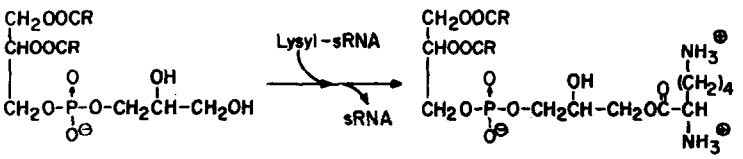
BIOSYNTHESIS OF AMINOACYL DERIVATIVES OF PHOSPHATIDYLGLYCEROL

Robert M. Gould* and W. J. Lennarz+
Department of Physiological Chemistry
The Johns Hopkins University School of Medicine
Baltimore, Maryland

Received January 22, 1967

Recent studies (Lennarz, et al., 1966; Lennarz, 1966) have demonstrated that the enzymatic synthesis of lysyl phosphatidylglycerol (lysyl-PG) in Staphylococcus aureus involves the transfer of a lysyl moiety from lysyl-sRNA to phosphatidylglycerol(PG), as shown below.‡



In view of reports on the occurrence of several different aminoacyl derivatives of PG in a variety of bacteria (cf. Macfarlane, 1964; Lennarz, in press), we have undertaken a more extensive study of the possible participation of aminoacyl derivatives of sRNA in the formation of these compounds. The results of this study indicate that:(1) the enzymatic synthesis of lysyl-PG from lysyl-sRNA occurs in a variety of microorganisms; (2) certain organisms have the enzymatic capacity to synthesize at least two different aminoacyl derivatives of PG from the appropriate aminoacyl sRNA derivatives.

Materials and Methods

Phosphatidylglycerol was isolated from S. aureus. Synthetic alanyl phosphatidylglycerol (alanyl-PG) was a gift from Dr. L.L.M. van Deenen. U-C^{14}-L-Amino acids were purchased from New England Nuclear Corp. With the exceptions noted below, all organisms were grown to late log phase in the following medium: 1% Bactopeptone, 1% yeast extract,

---

* Predoctoral recipient of Training Grant GM-00184

+ Lederle Medical Faculty Awardee

‡ The position of ester linkage in lysyl-PG has tentatively been assigned to the 3'-hydroxyl group of the glyceryl moiety (Bonsen, van Deenen and Lennarz, unpublished studies).

0.5% NaCl and 0.04% Na_2HPO_4, final pH adjusted to 7.2. S. aureus (Lennarz, et al., 1966), C. welchii (Cassidy, et al., 1965) and S. faecalis (ATCC 9790) (Vorbeck and Marinetti, 1965a) were cultivated as previously described. The particulate cell fraction containing aminoacyl-PG synthetase activity was obtained as previously reported (Lennarz, et al., 1966), except that the cells were ruptured by sonication.

All aminoacyl derivatives of sRNA were prepared with crude E. coli supernatant enzyme and E. coli sRNA (von Ehrenstein, in press).* Aminoacyl-PG synthesis was assayed as previously reported (Lennarz, et al., 1966). The water-soluble products resulting from acid hydrolysis (6 N HCl at 100° for 4-8 hrs) of the aminoacyl lipids were characterized by paper electrophoresis and paper chromatography. The two electrophoretic systems employed were: I. Plate electrophoresis on Whatman 3 MM for 1-1.3 hr at 100 v/cm (2.5% formic acid, 7.8% acetic acid, pH 1.85); II. Coolant bath electrophoresis on Whatman 3 MM for 1.75-1.9 hr at 6000 v (2.5% formic acid, 8.7% acetic acid, pH 1.90). Under the former conditions only lysine and alanine were completely resolved from all the other amino acids; the latter conditions gave resolution of all 14 amino acids employed in this study except leucine and isoleucine. Paper chromatography was performed on Whatman 1 impregnated with either (A), 0.067 M PO_4, pH 6.2, or (B), 0.067 M H_3BO_3-0.067 M KCl adjusted to pH 8.4. The developing solvent was phenol saturated with the same buffer (McFarren, 1951).

