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ON THE FUNCTION OF THE 2′-HYDROXYL GROUP IN THE PEPTIDYL tRNA TERMINUS DURING ELONGATION IN RIBOSOMAL PEPTIDE BIOSYNTHESIS

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ABSTRACT: Intramolecular ester amminolysis in 1,2-diglycyloxyethane, which was used in the present study to represent a bis(acyloxy)-tRNA adenylate terminus, was found to be so slow (5-10% yield after 8-10 hours at 195°) that the likelihood of bis(acyloxy)-tRNA as an intermediate in the ribosomal peptide biosynthetic process appears to be highly improbable.

Despite the fact that the 2′-hydroxyl group of the peptidyl tRNA adenylate terminus has been shown to be essential to the elongation stage (formation of the new peptide bond) of ribosomal peptide synthesis, no role has yet been attributed to the 2′-hydroxyl group in that process.[1,2] The present report presents evidence against a heretofore allowable role of the 2′-hydroxyl group: the PATHWAY A shown in Fig. 1, where Peptidyl-tRNA and New Aminoacyl-tRNA first undergo intermolecular transesterification to form Bis(acyloxy)-tRNA. The latter would then undergo intramolecular ester amminolysis to form New Peptidyl-tRNA. The evidence indicating the rejection of PATHWAY A rests upon the observation that intramolecular ester aminolysis in the model compound, **1**, used to represent Bis(acyloxy)-tRNA, was found experimentally to be so difficult to achieve that its reality in ribosomal peptide synthesis appears to be rather unlikely.

Because we wanted to extend our earlier studies of stereoselectivity during formation of the peptide bond[3,4] to the use of a model of the ribosomal process we chose amino acid esters of ethylene glycol to represent the aminoacyl adenylate terminus of tRNA. In order to use this model, however, it was first necessary to determine which mechanism, **PATHWAY A** or **PATHWAY B** (Fig. 1), actually represented the ribosomal process, for both mechanisms satisfied the experimental evidence, which required the presence of the 2′-hydroxyl group in the peptidyl tRNA terminus.[1]

We evaluated the relative merits of the two mechanisms by synthesizing the substrates[5] shown in Figure 2 and using them to compare the reactivities of the processes representing the two pathways.

For the process corresponding to **PATHWAY A** we observed the ability of **1** to react intramolecularly, forming the dipeptidyl ester, **2**. We also carried out an independent synthesis of **2** so as to have authentic material with which to compare and identify the product obtained from reaction of **1**. Compound **1** was obtained from **3** through treatment of the latter with a 30% solution of hydrogen bromide in glacial acetic acid for 5 mins. at room temperature. This procedure removed only the benzyloxycarbonyl, giving **1** after the initial reaction product, the hydrobromide, **4**, was treated with triethylamine. The ability of **1** (in dilute ethylene glycol solutions also containing two equivalents of triethylamine) to react intramolecularly (PATHWAY A) to give **2** was monitored, and it was only after 8-16 hours at 195° did the product **2** appear, produced in only 5-10% yield.

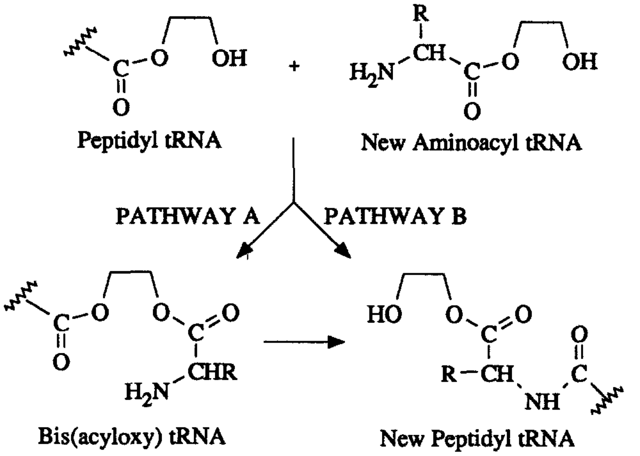


FIG 1—Schematic showing two mechanisms, **PATHWAY A** and **PATHWAY B**, leading to the formation of **New Peptidyl-tRNA** during the elongation stage of ribosomal peptide biosynthesis.

For a comparison with the intermolecular reaction (**PATHWAY B**), we monitored the reaction of 2-hydroxyethyl acetate, **5**, with nonylamine, **6**, which after 25 minutes at 40° gave about the same amount of product: 7% yield of the amide, **7**. Thus **PATHWAY A** appears to be a vastly more difficult process than **PATHWAY B**.

Although the natural ribosomal elongation process is attended by enzymes and other macromolecular auxiliaries, it is hard to imagine how anything could mitigate against the factors responsibile for the extreme unreactivity **1** and allow the **PATHWAY A** process to be a reasonable option.

