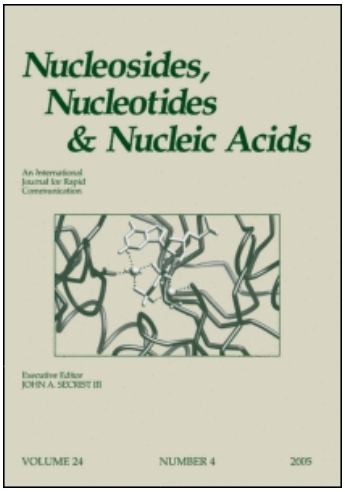

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T. Maltseva[a]; E. Usova[b]; S. Eriksson[b]; J. Milecki[a]; A. Földesi[a]; J. Chattopadhayaya[a]
[a] Department of Bioorganic Chemistry, Box 581, Biomedical Center, University of Uppsala, Uppsala, Sweden [b] Dept of Veterinary Medical Chemistry, Swedish University of Agricultural Sciences, Biomedical Center, Uppsala, Sweden

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THE NMR CONFORMATION STUDY OF THE COMPLEXES OF DEOXYCYTIDINE KINASE (dCK) AND 2′-DEOXYCYTIDINE/2′-DEOXYADENOSINE

T. Maltseva,[1] E. Usova,[2] S. Eriksson,[2,*] J. Milecki,[1,3] A. Földesi,[1] and J. Chattopadhayaya[1,†]

[1]Department of Bioorganic Chemistry, Box 581, Biomedical Center, University of Uppsala, S-751 23 Uppsala, Sweden
[2]Dept of Veterinary Medical Chemistry, Swedish University of Agricultural Sciences, Biomedical Center, Box 575, S-751 23, Uppsala, Sweden
[3]Faculty of Chemistry, A. Mickiewicz University, Grunwaldzka 6, 60-780 Poznan, Poland

ABSTRACT

The structures of the bound $^{13}C/^{2}H$ double-labelled 2′(R/S), 5′(R/S)-$^{2}H_2$-1′,2′,3′,4′,5′-$^{13}C_5$-2′-deoxyadenosine and the corresponding 2′-deoxycytidine moieties in the complexes with human deoxycytidine kinase (dCK) have been characterized for the first time by the solution NMR spectroscopy, using Transferred Dipole-Dipole Cross-correlated Relaxation and Transferred nOe experiments. It has been shown that the ligand adopts a South-type sugar conformation when bound to dCK.

INTRODUCTION

Deoxycytidine kinase (dCK) is a cytosolic enzyme that plays a key role in the activation of therapeutic nucleoside analogues by their 5′-phosphorylation (1). Nucleoside 5′-triphosphate *e.g.* ATP and UTP act as phosphate donors and a broad range of nucleosides serves as acceptors, including anticancer drugs such as

*E-mail: staffan.erikssoni@vmk.slu.se
†E-mail: jyoti@bioorgchem.uu.se

arabinosylcytosine, 2′,2′-difluorodeoxycytidine and 2-chlorodeoxyadenosine as well as antiviral compounds *e. g.* 2′,3′-dideoxycytidine (1). The Herpes kinases have broader substrate specificities for nucleoside analogues in that they phosphorylate acyclic and unnatural stereoisomeric forms. Some racemic compounds such as β-DL-($\pm$) and both isomers of 2′,3′-dideoxy-3′-thiacytidine (BCH-189; 3TC) are also phosphorylated by nucleoside kinases. A comprehensive study of the stereoisomeric specificities of the cellular deoxynucleoside kinase with four stereoisomers of natural deoxynucleoside was recently published (1).

We herein present our NMR evidences by the transferred Dipole-Dipole cross-correlated relaxation (CCR) (2) and transferred nOe (TRNOE) experiments, which show that the flexible pentose sugar moiety of both dAdo and dCyd in the bound state with dCK adopt a South-type conformation, which is most probably a "near transition state" conformation involved in the phosphate transfer reaction with ATP in vitro synthesis of 2′-deoxyribonucleoside 5′-monophosphate (3).

Recently the quantitative measurement of DD cross-correlation relaxation (2) based on the relaxation of multiple quantum coherences has shown that the ratio of the crosspeak intensities in the two experiments (cross and reference experiments) bears a direct relationship to the cross-correlated rate, ($\Gamma^{c}_{C_iH_iC_jH_j}$), and hence to the angle subtended by internuclear vectors. For the ribofuranosyl moiety in a nucleoside derivative, the determination of the sugar pucker amplitude is based on the ratio of the two cross-correlated rates: $\Gamma^{c}_{C_{1'}}H_{1'}C_{2'}H_{2'}/\Gamma^{c}C_{3'}H_{3'}C_{4'}H_{4'}$. For 2′-deoxynucleoside derivatives, this method could not be used because of DD cross-correlation between C2′H2′ and C2′H2″ vectors. Therefore chemospecific deuterium labelling at the C2′ was used to eliminate the cross-correlation effect of DD(^{13}C-^{1}H) from the ^{13}C relaxation rate of the methylene protons.

