

Interaction of human decapping scavenger with 5' mRNA cap analogues: structural requirements for catalytic activity

This article has been downloaded from IOPscience. Please scroll down to see the full text article.

2007 J. Phys.: Condens. Matter 19 285217

(<http://iopscience.iop.org/0953-8984/19/28/285217>)

View [the table of contents for this issue](#), or go to the [journal homepage](#) for more

Download details:

IP Address: 129.252.86.83

The article was downloaded on 28/05/2010 at 19:48

Please note that [terms and conditions apply](#).

Interaction of human decapping scavenger with 5' mRNA cap analogues: structural requirements for catalytic activity

Zbigniew M Darzynkiewicz¹, Elzbieta Bojarska¹, Joanna Kowalska¹,
Magdalena Lewdorowicz¹, Jacek Jemielity¹, Marcin Kalek¹,
Janusz Stepinski¹, Richard E Davis² and Edward Darzynkiewicz¹

¹ Department of Biophysics, Institute of Experimental Physics, Warsaw University, 93 Zwirki & Wigury Street, 02-089 Warsaw, Poland

² Department of Biochemistry and Molecular Genetics, University of Colorado Health Sciences Center, 12801 East 17th Avenue, Aurora, CO 80045, USA

E-mail: zdarzynk@fuw.edu.pl

Received 17 October 2006, in final form 4 December 2006

Published 25 June 2007

Online at stacks.iop.org/JPhysCM/19/285217

Abstract

The cap structure is a specific feature of the 5' end of mRNA which plays an important role in the post-transcriptional control in gene expression. A major step of gene regulation occurs at the level of mRNA turnover. Degradation of most eukaryotic mRNAs entails the removal of the cap structure in the various pathways. A human scavenger decapping enzyme (hDcpS) catalyses the cleavage of the residual cap structure m⁷GpppN and/or short oligonucleotides after the 3' → 5' exosome mediated digestion. In this paper we report a fluorescence study of association process of hDcpS with m⁷GMP, m⁷GDP and selected dinucleotide cap analogues resistant to enzymatic hydrolysis. The calculated values of association constants (K_{as}) and corresponding Gibbs free energies (ΔG°) depend on the type of substituents and their positions in the cap molecule, indicating which structural modifications are crucial for the catalysis.

1. Introduction

Eukaryotic mRNA is modified at its 5' end by a cap structure which plays an important role in many cellular processes including pre-mRNA splicing, its transport from nucleus to cytoplasm, efficient translation [1–3] and stabilization of mRNA against 5' → 3' exonucleolytic digestion [4]. Because of the important functions mediated by the cap, the level of mRNA in the cells needs to be tightly controlled.

There are two general pathways of eukaryotic mRNA degradation (3' → 5' and 5' → 3' directions), both initiated by shortening of the poly(A) tail and involving the cleavage of the cap structure [5, 6]. In the 5' → 3' decay, Dcp2 enzyme utilizes capped mRNA as a substrate and

hydrolyses the cap structure to generate m⁷GDP and 5' monophosphate mRNA. The exposed 5' end of the mRNA is degraded by exoribonuclease Xrn1, leading to a rapid decay of the remaining transcript. Degradation of mRNA in the 3' → 5' direction involves a complex of nucleases (exosom) which releases a free cap dinucleotide and/or capped oligoribonucleotides, hydrolysed further by DcpS scavengers, generating m⁷GMP [7]. The study of scavenger decapping activity indicates its strong preference for cap structure linked to mRNA containing fewer than 10 nucleotides.

The human decapping scavenger (hDcpS), a key enzyme in the mRNA 3' → 5' catabolic pathway, is a member of the HIT family of pyrophosphatases [8] containing in the active site a histidine triad with three histidines separated by hydrophobic residues (His-ϕ-His-ϕ-His). The two conserved histidines, His277 and His279, within the HIT motif are critical for decapping activity. The cleavage of cap structure catalysed by hDcpS proceeds through nucleophilic attack of His277 on the γ-phosphate group within the triphosphate bridge of substrates according to the mechanism proposed for the HIT protein family [9].

