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# GENETIC CODE CORRELATIONS: DIFFERENTIAL RATES OF NON-ENZYMATIC ACTIVATION OF HYDROPHOBIC AMINO ACIDS BY ATP

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SUMMARY: The relative rates of non-enzymatic activation of several hydrophobic amino acids by ATP have been found to bear an interesting relationship to the ordering of these amino acids in the genetic anticode. All of these hydrophobic amino acids (phe, leu, val, ile and met) have adenylic acid, the most hydrophobic nucleotide, as the central and most important member of their anticodons, and the ordering of their relative rates of non-enzymatic activation by ATP has been found not to correlate with the ordering of the hydrophobicities of the amino acids themselves, but rather with the ordering of the average estimates of the hydrophobicities of their respective anticodonic dinucleotides. These data suggest that the genetic code is based not just on hydrophobic relationships or affinities between amino acids and nucleotides, but perhaps more importantly, on the total reaction chemistry between amino acids and nucleotides.

## INTRODUCTION:

For a number of years, our research efforts have been directed toward an investigation of the chemistry of the component reactions of protein synthesis in the hope of gaining some insight into the molecular basis for the origin of that process, including the origin of the genetic code. We believe (1) that these two aspects of the living state arose simultaneously in evolution, and have subsequently evolved in concert. As part of our research program, we have systematically studied the chemistry of a variety of aminoacyl transfer reactions, including the spontaneous transfer of aminoacyl groups from adenylate anhydrides to imidazolides (2), and subsequently to the 2' -OH groups along the backbone of polyribonucleotides (3). The polymerization of amino acids esterified in this manner has also been observed (4). Further observations showed correlations of properties of amino acids with those of their anticodonic nucleotides (5,6,7), and led us to propose a model of primitive translation in which the four mononucleoside triphosphates served to select, organize and activate different classes of amino

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acids. In exploring this model, we turned to a study of the non-enzymatic activation of amino acids by nucleoside triphosphates.

Lowenstein et al. (8,9) were the first to study the chemical activation of carboxylic acids by ATP, and showed that both acetate and amino acids could be activated, provided that a divalent metal cation was present. $Be^{++}$, $Ni^{++}$ and $Ca^{++}$ were found to be the most effective in this regard. These workers used a technique developed by Lipmann and Tuttle (10) to estimate the extent of activation, trapping the activated species with hydroxylamine to form the hydroxamic acids, which then yields a brown color upon complexation with $Fe^{+++}$ ions ($\lambda_{max}$ = 495 nm). Using a similar technique, Ryan and Fox showed that ATP was the most effective nucleotide for the activation of glycine (11). We have previously shown (12) that the rate of chemical activation of carboxylic and amino acids by ATP varies directly as the $pK_a$ of the carboxyl group of the molecule being activated.

In the present paper, we report on our continuing studies of the non-enzymatic activation by ATP of a series of hydrophobic amino acids (phenylalanine, leucine, valine, isoleucine and methionine), all of which have adenylic acid as the central and most important nucleotide of their anticodons. The major questions were, (1) are there differences in the rates of reaction of these amino acids, and (2) if so, do the differences bear any relation to the genetic code? The results show an ordering of phe > leu > val > ile > met, the latter hardly being activated at all. This ordering is the same as the decreasing order of estimates of the hydrophobicities of the anticodons of the amino acids in the study.

MATERIALS AND METHODS:

Adenosine 5' -triphosphate (disodium salt), $MgCl_2 \cdot 6H_2O$, amino acids, hydroxylamine and hydroxamic acids were purchased from Sigma Chemical Co. and used without further purification.

For the activation studies, solutions containing 0.1M ATP, 0.2M $MgCl_2$, 0.4N hydroxylamine and 0.15M amino acids, pH 5, were incubated at 50°C and assayed for hydroxamate color essentially according to the method of Lipmann and Tuttle (10). At various times, 1.0 ml aliquots were withdrawn and placed in 1.0 ml 4N HCl. After addition of 1.0 ml of 10% $FeCl_3 \cdot 6H_2O$ in 0.1N HCl, the samples were read at 495 nm on a Gilford 240 spectrophotometer versus blanks

