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COMMUNICATION

Esterification of All Four Monoribonucleotides with Acetyl-D-L-valine Proceeds with a Preference for the D-Isomer but the D/L Ratio in the Products Declines as a Function of the Hydrophobicity of the Nucleotide

We recently reported that esterification of 5′-AMP with *N*-acetyl amino acids proceeds with a preference for D-amino acids, and the D/L ratio in products declines as the hydrophobicity of the amino acid declines. Using one amino acid, Ac-Val, we now show that esterification of all four nucleotides proceeds with a preference for the D-isomer and the preference declines as the hydrophobicity of the nucleotide declines. So, in both types of experiments, the preferences seem determined by hydrophobic interactions.

In recent years our research has been directed at understanding the chemistry of protein synthesis, mainly concentrating on the reactions of amino acids with 5′-AMP and involving both, the anhydride, in which the amino acid is attached to the phosphate, and the esters, in which the amino acid is attached to the 2′ and 3′ positions of the ribose. A number of selectivities have been observed in these studies, there being differences between amino acids and differences between D- and L-isomers of the same amino acid. These studies were recently summarized in a review (*2*). One of those studies showed that in a series of hydrophobic *N*-acetyl amino acids, the esterification of 5′-AMP favors the D-isomer and the preference for D is directly a function of the hydrophobicity of the amino acid. We were able to relate these preferences to the fact that esterification of 5′-AMP takes place at the 2′ position (*3*) and when the D-amino acid is in the 2′ position it has a greater affinity than the L-amino acid for the adenine ring (*4*). Thus the esterification transition intermediate at the 2′ position is more stabilized with the D-amino acid. Furthermore, we have shown that these interactions between amino acids and adenine are hydrophobic (*5–7*) and involve aliphatic as well as aromatic side chains. With a given hydrophobic amino acid, the affinity for the nucleotide declines as the hydrophobicity of the nucleotide declines (*5*). Conversely, with a given hydrophobic nucleotide, the affinity for the amino acid declines as the hydrophobicity of the amino acid declines (*6, 7*). This being so, one would predict that, because the nucleotides vary in hydrophobicity, AMP > GMP > CMP ≥ UMP, (*2*) the D/L ratio in the esterification of the four nucleotides with a single amino acid might decline as the hydrophobicity of the nucleotide decreases. The present report shows that to be so, the D/L ratio in the esterification products does decline as AMP > GMP > CMP = UMP. These data confirm that hydrophobic interactions between amino acids and nucleotides in aqueous solution can greatly influence the outcome of chemical reactions between the two types of molecule.

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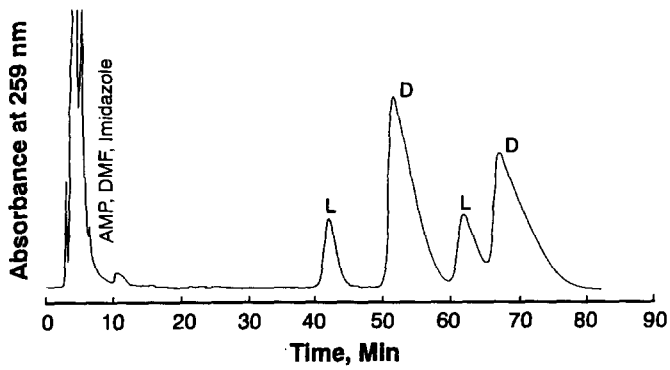

FIG. 1. HPLC separation of Ac-Val esters of 5′-UMP on a Phenomenex C-18 reverse phase column (3.9 mm × 30 cm) using 6% CH_3OH and 0.05 phosphoric acid (pH 2) as eluent at 1.0 ml per minute. The elution was monitored at 259 nm. The peaks were identified by separate esterifications with Ac-D- and L-Val. In our earlier work with 5′-AMP esters (*1*) the 2′ esters always eluted before the 3′. We do not know this to be the case with 5′-UMP esters, but for present purposes we only need to know which are the D- and which are the L-isomers. That the peaks in fact represent the esters was shown by collecting either peak and raising the pH to 7 for 20 min and rerunning the HPLC to show the appearance of the companion peak after the equilibration.

