Title
SIMULTANEOUS PEPTIDE AND OLIGONUCLEOTIDE FORMATION IN MIXTURES OF AMINO ACID, NUCLEOSIDE TRIPHOSPHATE, IMIDAZOLE, AND MAGNESIUM ION

Permalink
https://escholarship.org/uc/item/6s17w8rk

Author
Weber, A.L.

Publication Date
1976-12-01
SIMULTANEOUS PEPTIDE AND OLIGONUCLEOTIDE FORMATION IN MIXTURES OF AMINO ACID, NUCLEOSIDE TRIPHOSPHATE, IMIDAZOLE, AND MAGNESIUM ION


December 1976

Prepared for the U. S. Energy Research and Development Administration under Contract W-7405-ENG-48

For Reference

Not to be taken from this room
DISCLAIMER

This document was prepared as an account of work sponsored by the United States Government. While this document is believed to contain correct information, neither the United States Government nor any agency thereof, nor the Regents of the University of California, nor any of their employees, makes any warranty, express or implied, or assumes any legal responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by its trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof, or the Regents of the University of California. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof or the Regents of the University of California.
SIMULTANEOUS PEPTIDE AND OLIGONUCLEOTIDE FORMATION IN MIXTURES OF
AMINO ACID, NUCLEOSIDE TRIPHOSPHATE, IMIDAZOLE, AND MAGNESIUM ION

A. L. WEBER,* J. M. CAROON,** J. T. WARDEN,*** R. M. LEMMON, and
M. CALVIN

Laboratory of Chemical Biodynamics, Lawrence Berkeley Laboratory,
University of California, Berkeley, CA 94720

*Present address: The Salk Institute, San Diego, CA 92112
**Present address: Syntex Corporation, Palo Alto, CA 94304
***Present address: Biochemistry Program, Department of Chemistry,
Rensselaer Polytechnic Institute, Troy, N.Y. 12181
Simultaneous peptide and oligonucleotide formation was observed in reaction mixtures of amino acid, nucleoside triphosphate, imidazole, and MgCl$_2$. At 70° C in solutions that were evaporated to dryness the formation of peptide for phe and pro was greatest with CTP relative to ATP, GTP, and UTP. Lysine exhibited a preference for GTP and glycine for UTP. At ambient temperature in solution at pH 7.8, CTP was preferred by glycine, but at pH 8.7 UTP was preferred. The glycine nucleotide phosphoramidates were also detected and characterized in reactions at 40° C. The glycine-reaction preference for CTP at pH 7.8 and UTP at 8.7 suggested that the basicity of the nucleoside triphosphate was involved in increasing the peptide yield. CTP near neutrality is the most basic nucleoside triphosphate and the basic anionic form UTP could facilitate peptide formation at pH 8.7. These data, together with information on the complexing of poly(C) by GTP, led to the experimentally approachable hypothesis that GTP, by forming a basic triplex between the cytosine residues adjacent to the peptidyl adenosine and aminoacyl adenosine at the termini of two proto-tRNAs, would promote peptide bond synthesis between the aminoacyl residue and peptidyl residue.
I. Introduction

We have examined mixtures of amino acid, nucleoside triphosphate, imidazole, and MgCl₂ as a model system that allows investigation of (1) simultaneous appearance of oligopeptides and oligonucleotides, (2) reactions involved in the establishment of the protein biosynthetic pathways, and (3) possible specific chemical relationships between nucleotides and amino acids. Magnesium ion was included in the system because of its catalysis of amino acid adenylate anhydride formation from the amino acid and ATP (Lowenstein, 1958; Ryan & Fox, 1973). Imidazole was used because of its catalytic role in acyl and phosphoryl transfer reactions (Weber & Lacey, 1975; Ehler & Orgel, 1976; Stephen-Sherwood et al., 1974) and the condensation reactions of presumed amino acid imidazolides and nucleotide imidazolides (Weber & Lacey, 1974; Weimann et al., 1968; Ibanez et al., 1971). Peptide formation from glycine, ATP, imidazole, and MgCl₂ in the solid state has recently been reported (Lohrmann & Orgel, 1973; Sawai et al., 1975).