Many aspects of hDcpS activity regulation remain unknown. To gain insight into the formation and stability of the hDcpS–cap complex, binding studies using the human scavenger and cap analogues resistant to enzymatic hydrolysis were conducted by fluorescence titration experiments.

2. Materials and methods

Human DcpS (expressed in *E. coli*) was obtained according to [10]. The enzyme was stored at –80 °C in 20 mM Tris buffer (pH 7.5) containing 50 mM KCl, 0.2 mM EDTA, 0.5 mM PMSF and 20% glycerol. Cap analogues were synthesized as described earlier [11, 12].

The binding affinity of hDcpS for cap analogues was determined by monitoring the quenching of intrinsic Trp fluorescence. The time-synchronized-titration (TST) method was applied for this study [13]. The experiments were performed on LS-50B and LS-55 spectrofluorometers (Perkin Elmer Co., Norwalk, CT, USA) at 20 °C, in 50 mM TRIS/HCl, pH 7.6 containing 200 mM KCl, 0.5 mM EDTA and 1 mM DTT. Aliquots of 1 μl of the cap analogue solutions of increasing concentrations were added to 1 ml of the 0.1 μM DcpS solution. The fluorescence intensity was monitored at 340 nm (excitation at 280 nm) and corrected for sample dilution and inner filter effects. The equilibrium association constants have been derived from the fluorescence titration by fitting of the theoretical curve to the experimental data according to the equation

$$F = F_0 - [EL](\Delta f + f_L) + [L]f_L, \quad (1)$$

where F is measured fluorescence intensity, F_0 is the initial fluorescence intensity, Δf the difference between the fluorescence intensities of the apo-enzyme and the complex, and f_L the fluorescence intensity of the cap analogue. The equilibrium concentration of the cap–protein complex is given by

$$[EL] = \frac{[L] + [E_{\text{act}}]}{2} + \frac{1 - \sqrt{(K_{\text{as}}([L] - [E_{\text{act}}] + 1))^2 + 4K_{\text{as}}[E_{\text{act}}]}}{2K_{\text{as}}}, \quad (2)$$

where $[E_{\text{act}}]$ is the concentration of active protein. The numerical least-squares nonlinear regression was performed using ORIGIN 6.0 (Microcal Software Inc., USA). Obtained K_{as}^i values were association constants for single titration. Final K_{as} values were calculated as weighted averages of $\ln(K_{\text{as}}^i)$.

The Gibbs free energy of binding, ΔG° , was calculated as

$$\Delta G^\circ = -RT \ln K_{\text{as}}. \quad (3)$$

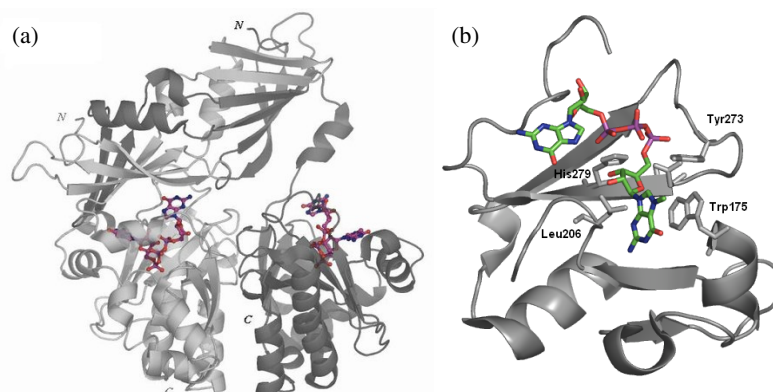


Figure 1. Ribbon diagrams generated by PyMOL (DeLano Scientific LLC, USA, [19]): (a) two m^7 GpppG molecules bound to DcpS dimer, with one molecule in closed (left) and the other in open (right) conformation; (b) cap-binding site with residues crucial in the binding process.

