.4 mM IPTG when OD$_{600}$ reached 0.5–0.8, and then, cells were further incubated at 18 °C overnight. After expression, the bacterial pellet was collected by centrifugation (7000g, 10 min) and was frozen at −80 °C. The frozen pellet was thawed on ice and then resuspended in ice-cold lysis buffer: 50 mM phosphate buffer pH 7.2, 150 mM NaCl, 1% Triton X-100, 20 mM imidazole. Suspension was sonicated and then centrifuged for 2 h. The supernatant was loaded on the HisTrapHP column (GE Healthcare Life Sciences). All separation by affinity chromatography was done with a gradient of imidazole 20–600 mM in 50 mM phosphate buffer pH 7.2. The DcpS protein was further purified and buffer exchanged: 50 mM Tris-HCl, 150 mM NaCl, 10% glycerol pH 7.5, by gel filtration (“Superdex 200 10/300 GL” GE Healthcare Life Sciences) using an ÄKTA protein purification system (GE Healthcare Life Sciences) and stored in −80 °C. A. suum DcpS was prepared according to the procedure described previously.\(^{23}\)

Decapping Assays. The hydrolytic susceptibility of dinucleotide cap analogues was examined in 50 mM phosphate buffer pH 7.2 containing 150 mM NaCl. Before each experiment, 2 mL of buffer solution containing the investigated compound was incubated at 20 °C for 10 min. The hydrolysis process was initiated by the addition of recombinant DcpS. To control the hydrolysis progress, 400 μL aliquots of the reaction mixture were withdrawn at different time intervals and incubated at 97 °C during 5 min to stop the reaction by heat inactivation of the enzyme. The samples were then subjected to analytical HPLC.

Analysis of Hydrolytic Stability of Cap Analogues. Analysis of hydrolysis products were done by the HPLC system (Agilent 1200 series) equipped with a reverse-phase Supelcosil LC-18-T column and UV−vis detector. Experiments were performed at 20 °C with a linear gradient of methanol in 0.1 M KH$_2$PO$_4$ (from 0 to 25% or from 0 to 40%) over 15 min at a flow rate of 1.0 mL/min. The changes of absorbance at 260 nm were monitored continuously during analysis.

Fluorescence Titration Analysis of Binding Affinity. DcpS-cap binding affinity was determined by monitoring the quenching of intrinsic DcpS Trp fluorescence. The experiments were performed on the LS-50B spectrophluorometer (PerkinElmer Co, Waltham, MA) in a quartz cuvette with an optical length of 4 mm for absorption and 10 mm for emission. All measurements were done at 20 °C, in 50 mM phosphate buffer pH 7.2 containing 150 mM NaCl. 1 μL aliquots of cap analogue solution of increasing concentration (from 2 μM to 2 mM) were added to 1.4 mL of DcpS solution (0.2 μM). The fluorescence intensity was monitored at 340 nm and corrected for sample dilution and inner filter effects. The equilibrium association constants for single titration curves were determined by fitting the theoretical dependence of the fluorescence intensity to the experimental data points. The final $K_a$ values were calculated as weighted averages of three independent titrations. The numerical least-squares nonlinear regression analysis was performed using ORIGIN 8.0 (Microcal Software, Inc.).
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REFERENCES

(1) Topisirovic, I.; Sv Hitkin, Y. V.; Sonenberg, N.; Shatkin, A. J. Cap and cap-binding proteins in the control of gene expression. Wiley Interdiscip. Rev.: RNA. 2011, 2, 277–298.

(2) Cougot, N.; van Dijk, E.; Babajko, S.; Serafin, B. Cap-tabolism. Trends Biochem. Sci. 2004, 29, 436–444.

(3) Ghosh, A.; Lima, C. D. Enzymology of RNA cap synthesis. Wiley Interdiscip. Rev.: RNA. 2010, 1, 152–172.

(4) Garneau, T.; Wilusz, J.; Wilusz, C. J. The highways and byways of mRNA decay. Nat. Rev. Mol. Cell Biol. 2007, 8, 113–126.

(5) Meyer, S.; Temme, C.; Wahle, E. Messenger RNA turnover in eukaryotes: pathways and enzymes. Crit. Rev. Biochem. Mol. Biol. 2004, 39, 197–216.

(6) Arribas-Layton, M.; Wu, D.; Lykke-Andersen, J.; Song, H. Structural and functional control of the eukaryotic mRNA decapping machinery. Biochim. Biophys. Acta 2013, 1829, 580–589.

