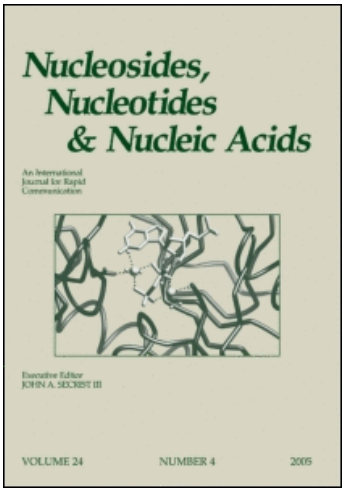

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INTERACTING PARTNERS OF M-PMV NUCLEOCAPSID-dUTPase

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□ *The nucleocapsid-dUTPase protein of Mason-Pfizer monkey virus is a truly bifunctional fusion enzyme. The exact role of this fusion protein in the viral life cycle is unclear. To explore its function, we started to identify interacting protein partners of the enzyme in vitro. Three viral proteins, integrase, capsid and nucleocapsid, were found to be capable of physical interaction with NC-dUTPase. Integrase protein is an important component within the preintegration complex; therefore the present results also suggest that NC-dUTPase might be associated with this complex.*

Keywords dUTPase; Nucleocapsid; Fusion protein; Preintegration complex

INTRODUCTION

dUTPase enzymes prevent incorporation of uracil into DNA via hydrolysis of dUTP into dUMP that is required for dTTP biosynthesis. In absence of dUTPase, the cellular dUTP/dTTP ratio gets elevated, leading to uracil-substituted DNA, futile cycles of excision repair, and finally cell death.[1,2] dUTPase is essential in pro- and eukaryotes, and contributes to infectivity in retroviruses.[3–5] Infectious retroviruses have three main genes coding for the virion proteins in the order: 5′ *gag-pol-env* 3′.[6] In Mason-Pfizer monkey virus (M-PMV), the prototype of betaretroviruses, an additional open reading frame (ORF) termed *pro*, carrying dUTPase and protease genes, is located between *gag* and *pol* ORFs. Ribosomal frame-shifts give rise to Gag-Pro and Gag-Pro-Pol polypeptides which both contain the fusion

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protein nucleocapsid (NC)-dUTPase. The two-domain protein NC-dUTPase is resistant to the retroviral protease cleavage during maturation of the retrovirus and it is present as a stable fusion protein both in the virion and in virus-infected cells.[7] The trimeric organization of dUTPase is maintained within the fusion protein.[7]

Our working hypothesis suggests that the homotrimeric structure of dUTPase may help organisation of the NC domains while NC segments may assist in the adequate localisation of the fusion protein in the vicinity of nucleic acids, thereby anchoring dUTPase activity to the site of reverse transcription and integration. In the present work, we addressed the question if NC-dUTPase may be capable of physical interaction with other viral proteins. Based on the involvement of dUTPase in DNA synthesis and repair, we investigated association capabilities with integrase (IN) that is part of the preintegration complex,[8] as a first step. The other studied proteins were capsid (CA), nucleocapsid (NC), capsid-nucleocapsid (CA-NC) fusion protein, p12, and matrix (MA).

MATERIALS AND METHODS

Proteins were produced as described before.[9–12] Far-Western dotblot was performed on nitrocellulose membrane and developed with primary antibody against dUTPase[7] and horseradish peroxidase linked secondary antibody. In surface plasmon resonance experiments, immobilized NC-dUTPase on CM5 chip was titrated with CA-NC.

