Reactions of Second Stage of Biosynthesis of Teichuronic Acid of Micrococcus lysodeikticus Cell Walls*

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A polysaccharide of alternating glucose and N-acetylmannosaminuronic acid residues is formed in the second stage of teichuronic acid biosynthesis by the particulate enzyme fraction of Micrococcus lysodeikticus. Di(N-acetylmannosaminuronyl)-N-acetylglucosaminyl carrier lipid, the third intermediate formed in the initial stage of teichuronic acid synthesis, serves as an acceptor for the sequential and alternating addition of glucose residues from uridine diphosphate glucose and N-acetylmannosaminuronic acid residues from uridine diphosphate N-acetylmannosaminuronic acid. Glucosyl-di(N-acetylmannosaminuronyl)-N-acetylglucosaminyl carrier lipid and N-acetylmannosaminuronylglucosyl-di(N-acetylmannosaminuronyl)-N-acetylglucosaminyl carrier lipid are intermediates formed by the action of the glucosyltransferase and the N-acetylmannosaminuronyltransferase, respectively. Both uridine diphosphate glucose and uridine diphosphate N-acetylmannosaminuronic acid produce uridine diphosphate in an amount stoichiometric with the incorporation of monosaccharide residues into teichuronic acid.

The transferases which catalyze the second stage of teichuronic acid synthesis are solubilized from the particulate enzyme fraction by Triton X-100. The glucosyltransferase can be selectively released at 5 to 10 mM magnesium ion, whereas at a concentration of 1 mM or less the N-acetylmannosaminuronyltransferase is also solubilized.

The in vitro biosynthesis of teichuronic acid by the particulate enzyme fraction from Micrococcus lysodeikticus requires uridine diphosphate N-acetyl-D-glucosamine, uridine diphosphate N-acetyl-D-glucosamine, and uridine diphosphate N-acetyl-D-glucosamine, respectively. Both uridine diphosphate glucose and uridine diphosphate N-acetylmannosaminuronic acid produce uridine diphosphate in an amount stoichiometric with the incorporation of monosaccharide residues into teichuronic acid.

The in vitro biosynthesis of teichuronic acid by the particulate enzyme fraction from Micrococcus lysodeikticus requires uridine diphosphate N-acetyl-D-glucosamine, uridine diphosphate N-acetyl-D-glucosamine, and uridine diphosphate N-acetyl-D-glucosamine, respectively. The biosynthesis occurs in two stages. In the first stage, UDP-GlcNAc, UDP-ManNAcUA, and a carrier lipid which is undecaprenol monophosphate are utilized in the formation of three intermediates the last of which is (ManNAcUA),GlcnAc carrier lipid (1). This paper shows that the second stage of teichuronic acid synthesis requires (ManNAcUA),GlcnAc carrier lipid, UDP-glucose, and UDP-ManNAcUA for the formation of the polymer of alternating residues of glucose and N-acetylmannosaminuronic acid. (ManNAcUA),GlcnAc carrier lipid serves as an acceptor for the sequential transglycosylation of glucose from UDP-glucose and N-acetylmannosaminuronic acid from UDP-ManNAcUA. This paper also describes the solubilization of the glycosyltransferases involved by extraction of the particulate enzyme fraction with Triton X-100.

EXPERIMENTAL PROCEDURES

Substrates - [14C]UDP-glucose was prepared by an exchange reaction of UDP-glucose with [14C]IMP catalyzed by the particulate enzyme fraction of Micrococcus lysodeikticus (4). [14C]UDP-[3H]glucose was a mixture of [14C]UDP-glucose and a commercial preparation of UDP-[3H]glucose. All other substrates were previously described (1, 2).

Particulate Enzyme Preparation - Cells of M. lysodeikticus ATCC 4698 harvested from 12 liters of midlog phase culture (grown with gyratory shaking at 37° in medium of 10 g of peptone and 5 g of NaCl/liter) were washed in a solution of 1 volume of 0.05 M Tris/acetate, pH 8.2, 1 mM magnesium acetate, 2 mM 2-mercaptoethanol (TMM buffer), and 1 volume of 0.1 M NaCl. The washed cells were resuspended in 200 ml of TMM buffer and digested for 2 h at room temperature with 50 mg of lysozyme, and 0.2 mg each of deoxyribonuclease and ribonuclease. The particulate enzyme fraction was recovered by centrifugation for 1 h at 48,000 x g, washed three times with water and recovered each time by centrifugation.

Reaction Mixtures and Assay Procedures - Component C was produced in reaction mixtures of 0.4 mM UDP-GlcNAc, 0.4 mM UDP-ManNAcUA, 50 mM Hepes, pH 8.2, 20 mM magnesium acetate, 2 mM 2-mercaptoethanol, and particulate enzyme fraction (5 to 10 mg of protein/ml) incubated at 37° usually for 3 h. Reaction mixtures of 50 ml were diluted to 1 ml with water and centrifuged 10 min at 12,000 x g to sediment the Component C enzyme complex. The pellet was washed to remove residual substrates by resuspension in 1 ml of water and centrifugation to recover the Component C enzyme complex.

