SYNTHESIS OF SERINE BY COXIELLA BURNETII

W. F. MYERS and D. PARETSKY

Department of Bacteriology, University of Kansas, Lawrence, Kansas

Received for publication May 15, 1961

ABSTRACT

MYERS, W. F. (University of Kansas, Lawrence) and D. PARETSKY. Synthesis of serine by Coxiella burnetii. J. Bacteriol. 82:761-763. 1961.—Enzyme preparations from suspensions of purified Coxiella burnetii are capable of synthesizing serine from formaldehyde and glycine-2-C\textsuperscript{14} in the presence of tetrahydrofolic acid. Evidence for the reaction is obtained by isolating and identifying radioactive serine and serine derivatives. The data suggest a role for folic acid in these rickettsiae, and extend the knowledge of host-independent biosynthetic competencies of these highly parasitic microorganisms.

Coxiella burnetii, the rickettsial agent of Q fever, has been shown to possess several enzyme systems (Paretsky et al., 1958; Consigli, Paretsky, and Downs, 1960), many in common with other rickettsiae (see review by Cohn, 1960). The demonstration by Myers (1958) of significant amounts of folic acid in C. burnetii suggested a study of the ability of this rickettsia to conduct reactions catalyzed by the folic acid series. Because of the fundamental importance of one-carbon transfers in living cells and the desirability of uncovering additional anabolic systems in rickettsiae, the hydroxymethylation of glycine to form serine was investigated. This reaction has been extensively studied in animal and microbial tissue, but has not been shown to occur in rickettsiae.

MATERIALS AND METHODS

Organism. C. burnetii, Nine Mile strain, was cultivated in embryonated eggs, harvested, and purified as previously described (Paretsky et al., 1958), with the following modifications: Prior to homogenization, 100 g of infected yolk sacs were suspended in 100 ml of sucrose-phosphate-glutamate solution, 0.7 m with respect to sucrose. All subsequent operations were performed in diluents prepared as previously described, except that they were 0.7 m with respect to sucrose. Finally, the rickettsiae were resuspended in 25 ml of 0.25 m sucrose, dispensed into screw-capped tubes, shell-frozen and stored in the dry-ice box. The rickettsial suspensions used in the present work had an LD\textsubscript{50} of 10\textsuperscript{-4.5} in 5-day-old embryonated eggs.

Enzyme suspension. Purified suspensions of rickettsiae were subjected to sonic oscillation for 45 min in a Raytheon 9 kc sonic oscillator operated at a plate voltage of 120 v; the suspension was then used as the enzyme source without further treatment.

Enzyme controls. Aliquots of the suspension placed in a boiling-water bath for 10 min were designated as “inactivated” enzyme. Yolk sacs of uninfected eggs underwent the same cycles of treatment used in extracting and purifying the rickettsiae.

THFA. One sample of tetrahydrofolic acid (THFA) was prepared from folic acid by the methods of Kisliuk (1957), and another was kindly supplied by F. Huennekens of the University of Washington. The THFA was stored under N\textsubscript{2}.

Formaldehyde. HCHO, analytical reagent grade 37%, was refluxed for 2 hr in 0.3 m H\textsubscript{3}PO\textsubscript{4} and distilled; the 97 C fraction was collected. HCHO was determined iodimetrically.

ATP-pyridoxal phosphate solution. This solution was 0.033 m with respect to adenosine triphosphate (ATP), 0.017 m with respect to pyridoxal phosphate, and 0.1 m with respect to tris(hydroxymethyl)aminomethane (tris) buffer, pH 7.2.

THFA-HCHO solution. This solution was 0.1 m with respect to HCHO, 0.014 m with respect to THFA, and contained 2 mg per ml potassium ascorbate.

Serine. Serine was separated from glycine by several procedures: (i) Serine was obtained by
four fractional crystallizations of the aqueous glycine-serine solution at 0 C, and finally by alcohol precipitation (Sickevitz and Greenberg, 1949). The tosyl derivative was prepared from a portion of the recrystallized serine (Cheronis and Entrikin, 1957). (ii) Chromatographic separation and identification was accomplished on Whatman no. 1 paper using a solvent mixture of n-butanol, methyl-ethyl-ketone, water, and ammonia (5:3:3:1) and the descending technique (Wolfe, 1957). (iii) Serine was isolated by ion-exchange chromatography, employing Dowex AG-50W-X5 resin, minus 400 mesh, hydrogen form, in a column (33 by 2.5 cm). The resin was prepared for use by successive washings of 1 N HCl, H2O, 1 N NaOH, H2O, 1 N HCl, and H2O. Serine was eluted from the column with 1 N HCl; the serine fractions were evaporated to dryness in vacuo. The serine was dissolved in water and the solution again evaporated.

Glycine-2-C14, Glycine-2-C14 with a specific activity of 49 μc per mg was obtained from Tracerlab, Inc., and was diluted with unlabeled glycine to 1 mg glycine containing 2 × 10^4 count/min per μmole.