(This figure is in colour only in the electronic version)

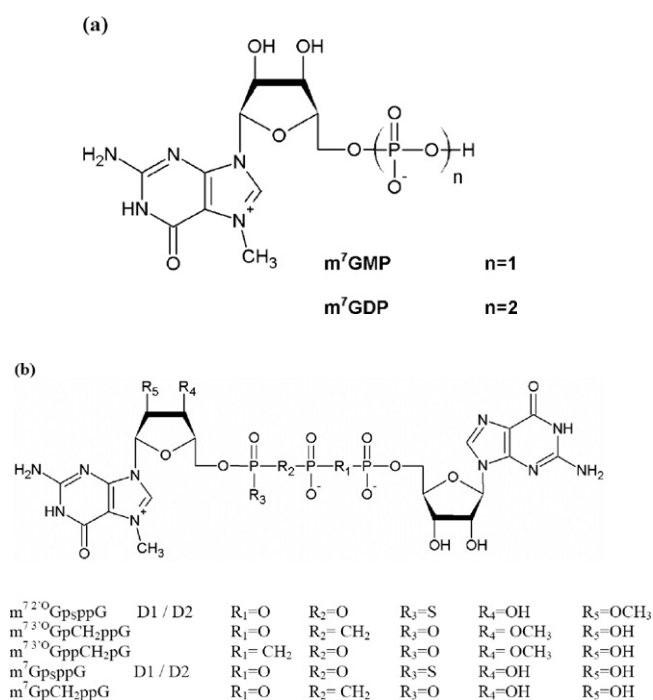
3. Results and discussion

The crystallographic data of human DcpS suggest a dynamic mechanism of the catalytic cycle [14, 15]. The structure of apo-hDcpS shows that the free protein forms a symmetric dimer which is different from the asymmetric dimer observed for the hDcpS–cap complex. Human scavenger with the bound cap analogues (m^7 GDP, m^7 GpppG, m^7 GpppA) creates an open non-productive complex and a closed productive complex during the catalytic cycle, which differ by a 30 Å movement in the N-terminal domain (figure 1(a)). This difference indicates that enzymatic reactions catalysed by hDcpS are accompanied by significant conformational changes of the protein upon the substrate binding, hydrolysis and product release.

To better understand the mechanism and regulation of hDcpS activity we have determined the binding affinity of human decapping scavenger for two mononucleotides (m^7 GMP and m^7 GDP) and non-hydrolysable dinucleotide cap analogues modified in the phosphate chain and ribose moiety of 7-methylguanosine (scheme 1).

The fluorescence titration curves of investigated cap analogues are presented in figure 2. The K_{as} and ΔG° values for complexes of the human scavenger with mono- and dinucleotides are collected in table 1. A significant difference has been observed between the association constants of the two mononucleotides. The binding affinity of hDcpS for m^7 GMP is sixfold weaker than that for m^7 GDP. These results indicate the importance of the second phosphate group for efficient binding with the enzyme. They are in agreement with the structural analysis of hDcpS complexes with cap analogues (m^7 GDP, m^7 GpppG, m^7 GpppA), showing that His279 makes direct contacts to the α , β phosphates in m^7 GDP and β , γ phosphates in dinucleotides [14, 15]. The weaker interaction of m^7 GMP with the enzyme is also caused by the conformational changes from the closed to the open configuration of the protein. The closed configuration stabilizes the substrate binding, while in the open configuration (which is adopted by m^7 GMP) the weaker binding enhances product release [15].

Dinucleotide cap analogue m^7 GpCH₂ppG exhibit stronger binding affinities as compared with m^7 Gp₅ppG (both D1 and D2 diastereoisomers) and m^7 GDP. The replacement of the pyrophosphate bridge oxygen between β - and γ -phosphorus atoms by a methylene group retains strong binding to hDcpS, higher than for the other investigated dinucleotide cap



Scheme 1. (a) Mononucleotide cap analogues m⁷GMP and m⁷GDP; (b) dinucleotide cap analogues. D1 and D2 are symbols for different stereoisomers [11].