The esterifications were carried out as is the previous study (*1*) based generally on the imidazolide method of Gottikh *et al.* (*8*) and involved a 1.7/1 ratio of nucleotide to amino acid. The *N*-acetyl amino acids were used to prevent peptide formation. The four nucleotides, Ac-Val, carbonyldiimidazole (CDI), and dimethyl formamide (DMF) were purchased from Sigma Chemical Co. and used without further purification. The imidazolides were prepared separately by reacting 1 mmol of racemic Ac-Val with 1.25 mmol of CDI in dry DMF (1.0 ml) for 5 min. The resulting concentration of *N*-acetylaminoacyl imidazolide was usually ~710 μmol/ml. Ten microliters of this mix (~7.1 μmol) was then added to 0.30 ml (12 μmol) of 0.04 M nucleotide solution (pH 7.0) and reacted for 10 s at 0° C. The reaction was stopped by addition of 0.1 of in 1 N HCl. The products were analyzed by reverse-phase HPLC after determining the isomeric identity of the peaks by (i) separate esterifications with Ac-D- and L-Val and (ii) collection of the 3′ peaks (presumed to be the second of each pair) and reequilibrating for 20 min at pH 7 followed by HPLC separation. This latter point establishes that the compound in the peak is an ester if it can be shown to reequilibrate and give the 2′ peak (and vice versa). A typical HPLC separation of Ac-Val UMP esters is shown in Fig. 1.

The D/L ratios for the esterification products of the various nucleotides are shown in Table 1. The data for 5′-AMP are from the previous publication (*1*) and are presented for comparison. There is an obvious decline in the D/L ratio as the hydrophobicity of the nucleotide decreases (Fig. 2). This decline as a function of hydrophobicity would seem to be explained by the facts that (*1*) esterification takes place at the 2′ position (*3*) and when in the 2′ position D-amino acids seem to be able to associate more strongly with the nucleotide base ring than the L-amino

TABLE I

D/L Ratio in the Esterification Products of the Ribonucleotides with Racemic Ac-Val Imidazolide

Nucleotide	D/L[a]
5′-AMP	6.1 ± 0.3[b]
5′-GMP	4.8 ± 0.1
5′-CMP	3.8 ± 0.2
5′-UMP	3.8 ± 0.1

[a] ±SD from three experiments.
[b] From (*1*).

acids, but the affinity of the hydrophobic amino acids for the nucleotide base declines as the hydrophobicity of the nucleotide base declines (4).

The present data then extend and confirm our earlier proposals that hydrophobic interactions can profoundly affect the course of chemical reactions in aqueous solution and indeed confirm the fact that hydrophobic interactions must have played a very important role in the origin of life as well as the origin of the code and protein synthesis (*10*).

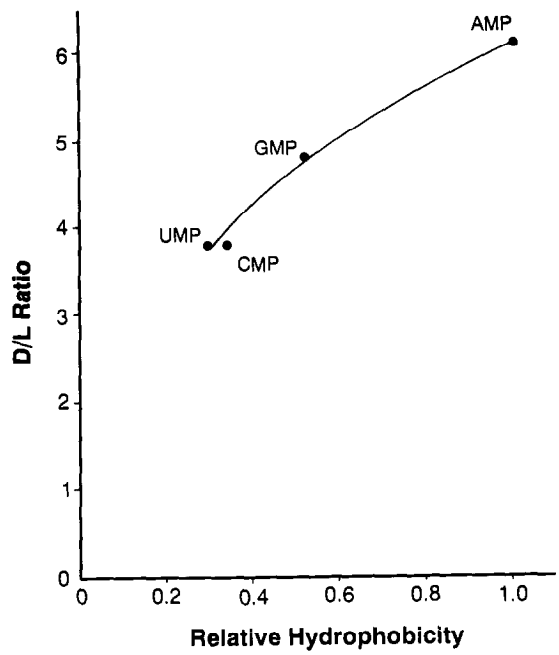

FIG. 2. A plot of the D/L ratio in the esterification products of the four ribonucleotides as a function of the relative hydrophobicity of the nucleotides as determined by Garel *et al.* (*9*).

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