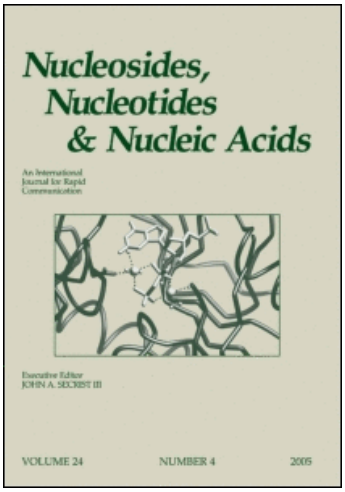

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Hydrolysis of some mRNA 5'-Cap Analogs Catalyzed by the Human Fhit Protein - and Lupin ApppA Hydrolases

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HYDROLYSIS OF SOME mRNA 5'-CAP ANALOGS CATALYZED BY THE HUMAN Fhit PROTEIN - AND LUPIN ApppA HYDROLASES

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ABSTRACT: Hydrolysis of the following four cap analogs: $m^7G(5')ppp(5')A$, $m^7G(5')ppp(5')m^6A$, $m^7G(5')ppp(5')m^{2'O}G$ and $m^7G(5')ppp(5')2'dG$ catalyzed by homogeneous human Fhit protein and yellow lupin Ap_3A hydrolase has been investigated. The hydrolysis products were identified by HPLC analysis and the K_m and V_{max} values calculated based on the data obtained by the fluorimetric method.

Decapping process is the key step in mRNA decay pathway because the transcript undergoes rapid degradation after the cleavage of a cap structure[1]. Various approaches were employed to control this process and several proteins were identified as decapping enzymes. It has been shown recently that Ap_3A hydrolase from yellow lupin seeds can cleave different dinucleoside triphosphates including cap analogs[2]. Some cap analogs were also among the potential substrates of the human Fhit (fragile histidine triad) protein which behaves as a typical dinucleoside 5',5'''-P^1,P^3-triphosphate hydrolase[3].

Human Fhit protein was overexpressed in *Escherichia coli*[4] and purified to homogeneity. Ap_3A hydrolase from yellow lupin seeds was isolated and purified to homogeneity as reported by Guranowski *et al.*[2]. Cap analogs were synthesized at the Department of Biophysics by the methods described elsewhere[5,6].

Hydrolysis catalyzed by the human Fhit protein was determined at 30°C in an incubation mixture, 1 ml final volume, containing 50 mM Hepes/NaOH (pH 6.8), 0.5 mM

$MnCl_2$, varied substrate concentration (10-75 μM) and rate-limiting amount of the enzyme. Hydrolysis catalyzed by the yellow lupin Ap_3A hydrolase (EC 3.6.1.29) was carried out at 37°C in an incubation mixture containing 50 mM Hepes/KOH (pH 8.2), 2 mM $MgCl_2$, 0.1 mM DTT, 10-75 μM m^7GpppA and rate limiting amount of the enzyme.

Time-dependent increase in fluorescence intensity at the emission maximum at 377 nm, with excitation at 294 nm (isosbestic point) has been recorded. The two kinetic parameters K_m and V_{max} were determined using the standard initial velocity method. The K_m values estimated for the Fhit protein are much higher [65 μM for m^7GpppA, 252 μM for m^7Gpppm^6A, 304 μM for $m^7Gpppm^{2'O}G$ and 274 μM for $m^7Gppp2'dG$] than the K_m for ApppA (1.3 μM). In case of the lupin Ap_3A hydrolase tested with m^7GpppA, the K_m value is 30 μM, whereas the K_m for ApppA is 1.2 μM. The V_{max} values estimated for the Fhit/Ap_3A hydrolase vary between 0.223 mol/s for m^7GpppA and 0.623 mol/s for $m^7Gppp2'dG$. Generally, the m^7G-containing dinucleoside triphosphates are much poorer substrates than ApppA or GpppG both for the human Fhit- and for the yellow lupin Ap_3A hydrolase. As concerns the preference of cleavage of the asymmetrical (hybrid) dinucleotides, it is clear that these two enzymes hydrolyze the triphosphate chain at the first phosphate from the bound m^7-guanosine.

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REFERENCES

1. Decker,C.J.; Parker, R. *Trends Biochem. Sci.* **1994,** *19*, 336-340

2. Guranowski, A.; Starzyńska, E.; Bojarska, E.; Stępiński, J; Darżynkiewicz, E. *Protein Expression Purif.*, **1996,** *8*, 416-422

3. Barnes, L. D.; Garrison, P.N.; Siprashvili, Z.; Guranowski, A.; Robinson, A.K.; Ingram, S.W.; Croce, C.M.; Ohta, M.; Huebner, K. *Biochemistry,* **1996,** *35*, 11529-11535

4. Brenner, Ch.; Pace, A. C.; Garrison, P.N.; Robinson, A.K.; Rosler, A.; Liu, X-H; Blackburn, G.M.; Croce, C.M.; Huebner, K.; Barnes, L.D. *Protein Engineering,* **1997,** *10*, 1461-1463

5. Stępiński, J.; Bretner, M.; Jankowska, M.; Felczak, K.; Stolarski, R.; Wieczorek, Z.; Cai, A-L.; Rhoads, R.E.; Temeriusz, A.; Darżynkiewicz, E. *Nucleosides Nucleotides,* **1995,** *14*, 717-720

6. Jankowska, M.; Stępiński, J.; Stolarski, R.; Wieczorek, Z.; Haber, D.; Darżynkiewicz, E. *Collect. Czech. Chem. Commun.*, **1996,** *61*, S197