(7) Grudzien-Nogalska, E.; Kiledjian, M. New insight into decapping enzymes and selective mRNA decay. Wiley Interdiscip. Rev.: RNA. 2017, 8, No. e1379.

(8) Kramer, S.; McMellen, A. G. The complex enzymology of mRNA decapping: Enzymes of four classes cleave pyrophosphate bonds. Wiley Interdiscip. Rev.: RNA. 2019, 10, No. e1511.

(9) Nagarajan, V. K.; Jones, C. I.; Newbury, S. F.; Green, P. J. XRN 5'→3' exonuclease: structure, mechanism and functions. Biochim. Biophys. Acta 2013, 1829, 590–603.

(10) McMellen, A. G. The nudix hydrolase superfamily. Cell. Mol. Life Sci. 2006, 63, 123–143.

(11) McMellen, A. G. Substrate ambiguity among the nudix hydrolases: biologically significant, evolutionary remnant, or both? Cell. Mol. Life Sci. 2013, 70, 373–385.

(12) Milac, A. L.; Bojarska, E.; Wypijewska del Nogal, A. Decapping Scavenger (DcpS) enzyme: Advances in its structure, activity and roles in the cap-dependent mRNA metabolism. Biochim. Biophys. Acta, Gene Regul. Mech. 2014, 1839, 452–462.

(13) Taverniti, V.; Serafin, B. Elimination of cap structures generated by mRNA decay involves the new scavenger mRNA decapping enzyme Aph1/FHTI together with DcpS. Nucleic Acids Res. 2015, 43, 482–492.

(14) Lima, C. D.; Klein, M. G.; Hendrickson, W. A. Structure-based analysis of catalysis and substrate properties definition in the HIT protein family. Science 1997, 278, 886–890.

(15) Piecyk, K.; Darzynkiewicz, Z. M.; Jankowska-Anyszka, M.; Ferenc-Mrozek, A.; Stepinski, J.; Darzynkiewicz, E.; Bojarska, E. Effect of different N7 substitution of dimethylcap analogs on the hydrolytic susceptibility towards scavenger decapping enzymes (DcpSs). Biochem. Biophys. Res. Commun. 2015, 464, 89–93.

(16) Liu, S.-W.; Jiao, X.; Liu, H.; Gu, M.; Lima, C. D.; Kiledjian, M. Functional analysis of mRNA scavenger decapping enzyme. RNA 2004, 10, 1412–1422.

(17) Cohen, L. S.; Mikhail, C.; Friedman, C.; Jankowska-Anyszka, M.; Stepinski, J.; Darzynkiewicz, E.; Davis, R. E. Nematode mGpppG and m7GpppG decapping: Activities in Ascaris embryos and characterization of C. elegans DcpSs. Adv. 2004, 10, 1609–1624.

(18) Malys, N.; McCarthy, J. E. Dec2, a novel stress-induced modulator of mGpppX pyrophosphate activity that locates to P-bodies. J. Mol. Biol. 2006, 363, 370–382.

(19) Piecyk, K.; Davis, R. E.; Jankowska-Anyszka, M. Synthesis of N7-modified 7-methylguanosine 5′-monophosphates as nematode translation inhibitors. Bioorg. Med. Chem. 2012, 20, 4781–4789.

(20) Piecyk, K.; Lukaszewicz, M.; Darzynkiewicz, E.; Jankowska-Anyszka, M.; Trizazole-containing monophosphate mRNA cap analogs as effective translation inhibitors. RNA 2014, 20, 1539–1547.

(21) Kocmi, I.; Piecyk, K.; Rudzinska, M.; Niedzwiecka, A.; Darzynkiewicz, E.; Grzela, R.; Jankowska-Anyszka, M. Modified ARCA analogs providing enhanced translational properties of capped mRNAs. Cell Cycle 2018, 17, 1624–1636.

(22) Piecyk, K.; Niedzwiecka, A.; Ferenc-Mrozek, A.; Lukaszewicz, M.; Darzynkiewicz, E.; Jankowska-Anyszka, M. How to find the optimal partner—studies of snurportin 1 interactions with U snRNA 5′-TMG-cap analogues containing modified 2- amino group of 7-methylguanosine. Bioorg. Med. Chem. 2015, 23, 4660–4668.

(23) Wypijewska, A.; Bojarska, E.; Lukaszewicz, M.; Stepinski, J.; Jemielity, J.; Davis, R. E.; Darzynkiewicz, E. 7-methylguanosine diphosphate (mGDP) is not hydrolyzed but strongly bound by scavenger decapping enzymes. Gene Regul. Mech. 2011, 4, No. e1511.