Results and Discussion

Studies with Alanyl- and Lysyl-sRNA. In view of previous reports on the occurrence of alanyl-PG, as well as lysyl-PG in C. welchii (Macfarlane, 1962), a preliminary study was undertaken of the enzymatic synthesis of these compounds in C. welchii, as well as in a variety of other bacteria. As shown in Table I, the particulate fractions obtained from extracts of four of the organisms tested formed lysyl-PG from added lysyl-sRNA, whereas only one, C. welchii, formed alanyl-PG as well as lysyl-PG from the corresponding aminoacyl sRNA derivatives. Enzymatic formation of neither of these lipids was detected in extracts of E. coli, S. lutea, or M. lysodeikticus. The enzyme system catalyzing the formation of alanyl-PG in C. welchii has the same general properties as the lysyl-PG synthetase previously studied in S. aureus; maximal synthesis of alanyl-PG requires exogenous PG and intact alanyl-sRNA (Table II). Enzymatically formed C^{14}-alanyl-PG was isolated from large scale incubations and shown to be chromatographically identical with synthetic alanyl-PG in three different thin layer chromatographic systems (Lennarz,

* Unpublished studies indicate that, at least in S. aureus, lysyl-PG synthetase does not show species specificity with regard to the source of lysyl-sRNA.

Table I

Survey of Several Organisms for Lysyl- and Alanyl-PG Synthetase Activity

Organism	LysylPG (μμmoles formed/mg protein)	AlanylPG (μμmoles formed/mg protein)
Staphylococcus aureus	369	< 1
Bacillus megaterium KM	128	< 1
Bacillus cereus (ATCC 9139)	118	< 1
Clostridium welchii (ATCC 3624)	194	213
E. coli B	< 1	< 1
Sarcina lutea (ATCC 533)	< 1	< 1
Micrococcus lysodeikticus (ATCC 4698)	< 1	< 1

Incubation at 30° for 30 min with 0.5-1.25 mg of particulate enzyme and (in μmoles) Tris-maleate, pH 7.0, 5.0; Tris·HCl, pH 7.0, 1.0; KCl, 100; fatty acid salt, 0.6; S. aureus PG, 0.425; and C^{14}-lys-sRNA (368 μμmoles) or C^{14}-ala-sRNA (304 μμmoles) in a final volume of 0.4 ml.

Table II

Requirements for Alanyl-PG Synthesis by C. welchii Particulate Fraction

Conditions	Alanyl-PG cpm	Alanyl-PG μμmoles
Complete system	6210	250
" " , minus PG	2475	100
" " , boiled enzyme	30	0.12
" " , but preincubated with 1 μg RNase	88	0.35
" " , minus C^{14}-ala-sRNA plus C^{14}-alanine	20	0.08

Conditions as in Table I except that 1.25 mg of protein and 304 μμmoles of C^{14}-ala-sRNA or 800 μμmoles of C^{14}-alanine were used. Preincubation with RNase was performed for 5 min at 30° in the absence of PG and particulate enzyme.

et al., 1966). The radioactive product obtained by acid hydrolysis of the C^{14}-lipid was identified as alanine by electrophoresis (System I, $R_{lys} = 0.67$) and paper chromatography (System A, $R_f = 0.45$). Treatment of C^{14}-alanyl-PG with neutral hydroxylamine (Lennarz, et al., 1966) yielded C^{14}-alanine hydroxamate, as identified by electrophoresis (System I, $R_{lys} = 0.87$) and by paper chromatography (System A, $R_f = 0.14$).

Studies with aminoacyl-sRNA. These findings with lysyl- and alanyl-sRNA prompted a more extensive investigation of the possible formation of other aminoacyl derivatives of PG. sRNA was charged with a mixture of 14 amino acids of high specific activity (40 μc/μatom), and a portion of the resulting aminoacyl-sRNA mixture was hydrolyzed and analyzed by electrophoresis. The following C^{14}-amino acids were found to be covalently

linked to sRNA: Alanine, arginine, aspartate, glutamate, histidine, isoleucine and/or leucine, lysine, phenylalanine, proline, serine, threonine, tyrosine and valine.