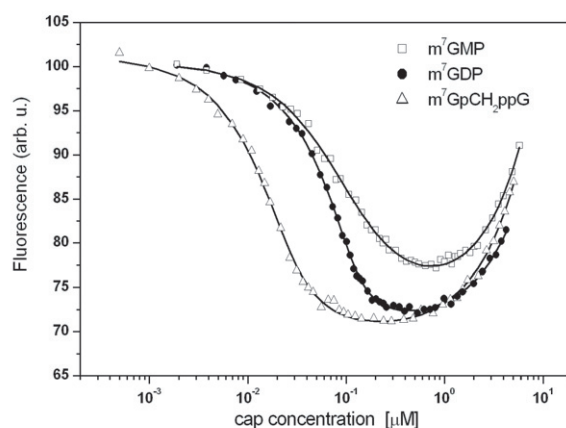


Figure 2. Titration curves describing hDcpS–cap interactions. The experiments were performed at 20 °C in 50 mM Tris/HCl, pH 7.6, 0.5 mM EDTA and 1 mM DTT (an increasing fluorescence signal at higher cap concentrations originates from emission of the free cap analogues in solution).

analogues. The almost two-fold smaller affinity constants obtained for m⁷GpsppG indicate that steric hindrance and charge distribution changes induced by oxygen to sulfur replacement in the phosphate bridge significantly weaken the cap analogues' binding with hDcpS. The absolute configurations of D1 and D2 diastereoisomers have not been yet ascribed, so it is difficult to discuss details of their steric effects on binding with hDcpS.

Table 1. Equilibrium association constants (K_{as}) and binding Gibbs free energies (ΔG°) for the cap–DcpS complexes at 20 °C.

Cap analogue	K_{as} (μM^{-1})		ΔG° (kcal mol ⁻¹)	
m ⁷ GPM	19	±1	-9.77	±0.03
m ⁷ GDP	114	±8	-10.80	±0.04
m ⁷ 2' ^O Gp _S ppG D1	45	±2	-10.26	±0.03
m ⁷ 2' ^O Gp _S ppG D2	14.8	±0.6	-9.61	±0.01
m ⁷ 3' ^O GpCH ₂ ppG ^a	43	±4	-10.23	±0.05
m ⁷ 3' ^O GppCH ₂ pG ^a	37	±3	-10.14	±0.05
m ⁷ Gp _S ppG D1	146	±6	-10.94	±0.02
m ⁷ Gp _S ppG D2	121	±10	-10.83	±0.05
m ⁷ GpCH ₂ ppG ^a	234	±14	-11.21	±0.03

^a Data from [16].

The analogues described above (m⁷GpCH₂ppG and two m⁷Gp_SppG stereoisomers) have modifications only in the phosphate bridge. Additional modification within ribose moiety by introducing the 2'^O- or 3'^O-methyl substituents results in a significant affinity constant decrease. For phosphorothioate-modified analogues the K_{as} constants are ~3 to ~9 fold lower (ΔG° less negative by 0.6 to 1.3 kcal mol⁻¹), depending on the stereoisomers. The K_{as} value for m⁷ 2'^OGpCH₂ppG is ~5 fold lower than for m⁷GpCH₂ppG. These results confirm the important role of the residues responsible for stabilizing the sugar ring of m⁷Guo moiety: Asp205, Lys207, Tyr217 and presumably His279. Weakening these interactions by steric hindrance significantly diminishes the binding affinity.

Furthermore, m⁷ 3'^OGppCH₂pG revealed itself to be a non-hydrolysed compound bound by hDcpS protein, while in contrast m⁷GppCH₂pG undergoes very slow hydrolysis [16]. This indicates the additive character of affinity decreasing effects. A methylene group replacing oxygen in the phosphate bridge affects the charge distribution and causes weaker interactions, while ribose ring modification of m⁷Guo creates steric hindrance for the enzyme active-site amino acids.

