AMINOACYL TRANSFER FROM ADENYLATE ANHYDRIDE TO THE 2'OH GROUPS ALONG THE BACKBONE OF POLYRIBONUCLEOTIDES

William E. White, Jr., James C. Lacey, Jr. and
Arthur L. Weber

Laboratory of Molecular Biology
University of Alabama School of Medicine
University Station
Birmingham, Alabama 35294

Received January 29, 1973

SUMMARY. This work reports the transfer of the N-acetylglycine from the adenylate anhydride to the 2'OH groups along the backbone of homopolyribonucleotides. This transfer involves an N-acetylglycylimidazole intermediate; no transfer was observed in the absence of imidazole, and the rate of transfer was different for the various polynucleotides: poly U > poly A > poly C = poly G = 0. These results suggest that catalysis is necessary for transfer of aminoacyl from adenylates to polyribonucleotides and the data are consistent with a model involving a histidine residue in the active site of aminoacyl-tRNA synthetases. They are also consistent with a model for primordial protein formation involving polymerization of amino acids which are attached at the 2'OH groups along the polyribonucleotide backbone.

INTRODUCTION. Even though knowledge of the chemistry of activated forms of amino acids is obviously important in understanding aminoacyl transfer in contemporary systems, relatively little has been reported in the literature on this subject. In addition to understanding the operation of contemporary systems, the importance of such studies relating to an understanding of the origin of the genetic coding system has been emphasized in recent years by the work of S. W. Fox and his collaborators (1-5).

We have investigated the transfer of N-acetylglycyl groups from their adenylate anhydrides to the 2'OH groups along the backbone of polyribonucleotides: 1) to better understand the mechanism of such transfers to the terminal -OH groups of tRNAs in the contemporary biosynthesis of proteins and 2) to demonstrate the possibility of a primitive peptide synthesizing system which forms peptides via aminoacyl ester intermediates of 2'OH groups along the backbone of polyribonucleotides. Our present

studies were made using an N-acetyl amino acid, N-acetyl glycine, in order to prevent the formation of peptides. Similar studies using free amino acids in this system are now in progress and include peptide bond formation from aminoacylated polyribonucleotides.

We have previously reported (6) that N-acetylglycyl adenylate anhydride can be converted almost quantitatively to N-acetylglycyl imidazole at pH 7 using 0.5 M imidazole at 5°C. A similar conversion had been reported by Jencks (7) for acetyl adenylate anhydride. In both studies there appeared to be significant amounts of adenylate ester formed, indicating that imidazole catalyzed the transfer of the acid function from anhydride to ester via an imidazole intermediate. Our earlier results are consistent with the idea that a histidine residue in the aminoacyl-tRNA synthetases might catalyze the transfer of aminoacyl groups from adenylate anhydride to tRNA. These results also suggest a mechanism whereby amino acids could be transferred from an activated nucleotide to the 2'OH groups along the backbone of RNAs as reported by Beljanski (8-19) for a bacterial peptide synthesizing system. We are interested in chemically exploring the nature of this latter system as an early evolutionary peptide synthesizing system in which genetic code relationships were first displayed. In essence the model we are exploring is as shown in Figure 1. This model was briefly discussed earlier (20). The basic idea is that aminoacyl intermediates of the 2'OH groups along RNAs allow side chain nucleotide "recognition" interactions and simultaneously allow the proximity of the phosphate group of the polynucleotide to activate the amino groups of the amino acid to attack the carbonyl group of a neighboring ester for peptide bond formation. The polynucleotide ribose phosphate backbone is seen as a "natural" catalyst for peptide formation via the 2'OH aminoacyl esters.

In the present publication we show that imidazole can catalyze the transfer of N-acetylglycine from the adenylate anhydride to the 2'OH groups of homopolyribonucleotides. Furthermore the ease of transfer is poly U >