Teichuronic acid was produced in reaction mixtures of 0.4 mM UDP-GlcNAc, 0.4 mM UDP-ManNAcUA, 50 mM Hepes, pH 8.2, 20 mM magnesium acetate, 2 mM 2-mercaptoethanol, and particulate enzyme fraction (5 to 10 mg of protein/ml) incubated at 37° usually for 3 h. Reaction mixtures of 50 ml were diluted to 1 ml with water and centrifuged 10 min at 12,000 x g to sediment the Component C enzyme complex. The pellet was washed to remove residual substrates by resuspension in 1 ml of water and centrifugation to recover the Component C enzyme complex.

Teichuronic acid was produced in reaction mixtures of 0.4 mM UDP-GlcNAc, 0.4 mM UDP-ManNAcUA, 50 mM Hepes, buffer solution of 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid and its sodium salt.
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UDP-ManNAcUA, 0.4 mM UDP-glucose, 50 mM Hepes, pH 8.2, 20 mM magnesium acetate, 2 mM 2-mercaptoethanol, and Component C enzyme complex (5 to 10 mg of protein/ml) by incubation at 37°C for the times indicated. Special alterations of reaction conditions and methods of analysis are described in the figure legends.

Reaction mixtures were inactivated with a 0.4 volume of isobutyl alcohol and subjected to paper chromatography in solvent System A. Dried chromatograms were either scanned with a radiochromatogram scanner or cut into 1-cm segments for quantitative determination. These results indicate that UDP-ManNAcUA, UDP-glucose, and Component C (but not UDP-GlcNAc) are required for the reactions of the second stage.

Scintillation solution A (4 g of 2,5-diphenyloxazole in 1 liter of toluene) was added dropwise to 20% to slightly beyond the equivalence point (as indicated by color change from green to yellow) followed by incubation at room temperature for 20 min. Methylated derivatives were recovered by partitioning between chloroform and water. The organic phase was concentrated to a syrup under reduced pressure below 40°C.

The permethylated materials were hydrolyzed by the method of Garegg and Lindberg (7). The syrup was dissolved in 72% sulfuric acid, and after 30 min at room temperature, 8 volumes of water were added to adjust the sulfuric acid concentration to 8%. After heating for 4 h at 100°C, the hydrolysate was neutralized with BaCO₃. BaSO₄ was removed by centrifugation and washed with methanol. The supernatants were pooled and concentrated. The methylated products were applied to Silica Gel G thin layer plates and developed twice in benzene:absolute ethanol:water:NH₄OH (200:47:15:1, v/v/v/v). The plates were scanned to locate radioactive compounds and subsequently incubated with UDP-ManNAcUA and UDP-glucose, yielding a Component C from teichuronic acid. Incubation of the particulate enzyme fraction with [G-3H]UDP-ManNAcUA and UDP-GlcNAc for 180 min to generate Component C. The addition of UDP-glucose is attributed to the incorporation of N-acetylmannosaminuronic acid into incomplete chains of teichuronic acid present in the particulate enzyme fraction.

Following addition of UDP-glucose, N-acetylmannosaminuronic acid incorporation was equimolar with the incorporation of [3H]glucose in a companion reaction mixture which was identical except that the labeled substrate was UDP-3H]glucose. Control reactions in which the initial stage requirements for the synthesis of Component C were not fulfilled did not permit extensive incorporation of N-acetylmannosaminuronic acid into the second stage of synthesis.

In a similar experiment, UDP-GlcN[14C]Ac was incubated with UDP-ManNAcUA and particulate enzyme fraction for 180 min to generate Component C. The addition of UDP-glucose at the beginning of the second incubation period resulted in a small but measurable incorporation of N-acetylglucosamine residues into chromatographically immobile product concomitant with the beginning of the second stage of teichuronic acid synthesis (Fig. 2, O—O). The extent of N-acetylglucosamine incorporation was about 2.5% of that observed for N-acetylmannosaminuronic acid and glucose under comparable conditions.