Each of two effects appear to be responsible for the significantly less reactivity of the **PATHWAY A** process. Both effects concern the eight-membered, tetrahedral intermediate, **8**, whose decomposition to product is the rate-limiting step.[6,7] First, the extra energy associated with the formation of an eight-membered ring[8] means that the intermediate **8** will be in low concentration compared to the corresponding tetrahedral intermediate of **PATHWAY B**.

The second effect which may also contribute to the very low reactivity of **PATHWAY A** concerns 9, the conformation of 8 needed for its stereoelectronic arrangement of bonds and orbitals favoring the decomposition of 8 into 2.[9] This conformation contains unfavorable interactions compared to other conformers of the set,[10] causing it to be in low concentration and therefore contributing to the slow rate of **PATHWAY A**.

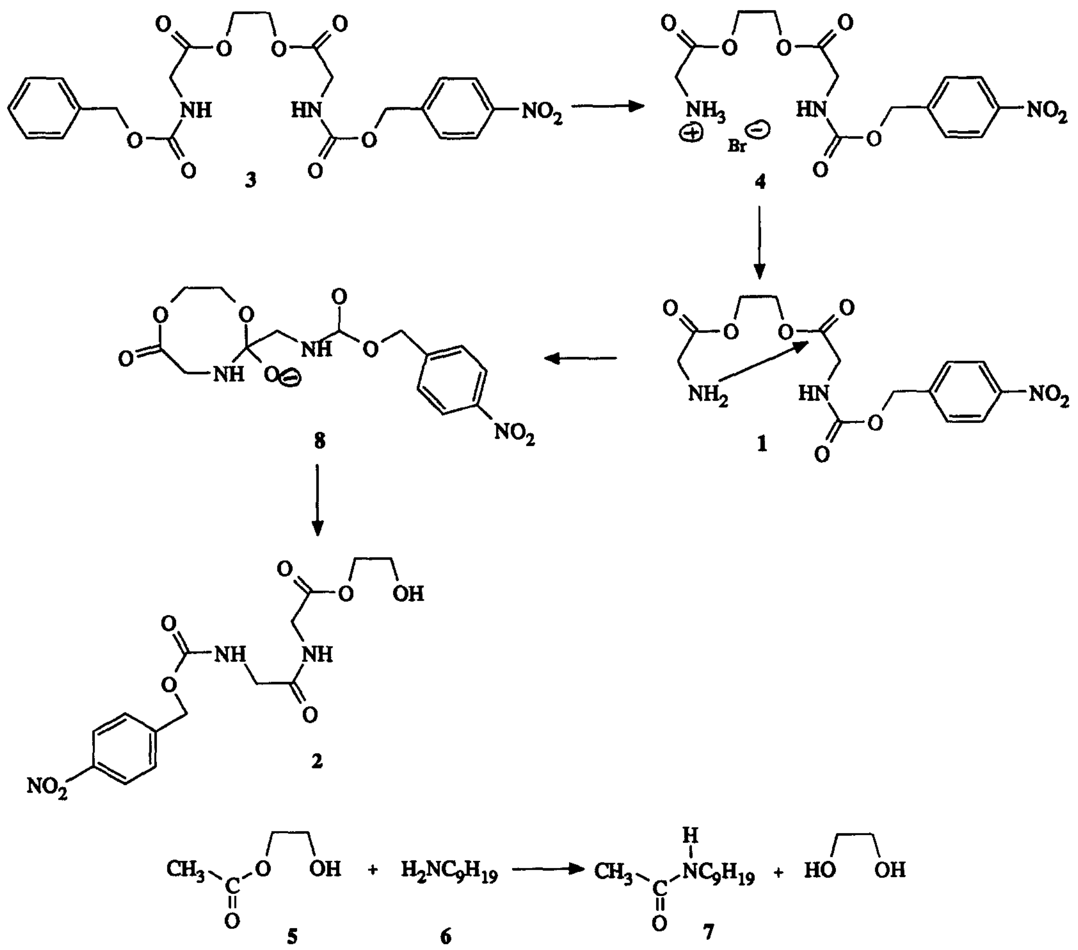
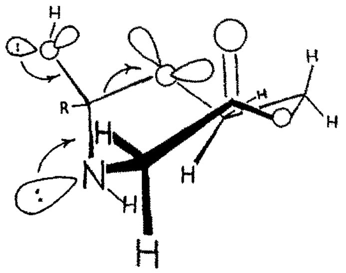


FIG 2—Substrates and reactions used to simulate the two mechanisms.

While the function of the essential 2'-hydroxyl group of the adenylate tRNA terminus is not that shown in PATHWAY A, other work in this laboratory indicates that the purpose of the hydroxyl group is at least to provide an electronic effect which contributes to the observed[1] fast rate of ribosomal peptide elongation.[11]



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5. All of the new compounds, **1**, **2**, **3** and **4**, were synthesized during the course of this work by procedures to be reported elsewhere. Each compound was purified and gave rise to satisfactory elemental analyses, infrared and nmr spectral data.

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