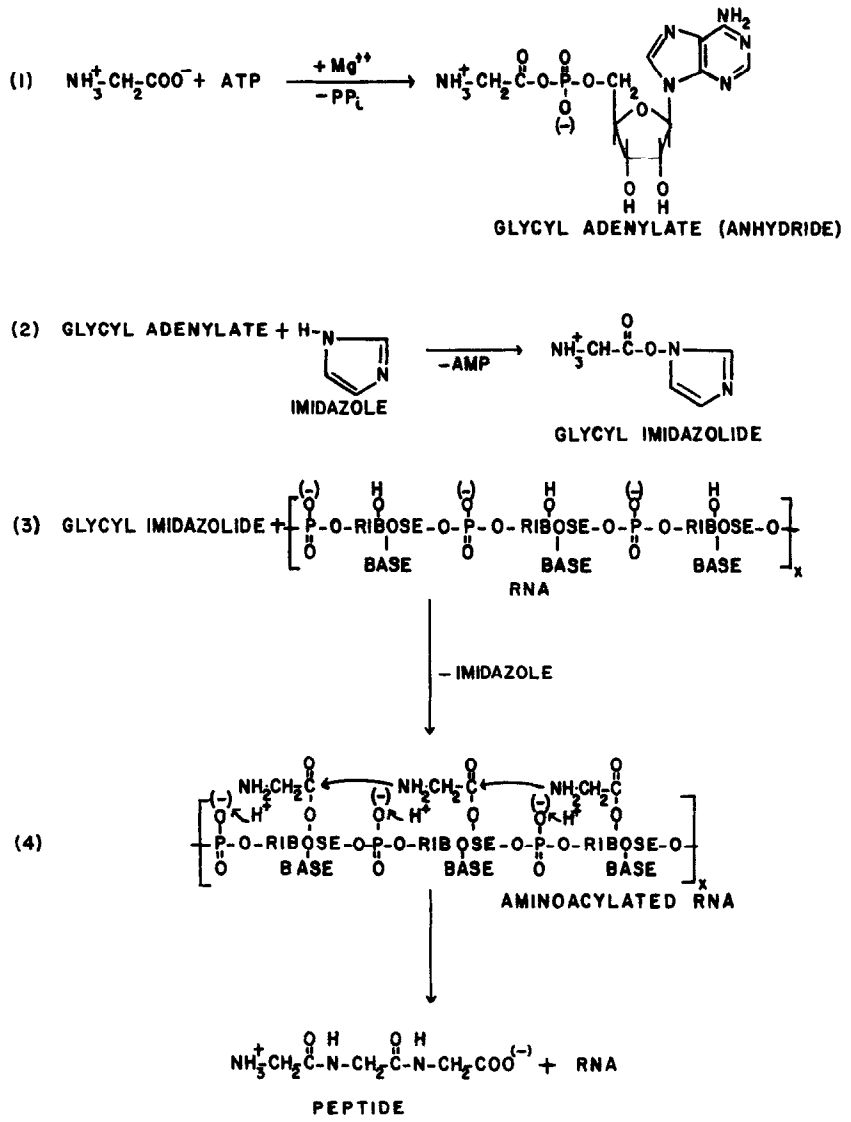

Figure 1. Model sequence for generation of peptides directly on the backbone of an RNA molecule. Using glycine as an example the steps are:

1) initial activation of the amino acid by reaction with ATP and formation of the adenylate
2) conversion of the aminoacyl adenylate to the amino-acyl imidazole
3) transfer of the aminoacyl groups to the 2'OH groups along the RNA backbone, and
4) reaction of the aminoacyl groups with each other, perhaps facilitated by the phosphate group abstracting a proton from the α- amino group of the amino acid.

Both imidazole and RNA are regenerated in the process and can be considered as catalysts.

poly A > poly G = poly C = 0. This ordering shows no obvious relationship to the genetic code but the heirarchy may be to some extent dependent on the degree of structuring of the homopolynucleotides in solution (21). Poly U is generally considered as being the least structured, however at 0-5°C considerable structure is present.

MATERIALS AND METHODS.

N-acetylglycyl adenylate anhydride was prepared by the method previously described (6), from N-acetylglycine (Sigma Lot 41C-3010) and 5' adenylic acid (Sigma Lot 47B-7270) and dissolved in H_2O, divided into one ml aliquots and kept at -70°C until use.

Approximately 20 mg of polynucleotide was dissolved in 0.4 ml of 1.5 M imidazole having an initial pH of 7.6. While the temperature was maintained at 5°C, 0.80 ml of N-acetylglycyl adenylate solution (∿ 0.1 M) was added and the pH dropped to 7. After 5, 10, and 15 min, 0.3 ml aliquots were taken and added to test tubes in an ice bath containing 3.0 ml of 1 M $BaCl_2$ in 0.1 M HCl to precipitate the polynucleotide. The precipitates were centrifuged, washed with another 3.0 ml of $BaCl_2$ solution and centrifuged again. The precipitates were analyzed for active acyl using the hydroxamate assay of Lipmann and Tuttle (22) except that they were incubated at 40°C for 30 minutes for formation of hydroxamate and color development was in 10% $FeCl_3 \cdot 6H_2O$ instead of 5% in 0.1 N HCl. The solutions were filtered through 0.45 μ millipore filters and the absorbance was read at 495 nm. The total active acyl in the reaction mixture was also monitored by analyzing 50λ aliquots taken at 5, 10, and 15 minutes.

The following polynucleotide potassium salts were used in this study: Poly U, Schwarz-Mann, Lot 1812-80 and Sigma Lot 1C-0640; Poly G, Sigma Lot 110C-2190; Poly C, Sigma Lot 119B-0280, and Poly A, Sigma Lot 110C-0960 and Schwarz-Mann Lot W2156. These polymers were completely soluble in the imidazole buffer.