In the present investigation peptide formation from amino acids that possess one homogeneous anticodon (namely, phe (AAA), pro (GGG), gly (CCC), and lys (UUU)) was examined in the solid state in the presence of the four nucleoside triphosphates -- ATP, GTP, CTP, UTP. This investigation was undertaken because of the correlation of hydrophobic properties of the amino acids and their anticodonic nucleotides, the hydrophobicity of which decrease in the sequence: Phe (ATP) > Pro (GTP) > Gly (CTP) > Lys (UTP) (Lacey & Weber, 1975). Previously, amino acids with similar polarities were shown to have similar codons (Woese et al., 1966; Woese, 1973) and polyanhydro amino acids were found to exhibit selective interactions with
polynucleotides (Fox et al., 1971; Fox, 1974). It was thought that amino acid polymerization might be facilitated when they were heated with the nucleotide having a similar hydrophobic character and resembling its anticodon nucleotides. In other words, phe might polymerize best with ATP, pro with GTP, gly with CTP, and lys with UTP. In addition, peptide formation was examined in solution at ambient temperature and at 40°C for glycine with the four nucleoside triphosphates.

2. Materials and Methods

L-Lysine, L-proline, phenylalanine trimer and tetramer, and glycine peptides were obtained from Sigma Chemical Co. Glycine, ATP (disodium), dinucleoside monophosphates, and DL-alanine were purchased from Calbiochem. Phenylalanine dimer, GTP (disodium), CTP (disodium), UTP (trisodium), poly(A), poly(G), poly(C), and 14C-L-lysine were purchased from Schwarz/Mann. Lysine peptides were obtained from Miles Laboratories and proline peptides from Vega-Fox. New England Nuclear supplied 14C-ATP, 14C-glycine, 14C-L-phenylalanine, and 14C-L-proline. L-Phenylalanine was purchased from the Aldrich Chemical Co. All those reactants were used as received.

2.1. Peptide formation in solutions evaporated to dryness at 70°C for each of the amino acids (phe, pro, gly, and lys) with each nucleoside triphosphate (ATP, GTP, CTP, UTP)

Three-tenths milliliter solutions that contained 0.10 M 14C-amino acid, 0.10 M nucleoside triphosphate (trisodium salt), 0.10 M MgCl2, and 0.30 M imidazole were heated in 13 x 100 mm open test tubes in an oven 24 hr at 70-75°C. An 0.10 ml solution of identical composition was allowed to stand 24 hr at ambient temperature in order to provide an unheated control.
attempt was made to control the pH during the reaction. Peptide formation was assayed by co-chromatography with standards obtained commercially in the two-dimensional paper chromatographic systems depicted in Fig. 1. For phe and gly the amino acids and peptides were visualized by autoradiography and the areas of the chromatogram containing these substances were cut out and counted in the liquid scintillation "cocktail" Aquasol-2. For pro and lys the amino acids were treated the same but due to low yield of radioactive peptides it was necessary to visualize their carrier peptides by spraying with ninhydrin and heating to 80°C for 5 minutes. Subsequently the ninhydrin-visualized spots were cut out and counted.

2.2. Peptide formation for glycine in solution at ambient temperature and at 40°C with each nucleoside triphosphate

Two-tenths ml solution that contained 0.10 M 14C-glycine, 0.10 M nucleoside triphosphate (tridosium salt), 0.10 M MgCl₂, and 0.30–1.2 M imidazole were allowed to stand at ambient temperature 3 days in sealed small vials (1 ml vol.) that had 5 μl chloroform added to prevent bacterial growth. The analysis of peptides was carried out as described earlier for glycine, except that glycine and its peptides were located on the chromatogram by their fluorescence under long-wave U.V. This fluorescence appeared after the chromatogram was dried in an air flow for 24 hr and intensified with extension of the drying time. A similar reaction was run at 40°C for 6 days with 0.6 M imidazole, except that the solution volumes were 0.10 ml.

In both the above experiments nucleotide-amino acid adducts were observed. These products were eluted from the chromatogram and rechromatographed. Their Rf values relative to glycine for the glycine adducts of AMP (ADP), GMP (GDP), CMP (CDP), and UMP (UDP) were respectively, 0.48 (0.20), 0.24 (0.09), 0.36 (0.13), and 0.55 (0.14). These adducts were eluted and
one-half of each treated with 0.1 N HCl for 1.5 hr and the other half treated with 0.1 N NaOH for 1 hr. Chromatography revealed that these substances were stable in base but hydrolyzed yielding glycine in acid.