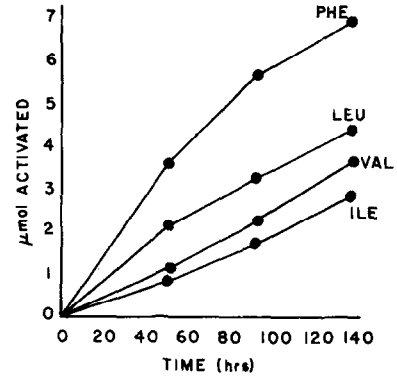


Fig. 1. Activation of a series of hydrophobic amino acids by ATP as a function of time at 50°C, pH 5. The reaction conditions and method of analysis are given in Materials and Methods. Although not included in this experiment, other studies have shown that methionine (the fifth A amino acid) shows little or no activation under these conditions, even after 140 hrs. This may reflect a real non-reactivity between this amino acid and ATP, although we suspect that methionine itself may be either unstable under the conditions of the reaction, or interfering with the assay.

containing 1.0 ml of a control treated identically except without ATP. Using commercial hydroxamic acids, it was determined that the presence of ATP in the hydroxamate assay does have a quenching effect on the development of color at 495 nm, and this was also corrected for in the values reported. The extinction coefficients of the various hydrophobic amino acid hydroxamates in the hydroxamate assay were found to be nearly identical, and the value used was 0.21 absorbance units/μmole/ml.

RESULTS AND DISCUSSION:

Fig. 1 shows the results of a time course study of the activation of the series of hydrophobic amino acids with $Mg^{++}$ -ATP at pH 5 and 50°C. Although we had previously confirmed the finding of Lowenstein et al. (9) that $Mg^{++}$ ions were relatively poor metal ion catalysts for the reaction at low temperatures (23-38°C), we later found that at 50°C, $Mg^{++}$ and $Be^{++}$ are very nearly equivalent in their catalytic effectiveness. This is interesting in view of the fact that $Mg^{++}$ is the metal cation of choice in many contemporary aminoacyl transfer reactions, including activation. $Mg^{++}$ is also the most prevalent divalent cation in seawater.

The data of Fig. 1 are surprising as well because our previous studies had shown that the rate of activation of a variety of carboxylic and amino acids was directly proportional to their carboxylic $pK_a$'s (12). This finding is consistent with the idea that it is the protonated form of a carboxylic

acid which is activated by ATP, since at a given pH, the higher the $pK_a$ the greater the amount of protonated form present. Phenylalanine, however, has the lowest $pK_a$ (1.83) of any of the hydrophobic amino acids employed, and yet its relative rate of activation is nearly twice that of leucine, which has a carboxylic group $pK_a$ of 2.36. Clearly, additional factors must be involved in controlling the rate of activation of the hydrophobic amino acids.

Perhaps the most remarkable feature of Fig. 1 is the fact that there are measurable differences in the reaction rates of these hydrophobic amino acids. Because ATP is the most hydrophobic nucleotide (6), it seems reasonable to predict that if a correlation is to be found, it would be that the most hydrophobic amino acid would react most rapidly, with the others decreasing as the amino acid hydrophobicity decreased. In fact, phe, the most hydrophobic of the set, was activated the fastest, but the rates of the other amino acids (phe > leu > val > ile > met) do not correlate with the ordering of their own hydrophobicities, which is phe > ile > leu > val > met. This latter ordering is based on an average of a collection of normalized values from the literature (6,13-16). Obviously either the measurements of hydrophobicity are not correct, or hydrophobicity is not the only parameter of importance when it comes to activation. While the rates of reaction do not correlate with the hydrophobicities of the amino acids themselves in several sets of data plotted separately, they do most frequently correlate (Fig. 2) with the average estimated hydrophobicities of their anticodon dinucleotides (see legend to Fig. 2). In some experiments the ordering of the rates of activation of leu and val are seen to be reversed; phe, however, always reacted most rapidly, and ile and met least rapidly. The average $r^2$ value for the plots in Fig. 2 is 0.97.