RESULTS. Figure 2 illustrates the transfer of the N-acetylglycyl moiety to the polynucleotides as monitored by an increase in hydroxamate color of the polynucleotide precipitates. The reaction rate heirarchy was poly U > poly A > poly C = poly G = 0. No transfer was observed in control experiments when imidazole was omitted from the reaction mixture.

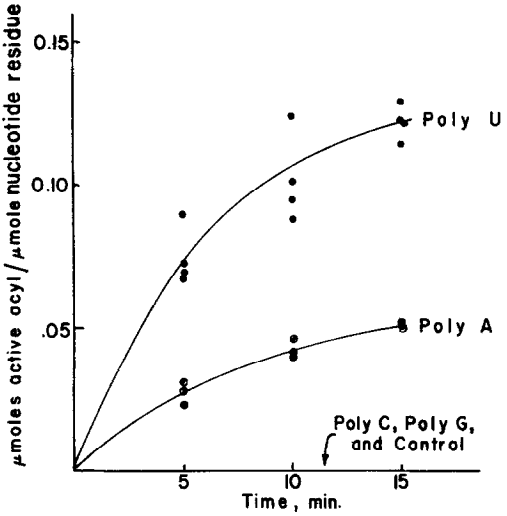

Figure 2. Transfer of N-acetylglycyl from the adenylate anhydride to the 2'OH groups of polyribonucleotides using 0.5 M imidazole as a catalyst, pH 7, 5°C with starting adenylate concentration about 0.1 M. Approximately 20 mg of polynucleotide was present in 1.2 ml reaction volume. Analysis for active acyl as described under Materials and Methods.

Figure 3 shows the decrease and plateau of the total hydroxamate color with time and indicates that hydrolysis of the N-acetylglycyl imidazole is a significant side reaction in aqueous solution.

After 15 minutes, 0.12 amino acids per nucleotide had been transferred to poly U while 0.05 amino acids per nucleotide were found on poly A. These values were calculated assuming the polynucleotide was quantitatively precipitated under the conditions employed. This was demonstrated to be true in separate experiments with the polynucleotides. Allowing the reaction

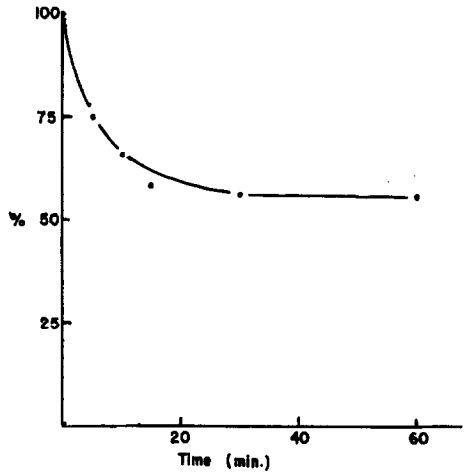

Figure 3. Percent of total active acyl remaining at various times at pH 7, 5°C with 0.5 M imidazole and starting adenylate concentration about 0.1 M. Hydroxamate assay for active acyl was as described under Materials and Methods.

to proceed for one hour instead of the usual 15 minutes had little effect because by 15 minutes hydrolysis and the formation of polynucleotide or AMP aminoacyl esters was complete.

Passage of the aminoacylated polynucleotide through a sephadex column showed that the active acyl came through the column with the polynucleotide fraction.

DISCUSSION. Our results show that the N-acetylglycyl moiety can be transferred from an adenylate anhydride to the 2'OHs along the backbone of polyribonucleotides and that a catalyst is required because the reaction proceeds unobservably slowly in the absence of imidazole. The N-acetylaminoacyl polynucleotide is also relatively stable in the presence of imidazole and water. Otherwise it could not be isolated by the method described above. It is not likely that the amino acids are reacting with the nucleotide ring substituents because work by Gottikh (23), Khorana (24) and by Knorre (25) show that in aqueous solution acylation takes place on the ribose moiety almost exclusively. The heirarchy that we observe for the transfer

of the amino acid to the polynucleotides is poly U > poly A > poly G = poly C, the latter two not receiving detectable amounts of amino acid. These results are comparable with the heirarchy poly U > poly A > poly C reported by Weber et al (4) when starting with the N-acetylaminoacyl imidazoles instead of the adenylates. Similarily Gottikh et al (23) reported an heirarchy of yields UMP > GMP = AMP > CMP of tBOC-alynyl esters of mononucleotides starting with the tBOC-alanyl imidazole. Gottikh and collaborators attributed these differences in yields to differences in basicity of the rings of the nucleotides. Whether the heirarchies are due to differences in degree of structuring or basicity or both remains to be determined.