2.3. ApAp$_n$ formation from ATP in the presence of ala or phe

Fifty microliter solutions of 0.80 M $^{14}$C-ATP (disodium salt), 0.80 M DL-alanine, 0.80 M MgCl$_2$, and 3.2 M imidazole were heated in 13 x 100 mm open test tubes in an oven 24 hr at 70-75° C. ApA formation was assayed by co-chromatography with standard ApA in the chromatographic system shown in Fig. 2. Chromatography was performed with untreated reaction product and with reaction product that was treated with acid phosphatase type IV obtained from the Sigma Chemical Co. The phosphatase treatment was done at ambient temperature for 3 hr with 0.9 mg/ml of enzyme, 0.18 mg/ml carrier ApA, and 5 mM of the nucleotide in the reaction mixture in 0.10 M ammonium phosphate at pH 4.8.

A similar reaction was performed with 0.2 ml solutions of 0.10 M ATP-$^{14}$C (trisodium salt), 0.10 M phenylalanine, 0.10 M MgCl$_2$, and 0.3 M or 0.6 M imidazole in open and sealed small vials (1.0 ml vol.) at several temperatures and for varying lengths of time. ApA was assayed as described above.

3. Results

Fig. 3 (a-d) depicts peptide formation for the four amino acids each of which possesses one homogeneous anticodon, phe (AAA), pro (GGG), gly (CCC), and lys (UUU). Peptide formation was carried out in the presence of Mg$^{+2}$, imidazole, and each of the nucleoside triphosphates ATP, GTP, CTP, UTP that resemble the homogeneous anticodons of these amino acids. As seen
in Fig. 3 (a, b) phe and pro, which are L-amino acids with uncharged side chains, yield with CTP the greatest amount of peptide for each degree of polymerization. Peptide formation was the lowest with ATP. The data for glycine (Fig. 3c) are more difficult to interpret because of formation of glycine products other than peptides. The yields of these products, which could be peptide adducts of the nucleotides or inorganic phosphate decrease in the sequence CTP (7.6%), GTP (2.3%), UTP (1.5%), ATP (0.5%). If the glyc peptides are bound as nucleotide or phosphate derivatives, the CTP and GTP values shown in Fig. 3c might be low. This contention is reinforced, as shown in Fig. 4, since CTP forms the most peptide in a similar reaction with glycine (0.3 M imidazole), except that the reaction was carried out in solution at ambient temperature.

As depicted in Fig. 3d, the basic amino acid L-lysine prefers GTP for peptide formation. An unknown product (Unk. A) is also shown because its ion-exchange behavior on cellulose phosphate suggests that the unknown is ε-linked dilysine. Fig. 1d shows that this substance migrated very close to the α-linked dilysine standard on cellulose phosphate. If this is the case then the ε-linkage is preferred in this reaction system.

Figure 4 shows the formation of diglycine in solution at ambient temperature for 3 days with Mg²⁺, imidazole, and the four nucleoside triphosphates. At pH 7.8 with 0.3 M imidazole, the most diglycine is formed with CTP. As the pH and imidazole concentration are increased, diglycine formation increases most with UTP. At pH 8.7, UTP is preferred significantly over the other nucleoside triphosphates. Spectrophotometric measurements of ATP (ADP) and AMP eluted from the chromatograms show that only about 2% of the ATP (ADP) has hydrolyzed to AMP during the reaction. The low yield
of peptides is due primarily to the slow reaction rate. If the remaining 98% of the ATP (ADP) were to react at the same efficiency, the yield of the reaction would be about 5%.

Figure 5 shows several controls of glycine peptide formation in a reaction mixture of glycine, ATP, Mg\(^{+2}\), and imidazole heated in sealed vials at 40\(^\circ\) C for 6 days. Omission of Mg\(^{+2}\) reduces the yield to 30% of that of the complete system. Substitution of 5\(^1\)-AMP for ATP or of 2,6-lutidine for imidazole reduces the yield to that of an unheated control that was frozen at -20\(^\circ\) C.

Table I shows diglycine formation in the same reaction shown in Fig. 5. Another product of the reaction, the nucleotide-glycine phosphoramidate, was detected by autoradiography. This product was characterized by being a U.V. quenching spot containing \(^{14}\)C-glycine that was stable in base and hydrolyzed in acid to yield free \(^{14}\)C-glycine. The data show that ATP yielded the least phosphoramidate, resulting in a high diglycine-phosphoramidate ratio.