The ability of extracts of various organisms to catalyze the incorporation of aminoacyl groups from the aminoacyl-sRNA mixture into lipid was tested as described in Table I except that 112,000 cpm of aminoacyl-sRNA was added. An extract of S. faecalis, an organism known to have aminoacyl lipids (Vorbeck and Marinetti, 1965), was also included in this survey. All preparations except those of E. coli, S. lutea and M. lysodeikticus were active in aminoacyl lipid synthesis, incorporation ranging from 6920 to 13,800 cpm. Boiled enzyme controls gave values ranging from 400 to 900 cpm. In order to characterize the amino acids incorporated into the lipids, the enzymatically formed C^{14}-lipids were isolated, hydrolyzed, and subjected to electrophoresis. The results obtained with B. cereus extract are shown in Fig. 1. Only lysine was significantly incorporated into the lipids. The small amount of radioactivity evident in the region where 10 amino acids

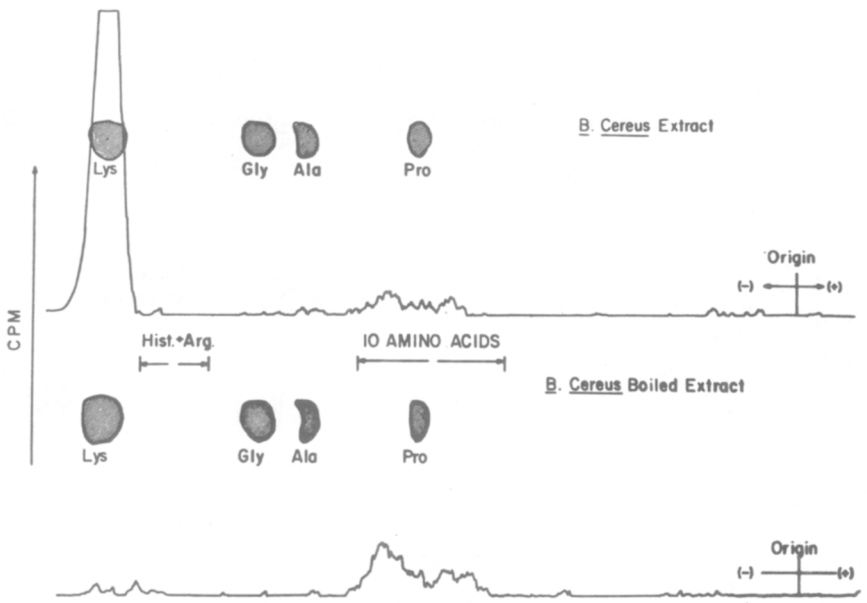


Fig. 1. Electrophoretogram (System 1) of the amino acids incorporated into lipids by the particulate fraction of B. cereus.

* Only a low level of C^{14}-histidinyl-sRNA was present. The levels of the various C^{14} aminoacyl-sRNA's differed, but were of the same order of magnitude. However, in view of these variations, the results on aminoacyl phospholipid synthesis can only be considered qualitatively.

are found under these electrophoretic conditions evidently was not due to aminoacyl phospholipid synthesis, inasmuch as the same region contained radioactivity when the "products" of the boiled extract were analyzed by electrophoresis. Similar results were obtained upon electrophoretic analysis of the hydrolysates of the aminoacyl lipids formed by extracts of S. aureus, B. megaterium and C. welchii. Extracts from all three organisms catalyzed the formation of lysyl-PG; in addition, C. welchii extract produced alanyl-PG. These results complement the earlier studies with lysyl- and alanyl-sRNA; moreover, they demonstrate the high specificity of the reaction for these two aminoacyl sRNA derivatives under the assay conditions employed.

