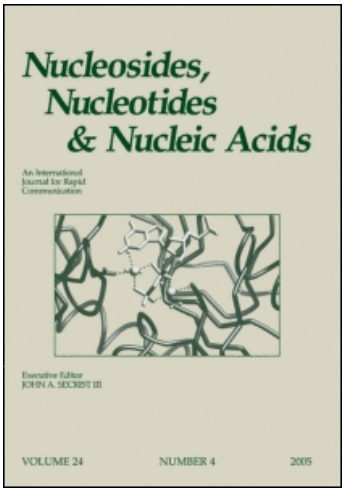

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

New Evidences for a Regulation of Deoxycytidine Kinase Activity by Reversible Phosphorylation

C. Smal[a]; L. Bertrand[b]; E. Van Den Neste[ac]; S. Cardoen[a]; M. Veiga-da-Cunha[a]; S. Marie[a]; V. Race[a]; A. Ferrant[c]; G. Van den Berghe[a]; F. Bontemps[a]
[a] Laboratory of Physiological Chemistry, Christian de Duve Institute of Cellular Pathology, Brussels, Belgium [b] Hormone and Metabolic Research Unit, Christian de Duve Institute of Cellular Pathology, Brussels, Belgium [c] Department of Hematology, Cliniques universitaires Saint-Luc, Université catholique de Louvain, Brussels, Belgium

Online publication date: 27 October 2004

To cite this Article Smal, C. , Bertrand, L. , Neste, E. Van Den , Cardoen, S. , Veiga-da-Cunha, M. , Marie, S. , Race, V. , Ferrant, A. , Berghe, G. Van den and Bontemps, F.(2004) 'New Evidences for a Regulation of Deoxycytidine Kinase Activity by Reversible Phosphorylation', Nucleosides, Nucleotides and Nucleic Acids, 23: 8, 1363 — 1365
To link to this Article: DOI: 10.1081/NCN-200027620
URL: http://dx.doi.org/10.1081/NCN-200027620

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS
Vol. 23, Nos. 8 & 9, pp. 1363–1365, 2004

New Evidences for a Regulation of Deoxycytidine Kinase Activity by Reversible Phosphorylation

C. Smal,[1,*] L. Bertrand,[2] E. Van Den Neste,[1,3] S. Cardoen,[1] M. Veiga-da-Cunha,[1] S. Marie,[1] V. Race,[1] A. Ferrant,[3] G. Van den Berghe,[1] and F. Bontemps[1]

[1]Laboratory of Physiological Chemistry and [2]Hormone and Metabolic Research Unit, Christian de Duve Institute of Cellular Pathology, Brussels, Belgium
[3]Department of Hematology, Cliniques universitaires Saint-Luc, Université catholique de Louvain, Brussels, Belgium

ABSTRACT

Recent studies indicate that deoxycytidine kinase (dCK), which activates various nucleoside analogues used in antileukemic therapy, can be regulated by post-translational modification, most probably through reversible phosphorylation. To further unravel its regulation, dCK was overexpressed in HEK-293 cells as a His-tag fusion protein. Western blot analysis showed that purified overexpressed dCK appears as doublet protein bands. The slower band disappeared after treatment with protein phosphatase lambda (PP λ) in parallel with a decrease of dCK activity, providing additional arguments in favor of both phosphorylated and unphosphorylated forms of dCK.

Key Words: Deoxycytidine kinase; HEK-293 cells; Protein phosphatase; Protein dephosphorylation.

*Correspondence: C. Smal, Laboratory of Physiological Chemistry, Christian de Duve Institute of Cellular Pathology, Brussels, Belgium.

DOI: 10.1081/NCN-200027620
1525-7770 (Print); 1532-2335 (Online)

INTRODUCTION

Deoxycytidine kinase (dCK) is a key enzyme supplying the cells with deoxyribonucleotides via the salvage pathway. Because of its broad substrate specificity, dCK is able to phosphorylate and activate many deoxynucleoside analogues used against leukemias, solid tumours and viral infections. In recent years, various genotoxic agents, including 2-chloro-2′-deoxyadenosine,[1] aphidicolin,[2] and UV light,[3] have been shown to enhance dCK activity in leukemic and normal lymphocytes without changing the level of dCK protein. These observations led to the hypothesis that dCK activity can be regulated by post-translational modification, most probably by reversible protein phosphorylation. To analyse its regulation in more details, dCK was overexpressed in eukaryotic cells (HEK-293, human embryonic kidney cells) as a His-tag fusion protein.