In affinity studies (17) using several different experimental systems, phe was consistently found to have the greatest affinity for adenine derivatives of any of the amino acids employed in this study. Because of the

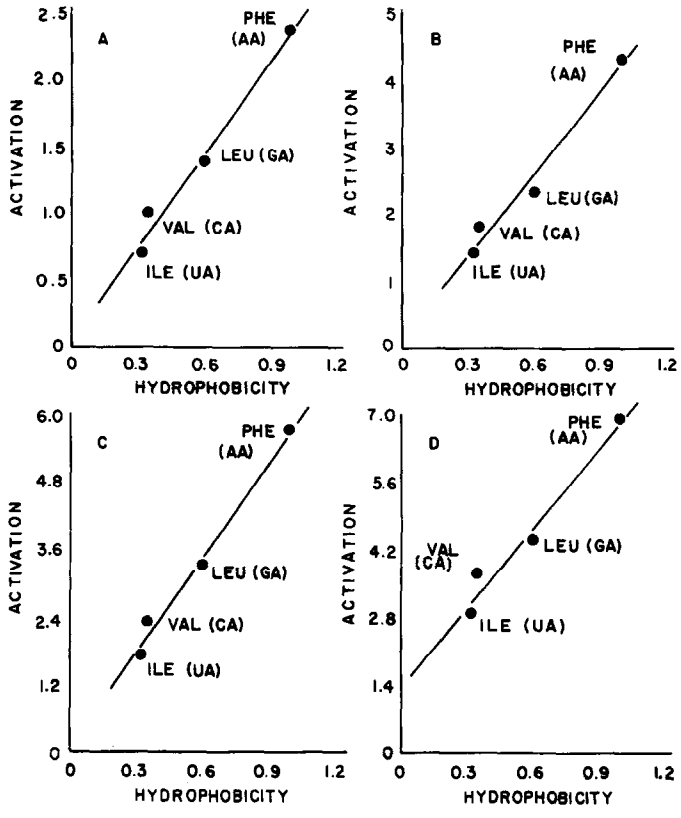


Fig. 2. μmoles hydrophobic amino acids activated by ATP at various temperatures and times as a function of the average hydrophobicity of their major anticodon dinucleotides (shown 3' → 5'). The reaction conditions are given in Materials and Methods. The hydrophobicities of the dinucleotides are normalized average values of those determined by Garel et al. (16), and of the reciprocal hydrophilicities determined by Weber and Lacey (6) and Barzilay et al. (18). Only the GA (3' → 5') anticodon dinucleotide was used for leucine. A) 170 hours, 37°, $r^2$ = 0.976; B) 260 hours, 37°, $r^2$ = 0.967; C) 95 hours, 50°, $r^2$ = 0.985; D) 140 hours, 50°, $r^2$ = 0.966.

consistently faster reaction of phe with ATP in the present system, we suggest that affinity between the amino acid and ATP may affect the reaction rate. Preliminary studies on the entropies of transition state formation ($T\Delta S^*$) for the reaction support this idea. Nevertheless, much more extensive studies on affinity measurements, the energy of transition state formation and actual peptide bond formation will be required before the idea can become more than a suggestion.

We further suggest that the phenomenon is a general one: that is, that selective affinities between all amino acids and nucleotides could

have resulted in selectively accelerated chemical reactions between these two molecular species, allowing for selective incorporation of amino acids into peptides and, thus, serving as the basis for the origin of the genetic code.

Finally, some qualifying remarks with respect to this work should be mentioned. First, we do not wish to overemphasize the ordering of activation (phe > leu > val > ile > met) at the expense of stressing the fact that phe, the most hydrophobic amino acid of this series, and which shows the greatest affinity for adenine derivatives (and which has AA as its only anticodonic dinucleotide), is always activated most rapidly by ATP under a variety of conditions. Second, although the results reported in this paper are quite reproducible and offer little reason to question the fact that the color development observed is due to aminoacyl hydroxamates, we have not rigorously correlated color development with the amount of isolated hydroxamate in any of the activation experiments. Ryan and Fox (11), however, have identified radioactive glycine hydroxamate following incubation of [$^{14}$C]-glycine in the presence of ATP, hydroxylamine and $MnCl_2$, and we have qualitatively identified amino acid hydroxamates by chromatography in several experiments.

Another essential point to be mentioned is that, although we have assumed, as have others (8,9,11), that the formation of the amino acid hydroxamates proceeds via the formation of either aminoacyl phosphates or aminoacyl adenylates, such a mechanism has not been rigorously demonstrated by anyone. Thus, use of the word activation in this paper is intended to convey the idea of the formation of activated intermediates, caused by the presence of ATP, and leading to the formation of hydroxamate color without specifying the intermediate. Regardless of the exact nature of the intermediate, ATP is required for the reaction and different amino acids respond differently.

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