However, studies with S. faecalis have revealed that extracts of this organism catalyze the synthesis of one or more new aminoacyl lipids. Electrophoretic and paper chromatographic analysis of the products revealed lysine and a second amino acid with mobility identical to that of arginine (Fig. 2).* In the above experiments incubation of the various extracts and the aminoacyl-sRNA mixture were performed at pH 7.0. Preliminary studies on the effect of pH on aminoacyl lipid synthesis with extracts of S. faecalis indicate that low pH (5.0-5.5) slightly favors the synthesis of arginyl lipid; moreover, under these conditions two other aminoacyl lipids are formed in small amounts. One of these has been tentatively identified as an alanyl lipid; the other remains unidentified. It should be noted

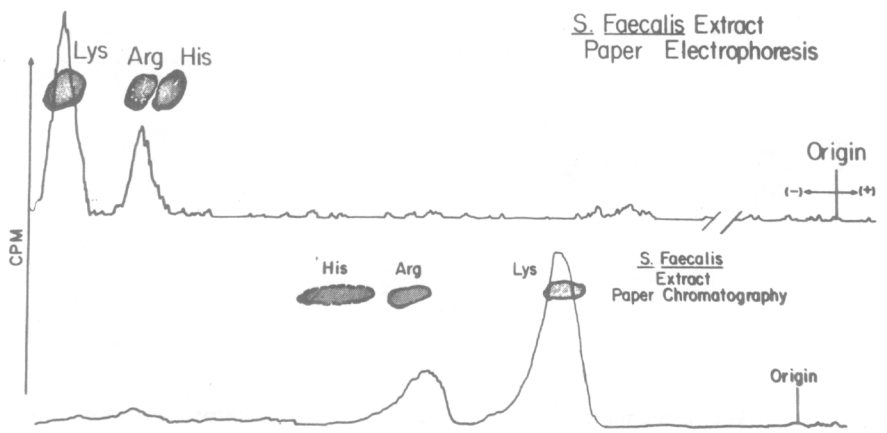


Fig. 2. Electrophoretogram (System II) and paper chromatogram (System B) of the amino acids incorporated into lipid by extracts of S. faecalis at pH 7.0.

* These results on arginyl lipid formation using a mixture of C^{14}-aminoacyl-sRNA derivatives have been confirmed in experiments with C^{14}-arginyl-sRNA.

that the aminoacyl lipids enzymatically formed in extracts of S. faecalis have not yet been characterized. Although it is reasonable to assume that they are aminoacyl derivatives of PG, this fact remains to be established.

Two observations indicate that further study will be necessary to define all the factors controlling the enzymatic formation of the various aminoacyl lipids: (a) aminoacyl lipid synthesis could not be detected in extracts of S. lutea, an organism known to contain these compounds (Huston, et al., 1965); (b) the type of aminoacyl lipid formed in vitro can vary with the incubation conditions employed. Nevertheless, the results reported in this paper indicate that aminoacyl-sRNA can participate in the synthesis of a variety of aminoacyl lipids.

Acknowledgment. This work was supported by USPHS Grant AI-06888-01.

References

Cassidy, J. T., Jourdian, G. W. and Roseman, S., J.Biol.Chem., 240, 3501 (1965).

Huston, C. R., Albro, P. W. and Grindey, G. B., J.Bacteriol., 89, 768 (1965).

Lennarz, W. J., in Paoletti, R. and Kritchevsky, D., (eds.), Advances in Lipid Research, 4, Academic Press, New York, in press.

Lennarz, W. J., Nesbitt, J. A., III, and Reiss, J., Proc.Nat.Acad.Sci., 55, 934 (1966).

Lennarz, W. J., Abstracts of 152nd Meeting of Am.Chem.Soc., New York, 1966, p. 153.

Macfarlane, M. G., in Paoletti, R. and Kritchevsky, D., (eds.), Advances in Lipid Research, 2, p. 91, Academic Press, New York, 1964.

Macfarlane, M. G., Nature, 196, 136 (1962).

McFarren, E. F., Anal. Chem., 23, 168 (1951).

von Ehrenstein, G., in Colowick, S. P. and Kaplan, N. O., (eds.), Methods in Enzymology, Academic Press, New York, in press.

Vorbeck, M. L. and Marinetti, G. V., J. Lipid Research, 6, 3 (1965a).

Vorbeck, M. L. and Marinetti, G. V., Biochem., 4, 296 (1965).