Figure 6 shows several experiments in which the formation of ApAp\(_n\) was assayed in reaction mixtures of amino acid, \(^{14}\)C-ATP, Mg\(^{+2}\), and imidazole. In Fig. 6a the amino acid was alanine and the reaction was carried out in the dry state at 70\(^\circ\) C for 1 day. ApAp\(_n\) formation was measured with phosphatase treatment as noted by the whole bar and without phosphatase treatment as represented by the striped area of the bar. The only significant formation of ApA is noted on samples treated with acid phosphatase. This indicates that the primary product is ApAp, ApApp, or ApAppp, which are hydrolyzed to ApA by phosphatase. The yields are very small, about 0.05%. Controls in which the reaction mixture was not heated or in which 5\(^1\)-AMP was substituted for ATP, or 2,6-lutidine for imidazole, show significantly less ApA. The \(^{14}\)C-ApA product was further characterized by digestion with snake venom phosphodiesterase to yield approximately 50% adenosine and 50% 5\(^1\)-AMP.
Fig. 6b shows ApApₙ formation at several temperatures in the solid state and in solution with phenylalanine. The data indicate that ApApₙ formation occurs only in the solid state.

4. Discussion

The oligonucleotide ApApₙ was formed in the solid state in mixtures of amino acid, ATP, imidazole, and MgCl₂. This reaction mixture also forms oligopeptides, as discussed below. Higher oligomers of Ap may be present but were not identified. As shown below, the oligonucleotide (ApApₙ) probably forms via the adenylate imidazolide (Weimann et al., 1968; Lohrmann & Orgel, 1973).

Peptide formation has been shown to occur in the solid state at 70° C in mixtures of each of the four amino acids, phe, pro, gly, and lys, and the four nucleoside triphosphates, ATP, GTP, CTP, and UTP, in the presence of Mg²⁺ and imidazole. As shown below for ATP, the mechanism of the solid state reactions at 70° C may involve the amino acid nucleotidyl anhydride (Katchalsky, 1973), which could be formed by reaction of the amino acid with either the nucleoside triphosphate (Lowenstein, 1958; Ryan & Fox, 1973) or nucleotide imidazolide (Lohrmann & Orgel, 1973; Sawai et al., 1975).

The amino acid nucleotide anhydride could react with (1) an amino acid to yield peptides (Katchalsky, 1973) or (2) imidazole to yield the amino acid imidazolide (Lacey & White, 1972), which subsequently reacts with an amino acid to yield peptides (Weber & Lacey, 1974) or with the ribose hydroxyl of a nucleotide to yield the ester (Weber & Lacey, 1975). The ester's behavior in the solid state is unknown but in solution it would
probably hydrolyze instead of yielding peptides (Weber & Lacey, 1976; Schuber & Pinck, 1974a).

Solution reactions at 40°C for 6 days with glycine yielded two stable products, glycine peptides and glycine nucleotide phosphoramidates. The absence of phosphoramidates of glycine peptides indicates that when the carboxyl group is activated the amino group is free and not protected as a phosphoramidate. The high ratio of diglycine to glycine nucleotide phosphoramidates in the reaction at 40°C suggests the possibility that ATP was selected in evolution to activate amino acids because of its preferential reaction with the carboxyl group of amino acids to yield the adenylate anhydride rather than reaction with the amino group to yield the adenylate phosphoramidate. A preference for ATP in activating amino acids has been reported (Ryan & Fox, 1973).

No anticodonic preferences were exhibited in peptide formation with the amino acids and the nucleoside triphosphates. Instead, CTP yielded the greatest amount of peptide for 2 of the 4 amino acids at 70°C in the solid state. CTP was also preferred by glycine at pH 7.8; however, UTP gave maximum yield of peptide at pH 8.7. These results suggest that the basicity
of the nucleotide to phosphate may enhance peptide formation. At pH 7.8, CTP (pK 4.8) is the most basic nucleoside triphosphate. At pH 8.7 the basic anionic form of UTP (pK 9.6) may be promoting peptide synthesis. At pH 8.7 the basic anionic form of GTP (pK 9.3) would also be present but no enhanced peptide formation was found, perhaps because of steric factors.

