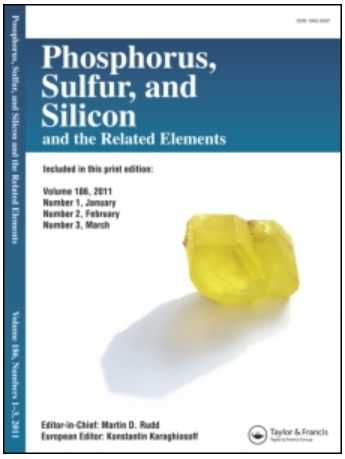

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Synthesis and Reactivity of Intermediates Formed in the T_4 RNA Ligase Reaction

Larry W. Mc Laughlin[a]
[a] Department of Chemistry, Boston College, Chestnut Hill, MA, U.S.A.

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SYNTHESIS AND REACTIVITY OF INTERMEDIATES FORMED IN THE T_4 RNA LIGASE REACTION

LARRY W. MC LAUGHLIN
Department of Chemistry, Boston College,
Chestnut Hill, MA 02167, U.S.A.

Abstract T_4 RNA ligase can be used efficiently for the synthesis of 3'-5' phosphodiester linkages between oligonucleotide blocks if the intermediate adenylated donor oligonucleotide is used. These adenylated oligonucleotides can be prepared using phosphorylating reagents activated by 1-hydroxybenzotriazole.

INTRODUCTION

T_4 RNA ligase catalyzes the synthesis of a 3'-5' phosphodiester between an "acceptor" oligonucleotide containing a free 3'-hydroxyl and a "donor" oligonucleotide containing a 5'-terminal phosphomonoester. The smallest substrates are NpNpN and pNp respectively. Enzymatic synthesis of the product involves three distinct steps:

$$E + ATP \rightleftharpoons E\text{-}AMP + PPi$$

$$E\text{-}AMP + pNp \rightleftharpoons E[A(5')pp(5')Np]$$

$$E[A(5')pp(5')Np] + XpYpZ \rightleftharpoons XpYpZpNp + E + AMP$$

The efficiency of the reaction appears to be dependent upon the nature of both the donor and the acceptor oligonucleotide (1,2). These substrate effects can be largely eliminated if the if the preadenylated form of the donor, $A(5')pp(5')(Np)_n$ is used.

SYNTHESIS

The desired T_4 RNA ligase intermediate takes the form $A(5')pp(5')(Np)_n$. It contains both a P^1,P^2-dialkylpyrophosphate and a 3'-terminal phosphomonester. The simplest adenylated intermediates, where n = 1, can be prepared in high yield by a combined

chemical/enzymatic synthesis[3].

A general chemical synthesis of these intermediates involves initially the preparation of a fully protected derivative containing both 3' and 5' terminal phosphorus moieties. The simplest derivative prepared was $R^1,R^2OP(O)(5')T(3')P(O)OR^3,OR^4$, where R^1= morpholino, R^2 = benzotriazolyl, R^3 = 2-(4-nitrophenyl)ethyl and R^4 = 2,4-dichlorophenyl. The use of four different protection groups on phosphorus allows selective introduction of an AMP residue at the 5' terminus and subsequent generation of the 3'-terminal phosphomonoester. Using this procedure both A(5')pp(5')Tp and A(5')pp(5')GpGpGp have been prepared.

REACTIVITY

It has been shown previously that T_4RNA ligase will use pCp more efficiently than pGp in the joining reaction. This appears to result largely from the adenylation step since pCp is adenylated much more efficiently than pGp[3]. However, if the enzyme is offered the adenylated derivatives A(5')pp(5')Cp and A(5')pp(5')Gp the rate of phosphodiester formation is dramatically increased and is similar for both intermediates. Similar increases in reaction efficiencies are observed when the chemically synthesized A(5')pp(5')Tp and A(5')pp(5')GpGpGp are used in place of pTp and pGpGpGp.[4]

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