RESULTS AND DISCUSSION

To investigate the interactions between NC-dUTPase and viral proteins, the technique of far-Western dotblot was used first as a qualitative method (Figure 1). The different proteins were incubated with NC-dUTPase. Four

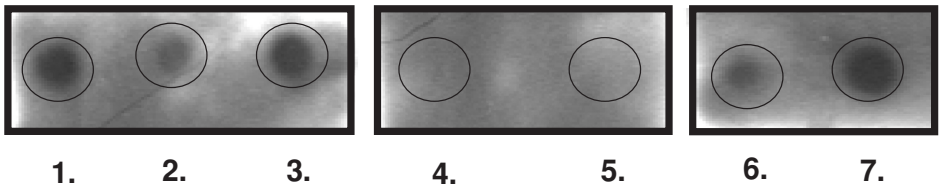

FIGURE 1 Far-Western dotblot. Proteins (1-IN, 2-CA, 3-CA-NC, 4-p12, 5-MA, 6-NC, 7-NC-dUTPase, this latter as a positive control) were applied to the membrane in equivalent amounts (0.6 μg each). Black circles indicate the spots of each protein on the membrane. Following incubation with NC-dUTPase and extensive washes, the blots were developed with antibody against dUTPase.[7] Retention of NC-dUTPase on the applied protein is indicated by the positive immune reaction, while negative immune reactions indicate that NC-dUTPase does not bind to the protein applied to the negative spot. Reactions were run in triplicate with practically indistinguishable results.

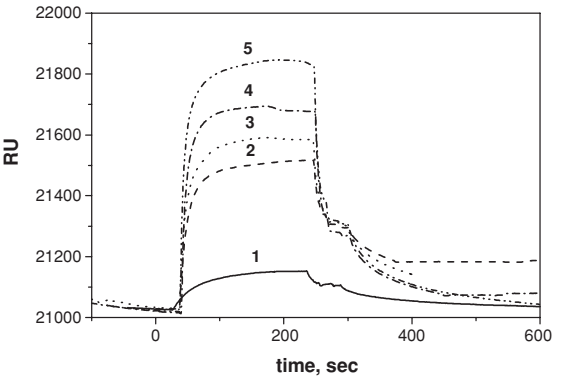

FIGURE 2 Interaction of NC-dUTPase and CA-NC followed by surface plasmon resonance. Concentrations of injected CA-NC (in 10 mM HEPES pH 7.4, 150 mM NaCl, 3 mM EDTA, 0.005% (v/v) Surfactant p20) were: 0.001 mg/ml (curve 1, solid), 0.005 mg/ml (curve 2, dashed), 0.01 mg/ml (curve 3, dotted), 0.02 mg/ml (curve 4, dash-dot), 0.03 mg/ml (curve 5, dash-dot-dot).

proteins showed positive reactions: IN, CA, NC, and the recombinant fusion protein CA-NC. No interaction could be visualised for p12 and MA proteins.

The far-Western dotblot experiments indicated several positive reactions alluding to potential physical interactions in a qualitative fashion. In order to quantify results, the interaction between CA-NC and NC-dUTPase was also investigated by surface plasmon resonance. NC-dUTPase was immobilized on surface of the sensor chip and the CA-NC was injected upon this surface in increasing concentrations. The association and dissociation curves show obvious signs of the interaction between the two fusion proteins (Figure 2). The binding constants are calculated from the fitted curves (Table 1).

CONCLUSIONS

Results from two independent techniques indicated in agreement that the fusion protein NC-dUTPase may form a macromolecular complex with CA-NC, an engineered fusion protein, with an apparent binding constant of $\sim 10^7$ 1/M. The interactions between CA and NC-dUTPase as well as between IN and NC-dUTPase were suggested by far-Western dotblot experiments. These interactions could provide some suggestions as to the

TABLE 1 Parameters Characterizing the Complexation of NC-dUTPase and CA-NC

k_{assoc} (1/s/M)	k_{diss} (1/s)	K_A (1/M)	K_D (M)
$10.4 \pm 5 \times 10^4$	$1.15 \pm 0.0017 \times 10^{-2}$	$8.6 \pm 4 \times 10^6$	$1.4 \pm 0.6 \times 10^{-}7$

role and localisation of the enzyme during the viral life cycle. Both of the techniques used provided reproducible results and required minute amounts of proteins, and can therefore be recommended for systematic in vitro investigations. These in vitro results need to be assessed within the cellular milieu and also within the virion particles in order to draw conclusions possibly relating to physiological consequences.

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