MATERIALS AND METHODS

The coding region of the dCK cDNA from human lymphocytes was amplified by PCR, and cloned in vectors for high-level expression in mammalian cells to produce recombinant proteins with a N-terminal (pEF6/His) or C-terminal (pEF6/*myc*-His) polyhistidine tag. These recombinant proteins were overexpressed in HEK-293 cells using the calcium phosphate precipitation transfection method and purified by affinity chromatography with agarose-cobalt resin. Activity of dCK was determined with 10 μM [5-^{3}H]deoxycytidine as substrate. For dCK dephosphorylation analysis, purified recombinant dCK was incubated with PP λ (3.3 U/μl) during 30 min at 30°C. Western blotting was performed with monoclonal antibodies against poly-His (1/2000) in PBS-T (0.1%) with 5% powder milk.

RESULTS AND CONCLUSION

The dCK activity measured after overexpression of dCK as a His-tag fusion protein was higher with His C-terminal fusion protein (∼90-fold higher than in untransfected cells) than with His N-terminal fusion protein (∼35-fold). After purification, SDS-PAGE electrophoresis and immunoblotting, dCK appeared as doublet protein bands, possibly due to two different phosphorylation levels resulting in a mobility-shifted signal (Fig. 1). To verify this hypothesis, purified overexpressed dCK was treated with PP λ, a non-specific protein phosphatase. This treatment led to a profound decrease of

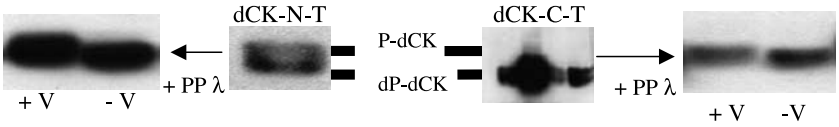

Figure 1. Mobility-shift of the recombinant dCK proteins before and after treatment with PP λ. V: vanadate 1 mM; P-dCK and dP-dCK: phosphorylated and dephosphorylated dCK.

dCK activity (~87%) after 30 min of incubation (not shown). This effect was reproducibly observed with HEK-293 crude extracts containing overexpressed dCK and also with native lymphocyte dCK. This fall of dCK activity was accompanied by a slight increase of the electrophoretic mobility of the recombinant enzyme after immunoblotting. When vanadate (V) was added to inhibit PP λ, this mobility-shift was prevented (Fig. 1).

These results are additional arguments in favour of a regulation of dCK activity by reversible phosphorylation, since its treatment with PP λ increases its electrophoretic mobility and decreases its activity in parallel.

REFERENCES

1. Sasvari-Szekely, M.; Spasokoukotskaja, T.; Szoke, M.; Csapo, Z.; Turi, A.; Szanto, I.; Eriksson, S.; Staub, M. Activation of deoxycytidine kinase during inhibition of DNA synthesis by 2-chloro-2′-deoxyadenosine (Cladribine) in human lymphocytes. Biochem. Pharmacol. **1998**, *56*, 1175–1179.
2. Csapo, Z.; Sasvari-Szekely, M.; Spasokoukotskaja, T.; Talianidis, I.; Eriksson, S.; Staub, M. Activation of deoxycytidine kinase by inhibition of DNA synthesis in human lymphocytes. Biochem. Pharmacol. **2001**, *61*, 191–197.
3. Van Den Neste, E.; Smal, C.; Cardoen, S.; Delacauw, A.; Frankard, J.; Ferrant, A.; Van den Berghe, G.; Bontemps, F. Activation of deoxycytidine kinase by UVC irradiation in chronic lymphocytic leukemia B-lymphocytes. Biochem. Pharmacol. **2003**, *65*, 573–580.