RESULTS

Three 15-ml capped serum bottles were flushed with N2. To each bottle was added 0.5 ml of 1 M glycine 2-C14, 0.5 ml of the THFA-HCHO solution, 0.3 ml of ATP-pyridoxal phosphate mixture, and 0.7 ml of 0.1 M tris buffer, pH 7.2. To the first bottle was added 3.0 ml of normal yolk sac that had been treated by sonic oscillation; to the second, 3.0 ml boiled C. burnetii that had been treated by sonic oscillation, and to the third bottle, 3.0 ml of the C. burnetii enzyme preparation. The C. burnetii preparations contained 0.53 mg N/ml, whereas the normal yolk sac had no detectable N. All additions were made under N2. The vessels were incubated for 2 hr at 35 C with constant shaking, and the reactions stopped by the addition of 0.5 ml of 40% trichloroacetic acid. After centrifuging the precipitated protein, the solutions were evaporated to dryness. Carrier D,L-serine was added as follows: vessel 1, 444.8 mg; vessel 2, 453.0 mg; vessel 3, 465.8 mg; 2 ml of hot water were added to each vessel to dissolve the serine. Serine was separated from glycine by fractional crystallization as described. p-Toluenesulfonates were prepared directly from the recovered recrystallized serine, and had corrected melting points of 212 to 214, 214 to 216, and 213 to 214 C for vessels 1, 2, and 3, respectively. The p-toluenesulfonates prepared from serine recovered by recrystallization; C, prepared from serine recovered by ion-exchange chromatography. Sample counts are corrected for background and self-absorption.

The data presented in Table 1 show that glycine is converted to serine by C. burnetii preparations. From an average value of 18 × 10^4 counts/min of the p-toluenesulfonates and glycine substrate with 2 × 10^4 counts/min, it may be assumed that about 9 μmoles of serine were synthesized in this experiment, or about 18 μmoles per mg rickettsial protein.

A second experiment employed identical substrate conditions and duplicate vessels, but a sonically treated solution containing 0.55 mg per ml rickettsial N was used. The recovered recrystallized serine was chromatographed in duplicate on paper, by use of 0.25 mg serine. One strip was sprayed with ninhydrin to reveal the position of the serine; the Ioci were excised from the duplicate strips, and eluted with water. The eluates were dried and counted, and showed 794 and 611 count/hr above background. Serine from the control vessels showed 0 count/min above background.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Vessel</th>
<th>Count/min</th>
<th>Count/min corrected for boiled control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serine</td>
<td>1</td>
<td>22,568</td>
<td>22,568</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>29,364</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>92,349</td>
<td></td>
</tr>
<tr>
<td>p-Toluene-sulfonate S</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>16,292</td>
<td>16,292</td>
</tr>
<tr>
<td>p-Toluene-sulfonate C</td>
<td>1</td>
<td>3,208</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3,806</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>24,066</td>
<td>20,260</td>
</tr>
</tbody>
</table>

Vessel 1, normal yolk sac; 2, boiled enzyme; 3, enzyme system. p-Toluenesulfonate S, prepared from serine recovered by recrystallization; C, prepared from serine recovered by ion-exchange chromatography. Sample counts are corrected for background and self-absorption.
DISCUSSION

The THFA-dependent hydroxymethylation of glycine has been demonstrated in a wide variety of animal tissues and microorganisms (see especially Kisliuk and Sakami, 1955; Blakely, 1954; Rabinowitz, 1960).

The demonstration of folate acid in rickettsiae (Kleinschmidt, Holmes, and Behrens, 1956; Myers, 1958) and in the psittacosis group (Colon and Moulder, 1958; Colon, 1961) is suggestive of the presence of folate acid-mediated reactions in these organisms. The data presented in this paper support this view, at least for C. burnetii. The incorporation of labeled glycine into typhus rickettsiae protein (Bovarnick and Schneider, 1960) likewise tends to support this position, although the labeled moiety of the protein was not determined. The evidence presented here for serine biosynthesis in C. burnetii extends the existing information on the anabolic competencies of supposedly obligate intracellular parasites (Paretsky et al., 1958; Bovarnick, Schneider, and Walter, 1959; Fujita, Kohno, and Shishido, 1959; Schmidt et al., 1961). The further roles of both THFA and glycine in synthesis of rickettsial purines is now under investigation (Schmidt et al., 1961). The presence of vitamins in rickettsiae and related parasitic organisms could serve as a guide to establishing other as yet unde demonstrated reactions, and thus may aid in elucidating the biochemical nature of rickettsial parasitism.

ACKNOWLEDGMENTS

The authors are grateful to F. Huennekens of the University of Washington for a gift of THFA, to Fred Jones of the University of Kansas for synthesis of THFA, and to Karen R. New for her valuable technical assistance.

Supported by grants from the Office of Naval Research [Nonr 538 (10)], the National Institutes of Health (E1338), and The National Science Foundation (G11466).

LITERATURE CITED


