5'-ADENYLYLATION AND 5'-URIDYLATION OF N-ACETYL TYROSINE ETHYL ESTER: A GENERAL SYNTHESIS OF 5'-NUCLEOTIDYLATED TYROSYL RESIDUES*

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Summary

A synthetic procedure for nucleotidylated tyrosine derivatives using 1,1'-carbonyldiimidazole as a condensing agent is demonstrated by the synthesis of the adenylyl and uridyl derivatives of N-acetyl tyrosine ethyl ester. The spectral properties, susceptibility to snake venom phosphodiesterase, and alkaline stability are fully compatible with the formation of an O-nucleotidyl tyrosyl residue.

In 1968, Shapiro and Stadtman (1) demonstrated that a specific tyrosyl residue on the Escherichia coli glutamine synthetase is the site of 5'-adenylylation, a unique chemical modification now known to profoundly affect the nitrogen metabolism of many Gram-negative bacteria (2,3). Proof that the 5'-adenylyl group is bound in a phosphodiester linkage to the phenolic hydroxyl of this tyrosyl residue was obtained by comparing the ultraviolet absorption spectrum of the isolated adenylylated peptide at neutral and alkaline pH, before and immediately after removal of the adenylyl moiety by the action of snake venom phosphodiesterase. Only after removal of the adenylyl group did alkali cause an absorption spectral difference exactly coincident with that of an ionized tyrosyl residue (1). In subsequent studies, Heinrikson and Kingdon (4) determined the sequence of a larger 21-amino acid tryptic peptide, and these investigators confirmed that the location was indeed as suggested earlier (1). More recently,

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the site of uridylation of the so-called P_{II} regulatory protein, which also is involved in the cascade of enzyme-catalyzed reactions controlling the glutamine synthetase, has also been shown to be a tyrosyl hydroxyl (5). Except for preliminary evidence of adenylylation of an E. coli aspartokinase isozyme (6), there are no other known systems involving nucleotidylation reactions of this sort. Moreover, relatively little is known about the chemistry and enzyme mechanisms of the adenylyl and uridyl transfer enzymes catalyzing the formation of this interesting new class of phosphodiesters.

Preliminary to the characterization of the chemical properties of such adenylylated and uridylated tyrosyl residues as well as a broader search for similar modification reactions in mammalian enzyme regulation, we have developed a general route to the synthesis of such derivatives in rather high yield.

RESULTS AND DISCUSSION

The procedure involves the sequential reaction of 1,1′-carbonyldiimidazole (CDI) with the tri-n-octylammonium salt of the corresponding nucleoside-5′-monophosphate and N-acetyl tyrosine ethyl ester. Approximately 1 mmole of the nucleotide (free acid) is suspended in a dry pyridine solution containing 2 mmoles of tri-n-octylamine. The resulting tri-n-octylammonium salt is washed three times with cold dry pyridine and is then dissolved in 5 ml dry dimethylformamide (DMF). After the addition of a 1.5-2.0-fold molar excess of CDI, the entire sealed reaction mixture is vigorously agitated on a wrist-shaker for 2 to 3 hours, whereupon a 2-fold molar excess of the tyrosine derivative is added. After the well stoppered reaction mixture is held at room temperature overnight, the solution is concentrated under vacuum to approximately a 1 ml volume. This material is then applied to a DEAE-cellulose column (Whatman DE-52, free base form) and eluted by a linear gradient from 0 to 0.1 M tri-ethylammonium bicarbonate at pH 7. The desired product, which elutes at approximately 0.02 M buffer, contains

contaminating levels of the unreacted tyrosyl derivative and imidazole. After lyophilization, the complete removal of these contaminants is accomplished by passage of a 50% ethanol solution of the product over a small column of Dowex-1 (chloride form). The product, which apparently is the only material bound, is eluted with 1% ammonium bicarbonate, and this buffer is easily removed by repeated lyophilization. Yields of recovered product vary from 50% to 70%.

The identification of the reaction products as the 5-adenylyl and 5′-uridyl derivatives of N-acetyl tyrosine ethyl ester rests upon the following criteria. At 260 nm, the AMP and UMP derivatives had weight equivalent extinction coefficients of 22 and 16 Lg^{-1} cm^{-1}, respectively, which correspond quite well to expected absorptivities of 23 and 18 Lg^{-1} cm^{-1}. These data exclude the possible formation of P^1,P^2-di(adenosine-5′)diphosphate or P^1,P^2-di(uridine-5′)diphosphate by condensation of two molecules of the corresponding nucleoside-5′-monophosphates. The spectra of these products are shown in Fig.1. As observed by Shapiro and Stadtman (1) under similar conditions, treatment with 0.6 N KOH at 50°C gave only 25% hydrolysis after 1 hour. Phosphate analysis of the hydrolyzed product (7) is also consistent with the formation of such products. The most compelling evidence, however, rests upon the action of snake venom phosphodiesterase as described elsewhere (1). The treatment of our products with this enzyme resulted in a difference spectral peak at 292 nm corresponding to the free phenoxide ion of the tyrosyl derivative. In addition, the action of this enzyme was monitored by chromatography on DEAE-cellulose ion exchange paper (0.2% ammonium bicarbonate buffer, pH 9.2) which clearly resolves the nucleotidylated tyrosyl derivative from the unmodified nucleotide. It is of interest to note that prior to addition of enzyme, the isolated products are completely free of the nucleoside-5′-monophosphate; however, after prolonged storage of 1-2 months, some hydrolysis is detectable.

The facile synthesis of the 5′-adenylyl and 5′-uridyl derivatives of

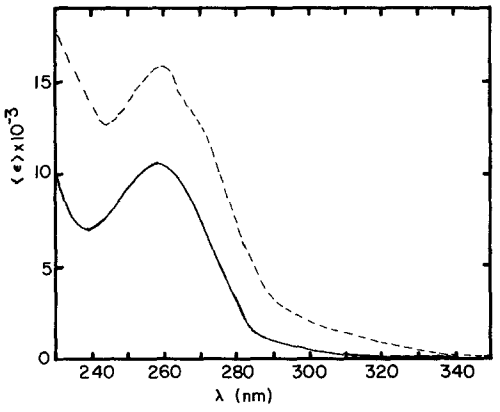

Fig. 1. Ultraviolet absorption spectra of 5′-O-adenylyl, N-acetyltyrosine ethyl ester (dashed line) and 5′-O-uridyl, N-acetyltyrosine ethyl ester (solid line) in 1% ammonium bicarbonate (pH 7.0). The values plotted on the ordinate indicate the observed molar absorptivities under these conditions.

N-acetyl tyrosine ethyl ester suggests that the reaction conditions disfavor the undesired formation of modifications on the amino and hydroxyl groups of the purine and pyrimidine bases. It is likely that these functional groups are not sufficiently nucleophilic to displace imidazole from the imidazolidate intermediate formed by the action of the condensing agent upon the nucleoside monophosphate (7). In any case, the reaction as described proceeds without significant competing reactions, and this convenient synthetic route should prove valuable in the study of such nucleotidyl transfer reactions.

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