The possibility that the basicity of CTP promotes peptide formation, together with the observed presence of 2 cytidine residues in the universal CCA terminus of t-RNAs, led to an examination of the literature on the properties of poly(C). At pH 6.0 poly(C) begins to form a hemiprotonated duplex of parallel strands that is most stable at pH 4.5 (Guschlbauer, 1975; Langridge & Rich, 1963). Protonated triplexes with a C, C+, G stoichiometry form between poly(C) and poly(G), oligo G, GMP, GTP, and guanosine (Thiele & Guschlbauer, 1971; Howard et al., 1964; Sarocchi et al., 1970; Ts'o & Huang, 1968). In complexes between poly(C) and 3'-GMP the triplex begins to form at pH 6.6 and triplex formation is complete at pH 5.15 (Sarocchi et al., 1970). GTP has been shown to form a 2C-1G triplex with poly(C) at room temperature at pH 7.0 (Ts'o & Huang, 1968). The formation of this GTP triplex is accompanied by a phase transition in which the complex precipitates.

This information has led us to the hypothesis that GTP could promote peptide bond formation between primitive peptidyl t-RNA and aminoacyl t-RNA species by formation of a protonated triplex with the cytosine residues near the terminus of these primitive t-RNAs, as depicted below. This primitive system may have had more than two cytosine residues near the terminus in order to promote GTP binding.
As shown in Fig. 7 this hypothesis is reinforced by the excellent positioning of the amino group of the amino acid ester of the terminal adenosine of one strand near the carbonyl group of the peptidyl ester of the terminal adenosine of the second strand. This positioning is afforded by the 2C-IG triplex of GTP with the final cytidine residues of the two strands. The proton involved in the 2C-IG triplex, which lies below the terminal adenosine residues, is denoted by (+) in Fig. 7. It is possible that the amino acid ester with a pK of 7.5 – 7.8 (Schuber & Pinck, 1974b; Wolfenden, 1963) provides the proton necessary for formation of the terminal protonated 2C-IG triplex. This possibility depends upon the basicity of triplex with GTP. The formation of the poly(C)-GTP triplex at room temperature and at pH 7.0 suggests that the basicity of the triplex is sufficient to bring about delocalization of the proton on the amino acid (i.e., the proton is shared by the triplex and amino group of the amino acid).

This hypothesis is presented because of the three possible modes by which it could promote peptide bond synthesis with the esters of
oligonucleotides. The triplex (1) positions the amino acid and peptidyl ester for reaction, (2) provides a basic environment for deprotonating the amino acid, and (3) removes the reactants from solution and contact with water. This model is consistent with the involvement of GTP in the binding of aminoacyl t-RNAs to the peptidyl transferase site in contemporary protein biosynthesis (Haselkorn & Rothman-Denes, 1973), but the contemporary system probably does not use triplex formation between GTP and the cytosine residues of the t-RNAs in peptide bond formation.

Acknowledgement
This research was supported by the U.S. Energy Research and Development Administration.

Tribute
The question of the origin of life and the evolutionary system that led to it was one of great interest to Professor Onsager. Although I never heard him express it in the terms I think of it, his principal theorem in irreversible thermodynamics to the effect that the rate of entropy increase should be minimized in an open system seems to me to be the underpinning of the development of living systems on the Earth. It is only with the appearance of the organized energy transducing systems that are living things that the rate of entropy increases, at least in the locale of the Earth's surface, could be minimized.

Melvin Calvin
References


References (continued)

Schuber, F. and Pinck, M., 1974b, On the chemical reactivity of the aminoacyl-tRNA ester bond. II. Aminolysis by tris and diethanolamine, Biochimie 56, 391.
Thiele, D. and Guschlbauer, W., 1971, Protonated polynucleotide structures. IX. Disproportionation of Poly (G)·Poly(C) in acid medium, Biopolymers 10, 143.
References (continued)

Ts'o, P.O.P, and Huang, W. M., 1968, Physicochemical basis of the recognition process in nucleic acid interactions. II. Interactions of polyuridylic acid and polycytidylic acid with nucleoside mono- and triphosphates, Biochemistry 7, 2954.


Legends to Figures

Fig. 1. Two dimensional chromatography of the homopeptides of (a) phenylalanine, (b) proline, (c) glycine, and (d) lysine. Chromatographic solvents: (1): n-butyl alcohol, acetic acid, water (4:1:5 v/v), upper phase, 18 hr.; (2): saturated ammonium sulfate, formic acid, water (3:2:6 v/v), 6 hr.; (3): n-butyl alcohol, acetic acid, water (4:1:5 v/v), upper phase, 18 hr.; (4): saturated ammonium sulfate, formic acid, water (20:1:4 v/v), 6 hr.; (5): n-propyl alcohol, water (3:1 v/v), 48 hr.; (6): n-propyl alcohol, 1 M potassium acetate (3:1 v/v), 48 hr.; (7): 0.4 M potassium acetate, 3.5 hr.; (8): n-propyl alcohol, 1 M potassium acetate (7:3 v/v), 24 hr. Phe, pro, and gly chromatographed on Whatman #1; lys chromatographed on Whatman P81 (cellulose phosphate).