Both β - γ methylene and phosphorothioate dinucleotide cap analogues are resistant to cleavage by hDcpS. Such modifications of substrate analogues of HIT proteins make them hydrolysis-resistant compounds [17, 18].

4. Conclusions

Affinity studies revealed the crucial role of β -phosphate for binding and stabilizing cap–DcpS complexes. Coherent results obtained for a group of non-hydrolysed cap analogues show that m⁷GpCH₂ppG presents the highest association constant, while dinucleotides with m⁷Guo sugar ring modifications are characterized by a significant affinity decrease.

Acknowledgments

This project was supported by the Polish Ministry of Science and Higher Education (2 P04A 006 28) and Howard Hughes Medical Institute Grant 55005604 (to ED).

References

- [1] Lewis J D and Izzaualde E 1997 *Eur. J. Biochem.* **247** 461–9
- [2] Furuichi Y and Shatkin A K 2000 *Adv. Virus Res.* **55** 1526–37

- [3] Gross J D, Moerke N J, von der Haar T, Lugovskoy A A, Sachs A B, McCarthy J E and Wagner G 2003 *Cell* **115** 739–50
- [4] Ross J 1995 *Microbiol. Rev.* **59** 423–50
- [5] Collier J and Parker R 2004 *Annu. Rev. Biochem.* **73** 861–90
- [6] Parker R and Song H 2004 *Struct. Mol. Biol.* **11** 121–7
- [7] Van Dijk E, Hir H L and Seraphin B 2003 *Proc. Natl Acad. Sci. USA* **100** 12081–6
- [8] Lima C D, Klein M G and Hendrickson W A 1997 *Science* **278** 286–90
- [9] Liu H, Rodgers N D and Kiledjian M 2002 *EMBO J.* **21** 4699–708
- [10] Cohen L S, Mikhli C, Friedman C, Jankowska-Anyszka M, Stepinski J, Darzynkiewicz E and Davis R E 2004 *RNA* **10** 1609–24
- [11] Kowalska J, Lewdorowicz M, Zuberek J, Bojarska E, Wojcik J, Cohen L S, Davis R E, Stepinski J, Stolarski R, Darzynkiewicz E and Jemielity J 2005 *Nucleosides Nucleotides Nucleic Acids* **24** 595–9
- [12] Kalek M, Jemielity J, Grudzien E, Zuberek J, Bojarska E, Cohen L S, Stepinski J, Stolarski R, Davis R E, Rhoads R E and Darzynkiewicz E 2005 *Nucleosides Nucleotides Nucleic Acids* **24** 615–20
- [13] Niedzwiecka A, Marcotrigiano J, Stepinski J, Jankowska-Anyszka M, Wyslouch-Cieszynska A, Dadlez M, Gingras A C, Mak P, Darzynkiewicz E, Sonnenberg N, Burley S K and Stolarski R J 2002 *Mol. Biol.* **319** 615–35
- [14] Gu M, Fabrega C, Liu S-W, Liu H, Kiledjian M and Lima C D 2004 *Mol. Cell* **14** 67–80
- [15] Chen N, Walsh A, Liu Y, Parker R and Song H 2005 *J. Mol. Biol.* **347** 707–19
- [16] Kalek M, Jemielity J, Darzynkiewicz Z M, Bojarska E, Stepinski J, Stolarski R, Davis R E and Darzynkiewicz E 2006 *Bioorg. Med. Chem.* **14** 3223–30
- [17] Brenner H, Pace H C, Garrison P N, Robinson A K, Rosler A, Liu X-H, Blackburn G M, Croce C M, Huebner K and Barnes L D 1997 *Protein Eng.* **10** 1461–3
- [18] Abend A, Garrison P N, Barnes L D and Frey P A 1999 *Biochemistry* **38** 3668–76
- [19] DeLano W L 2002 *The PyMOL Molecular Graphics System* (San Carlos, CA: DeLano Scientific)
<http://pymol.sourceforge.net/faq.html#CITE>