We here report (3) on the conformation of complexes of 2′(R/S), 5′(R/S)-$^{2}H_2$-1′,2′,3′,4′,5′-$^{13}C_5$-2′-deoxyadenosine and 2′-deoxycytidine with dCK by means of transferred Dipole-Dipole Cross-correlated Relaxation and Transferred nOe experiments.

The recombinant S8F dCK enzyme (3) is a dimer composed of two identical 30 kDa subunits. This mutation has only minor effects on the enzyme's kinetic behaviour. To perform the Cross-correlated relaxation (CCR) and Transferred NOE (TRNOE) experiments with samples of ligand-protein complexes at the same experimental and environmental conditions, we have used a mixture of 2′-deoxynucleoside consisting of 30% double ^{13}C/^{2}H labelled deoxynucleoside for dAdo and dCyd.

In the TRNOE experiments, the negative NOE signals were detected for the free nucleoside in solution, whereas an extensive set of positive NOE signals was detected when the nucleoside was mixed with dCK. The detection of the positive crosspeaks in 2D TRNOE spectra on the resonance of the free ligand allows us to conclude that both 2′-deoxynucleosides, dCyd and dAdo, are in fast exchange between the free and the weakly-bound states with dCK, which most probably represents a "near transition state" model for the transesterification reaction with ATP.

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The conformation of the bound-nucleosides in dCK, the sugar moieties in dAdo and dCyd were $^{13}C/^{2}H$ double-labelled in order to measure the CCR rates, enabling us to assess different endocyclic torsion angles. Two complementary spectra (cross and reference) (2) for each $^{13}C/^{2}H$ double-labelled dAdo and dCyd (5 mM) with dCK (0.1 mM) at 0°C have been obtained (3) showing the CCR crosspeaks between C1′H1′ and C2′H2′. These crosspeaks are observed in the cross-section through resonances of H1′ and ^{13}C2′ for complexes with either dAdo or dCyd with dCK. It is noteworthy that the crosspeaks found in the above CCR cross experiment are absent for free dAdo and dCyd (data not shown) because of their shorter correlation times in the free state. After scaling the intensity of the crosspeak in the corresponding reference experiments by 50 times (because a 1:50 ratio of the protein to ligand was used in the NMR experiment) for both 2′-deoxynucleosides, the ratios of I_{cross}/I_{ref} are found to be close to 1. The close to unity of the ratio of the cross and the reference peaks is due to the long correlation time of 2′-deoxynucleoside-dCK complex.

The qualitative analyses of the CCR experimental data for both dAdo and dCyd bound to dCK show that the ratio of I_{cross}/I_{ref} for C1′H1′-C2′H2′ is positive (3). On the other hand, the cross-correlated peaks for C3′H3′-C4′H4′ are very weak (3), and have opposite signs compared to the corresponding crosspeak in the reference experiments, and the ratio of I_{cross}/I_{ref} is close to zero or negative. Based only on the signs of $\Gamma^{c}_{C_{i'}H_{1'}C_{2'}H_{2'}}$ and $\Gamma^{c}_{C_{3'}H_{3'}C_{4'}H_{4'}}$ (2), it can be concluded that the sugar moiety of both dAdo and dCyd prefers to adopt a South-type conformation in the bound state with dCK. We have also subsequently performed (3) a qualitative analysis of the data obtained from TRNOE spectra, which has allowed us to assess the sugar conformation and the glycosyl torsion accurately.

CONCLUSION

(1) Based on the TRNOE and CCR experiments, the "near transition state" conformations of the weakly bound complexes between dAdo/dCyd with dCK have been identified and characterized. The TRNOE data shows that the aglycone of dCyd adopts the *anti* conformation and the sugar moiety is locked into the 2′-*endo* conformation in the dCK binding site.

(2) The TRNOE data for dAdo bound to dCK is consistent with the dAdo binding in two different sites on dCK, presumably at the dAdo site and the ATP binding site. The two bound dAdo molecules adopt two different combinations of aglycone and sugar conformations: one is a *syn*/South conformation and the another is an *anti*/South.

(3) The fact that in our NMR experiment on the dCK complex, no Mg^{2+} ion seems to be required as cofactor for the transesterification reaction supports the idea that ATP in the dCK active site is capable of adopting an active phosphate conformation necessary for activity as phosphate donor.

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