Fig. 2. Two dimensional chromatography of mono and oligonucleotides on Whatman #2. Chromatographic solvents: (1): 1 M ammonium acetate, 95% ethyl alcohol (4:1 v/v), 19 hr.; (2): sec-butyl alcohol, tert-butyl alcohol, water (44:7:49 v/v), 24 hr.

Fig. 3. Peptide formation in the dry state at 70° C (24 hr.) for each of the amino acids, phenylalanine, proline, glycine, and lysine with each of the nucleoside triphosphates, ATP, GTP, CTP, and UTP. Peptide values for phe, gly, and lys were not corrected because ambient temperature controls yielded insignificant amounts of peptides. Peptide values for proline were corrected by subtracting the peptide values observed in controls that stood 24 hr. at ambient temperature. During reaction the pH dropped from 7.6 to 6.9.
Legends to Figures (continued)

Fig. 4. Diglycine formation in solution at ambient temperature for 3 days with glycine in the presence of each nucleoside triphosphate. The diglycine values are corrected by subtraction of control values of reactions that did not contain the nucleoside triphosphate. These values were 0.041, 0.046, and 0.052 for 0.3 M, 0.6 M, and 1.2 M imidazole respectively.

Fig. 5. Control experiments on glycine peptide formation in solution in sealed vials at 40°C for 6 days. Striped bars = diglycine; open bars = triglycine.

Fig. 6. (a) ApA formation in ala solutions heated to dryness at 70°C for 24 hr. C-1 refers to the complete system. C-2 contained double the imidazole of the complete system. In the last two reactions an equivalent amount of 5'-AMP was substituted for ATP and 2,6-lutidine for imidazole. The striped portion of the bar graph represents ApA before acid phosphatase treatment; the whole bar represents ApA after phosphatase treatment.

(b) ApA formation (in the presence of phe) in the dry state and in solution at several temperatures. The first value at each temperature refers to reactions with 0.3 M imidazole; the second value refers to reactions with 0.6 M imidazole. Yields before and after phosphatase treatment are shown in the same way.

Fig. 7. Photograph of the amino acid ester of the 3'-hydroxyl of the terminal adenosine of CpA and the peptide ester of the 3'-hydroxyl of the terminal adenosine of an adjacent CpA with the C residues of the two di-nucleotides linked by GTP. (N) denotes hydrogen of amino group of amino acid, (C) denotes carbonyl group of peptide, and (+) denotes proton held by the 2C-IG triplex (it lies below the terminal adenosine residues). The
Legends to Figures (continued)

binding arrangement was that proposed by Howard et al., 1964, for the binding of GMP by parallel poly(C) strands.

Tables

TABLE 1

Diglycine and nucleotide-glycine phosphoramidate formation in solution of 40°C for 6 days with each nucleoside triphosphate.

<table>
<thead>
<tr>
<th></th>
<th>Diglycine</th>
<th>Nucleotide-Glycine phosphoramidate</th>
<th>Ratio of diglycine to phosphoramidate</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP</td>
<td>0.59</td>
<td>0.66</td>
<td>0.89</td>
</tr>
<tr>
<td>GTP</td>
<td>0.33</td>
<td>2.66</td>
<td>0.12</td>
</tr>
<tr>
<td>CTP</td>
<td>0.35</td>
<td>1.77</td>
<td>0.20</td>
</tr>
<tr>
<td>UTP</td>
<td>0.38</td>
<td>1.42</td>
<td>0.27</td>
</tr>
</tbody>
</table>
Fig. 1

XBL 767-6081
ATP or ADP

0 5'-AMP 0 Adenosine
or 2'-AMP

0 3'-AMP

0 ApA

0 ApApA

0 ApApApA


A

→ B

Fig. 2

XBL 767-6078
Fig. 3 (a, b)
LYSINE PEPTIDES (Percent Yield)

Fig. 3 (c, d)

GLYCINE PEPTIDES (Percent Yield)
Fig. 4
This report was done with support from the United States Energy Research and Development Administration. Any conclusions or opinions expressed in this report represent solely those of the author(s) and not necessarily those of The Regents of the University of California, the Lawrence Berkeley Laboratory or the United States Energy Research and Development Administration.