The data are consistent with the hypothesis that a histidine residue in the active site of aminoacyl-tRNA synthetases could be responsible for aminoacyl transfer in contemporary protein synthesis. The enzyme could form an aminoacyl-imidazole intermediate and thereby transfer the amino-acid from the high energy adenylate anhydride to the 3' terminal residue of the tRNA. A histidine residue could not catalyze peptide bond formation from aminoacyl-tRNA to any appreciable extent as the ester seems quite stable in the presence of imidazole. Also the selectivity shown makes it tempting to speculate that the uracil residue invariably adjacent to the 5' end of the anticodon in tRNAs might at some point in protein synthesis accept the amino acid or peptide moieties.

Beljanski's work (19) suggests another significance of these results. Using bacteria he isolated an enzymatic system which transfers amino acids to 2'OH groups along the backbone of RNAs. The extent to which such a system is used in contemporary organisms is not known, but it may have been significant in developing primordial systems. This system could have been an early step in the coevolution of the genetic code and the process of translation. The data presented previously (4-6) and the data in this publication are consistent with the model shown in Figure 1. However, we

have yet to demonstrate peptide bond formation and genetic code specificities in such a system. We also believe that such a system could have been retained by bacteria for synthesizing one or two ribosomal proteins. A major reason for believing this is that data by Iwata and Kaji (26) indicate that some of the E. coli ribosomal proteins are synthesized in a backwards direction, i.e. they grow by additions to the amino end instead of the carboxyl end.

Preliminary data show that the aminoacyl adenylate → aminoacyl imidazole → aminoacyl polyribonucleotide transitions also take place when the amino group is not blocked.

ACKNOWLEDGEMENTS. This work was supported by National Institutes of Health (NIH) Cancer Center Grant CA 13148, NIH General Research Support No. 5-S01-RR-05300-10 and an American Cancer Society Institutional Grant.

REFERENCES.

1) Krampitz, G. and Fox, S. W., Proc. Natl. Acad. Sci. 62:399 (1969).

2) Nakashima, T., Lacey, J. C., Jungck, J. R. and Fox, S. W., Naturwissenschaften 57:67 (1970).

3) Nakashima, T., and Fox, S. W., Proc. Natl. Acad. Sci. 69:106 (1972).

4) Weber, A. L., Lacey, J. C. and Fox, S. W., Fed. Proc. 30:1102 (1971) Abs.

5) Weber, A. L., Ph.D. Dissertation, U. of Miami (1973).

6) Lacey, J. C. and White, W. E., Biochem. Biophys. Res. Comm. 47:565 (1972).

7) Jencks, W. P., Biochim. Biophys. Acta 24:227 (1957).

8) Beljanski, Mirko., Compt. Rend. 250:624 (1960).

9) Beljanski, Mirko., Bull. Soc. Chim. Biol. 43:1017 (1961).

10) Beljanski, Mirko, Beljanski, Monique, and Lovigny, T., Biochim. Biophys. Acta 56:559 (1962).

11) Beljanski, Mirko. Biochem. Biophys. Res. Comm. 8:15 (1962).

12) Beljanski, Mirko and Beljanski, Monique. Biochim. Biophys. Acta 72:585 (1963).

13) Beljanski, Mirko, Fischer, C., and Beljanski, Monique., Compt. Rend. 257:547 (1963).

14) Beljanski, Mirko., Colloq. Intern. Centre Natl. Rech. Sci. No 124: 39 (1963).

15) Beljanski, Mirko., Biochem. Z. 342:392 (1965).

16) Beljanski, Mirko., Bull. Soc. Chim. Biol. 47:1645 (1965).

17) Beljanski, Mirko and Bourgarel, P., C. R. Acad. Sci., Paris, Ser. D. 264:1760 (1967).

18) Beljanski, Mirko and Bourgarel, P., C. R. Acad. Sci., Paris, Ser. D. 266:845 (1968).

19) Beljanski, Mirko, Fischer-Ferraro, C., and Bourgarel, P., Eur. J. Biochem. 4:184 (1968).

20) Lacey, J. C. and Fox, S. W., in Theory and Experiment in Exobiology Vol. 2 (A. Schwartz, ed.) Wolters-Nordhoff Pub., Groningen (1972) 35.

21) Yang, J. T. and Samejima, T., Prog. in Nucleic Acid Research 9:223 (1969).

22) Lipman, F. and Tuttle, L. C., J. Biol. Chem. 159:21 (1945).

23) Gottikh, B. P., Krayevsky, A. A., Tarussova, N. B., Purygin, P. P. and Tsilevich, T. L., Tetrahedron 26:4419 (1970).

24) Stuart, A. and Khorana, H. G., J. Biol. Chem. 239:3885 (1964).

25) Knorre, D. G. and Shamovsky, G., Biochim. Biophys. Acta 142:555 (1967).

26) Iwata, S. and Kaji, H., Proc. Natl. Acad. Sci. 68:690 (1971).