Component C is Acceptor for Synthesis of Teichuronic Acid - To demonstrate that Component C serves as an acceptor in the synthesis of teichuronic acid, radioactively labeled Component C was shown to assume the separation characteristics of teichuronic acid. Two separation methods gave the same result. Paper chromatography in solvent System A resolved Component C into a band (Rf, 0.6) from teichuronic acid (immobile). When [3H]ManNAcUA labeled Component C, prepared as its complex with the particulate enzyme fraction, was incubated with UDP-ManNAcUA and UDP-glucose, and subsequently subjected to paper chromatography, the radioactivity was retained at the origin. When either UDP-ManNAcUA or UDP-glucose was omitted from the incubation mixture, no radioactivity was found at the origin. Similar results have been obtained with [3H]GlcNAc-labeled Component C. Gel filtration in the presence of detergent also separated Component C from teichuronic acid. Incubation of the particulate enzyme complex, containing [14C]ManNAcUA-labeled Component C with UDP-ManNAcUA and UDP-glucose, yielded a product which shifted with reference teichuronic acid on Sepharose 6B (Fig. 3). Omission of either UDP-ManNAcUA or UDP-glucose prevented the label of Component C from assuming the elution properties of teichuronic acid.
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Sequential Addition of Glucose and N-Acetylmannosaminuronic Acid to Component C—Since Component C is formed in reaction mixtures which contain UDP-ManNAcUA but lack UDP-glucose, the first step in the second stage of teichuronic acid synthesis should utilize UDP-glucose. To demonstrate sequential addition of first a glucose residue and then an N-acetylmannosaminuronic acid residue to Component C, the following three reaction mixtures were prepared. In reaction mixture A, the particulate enzyme fraction was incubated with UDP-GlcNAc and \([G^\text{14}C]\)UDP-ManNAcUA to make \([G^\text{14}C]\)GlcNAc-ManNAcUA-labeled Component C. In reaction mixture B, unlabeled Component C was prepared and subsequently incubated with only UDP-[\text{14}C]glucose. In reaction mixture C, the two initial incubations were the same as in reaction mixture B but with unlabeled substrates and the final incubation was with only [\text{14}C]UDP-ManNAcUA. Following each incubation whether with unlabeled or with labeled substrates, the product particulate enzyme complex was recovered by sedimentation, resuspended, and again recovered by sedimentation to remove residual substrates. Each labeled complex was subjected to paper chromatography in solvent System A, and the radioactivity was determined at the origin.

Fig. 1. Incorporation of N-acetylmannosaminuronic acid residues. Reaction mixtures for the first incubation period (A, 0 to 180 min) contained particulate enzyme fraction (1 mg/ml) and the following substrates: O-O, UDP-GlcNAc and [G-\text{14}C]UDP-ManNAcUA (1900 cpm/nmol, 62% of the radioactivity in the ManNAcUA moiety); Δ—Δ, UDP-glucose and [G-\text{14}C]UDP-ManNAcUA; ▲—▲, [G-\text{14}C]UDP-ManNAcUA; ×—×, UDP-GlcNAc and [G-\text{14}C]UDP-ManNAcUA but with boiled particulate enzyme fraction; ●—●, UDP-GlcNAc and nonlabeled UDP-ManNAcUA (the radioactive substrate was UDP-[\text{14}C]glucose which was added at the start of the second incubation period). Substrates not already present were added at the beginning of the second incubation period (B, 180 to 240 min). During the second incubation period, all reaction mixtures contained all three substrates, UDP-GlcNAc, UDP-ManNAcUA, and UDP-glucose. Aliquots of the reaction mixtures were inactivated, subjected to paper chromatography in solvent System A, and the radioactivity was determined at the origin.

Fig. 2. Incorporation of N-acetylglucosamine residues. Reaction mixtures were similar to those of Fig. 1 except that the labeled substrate was UDP-GlcN[\text{14}C]Ac (7000 cpm/nmol). Reaction mixtures for the first incubation period (A, 0 to 180 min) contained UDP-GlcN[\text{14}C]Ac, particulate enzyme fraction (1 mg/ml), and the following substrates: O-O, UDP-ManNAcUA; ●—●, UDP-glucose; ×—×, UDP-ManNAcUA but with boiled particulate enzyme fraction; and Δ—Δ, none. Substrates not already present were added at the beginning of the second incubation period (B, 180 to 240 min). During the second incubation period, all reaction mixtures contained all three substrates, UDP-GlcNAc, UDP-ManNAcUA, and UDP-glucose.

Solubilization of Component C—[\text{3H}]GlcNAc-phosphoundecaprenol (Component D) and that of Reaction C is presumed to be ManNAcUA-GlcNAc-phosphoundecaprenol (Component E). The addition of N-acetylmannosaminuronic acid to Component C is dependent upon the prior addition of glucose, since little product of the mobility of Component E was observed in Reaction A which had been incubated with [G-\text{14}C]UDP-ManNAcUA.

Fig. 3. Incorporation of [\text{14}C]-labeled Component C into material having the same apparent molecular size as teichuronic acid as determined by gel filtration. Washed [\text{14}C]ManNAcUA-labeled Component C enzyme complex was added to reaction mixtures without any added substrates (Frame A), with UDP-ManNAcUA and UDP-glucose (Frame B), and with [G-\text{14}C]UDP-ManNAcUA and UDP-glucose (Frame B). After incubation for 1 h, the reaction mixtures were inactivated and totally solubilized with 1% sodium dodecyl sulfate, mixed with [\text{3H}]glucose-labeled teichuronic acid (Frames A and B, O–O), and applied to a Sepha-
rose 6B column which was equilibrated and developed with 0.1 M Tris/HCl, pH 8.2, 0.1 M LiCl, 0.5% sodium dodecyl sulfate. Radioactivity was determined in the effluent fractions by use of scintillation solution B and dual isotope liquid scintillation counting procedures.

Comparison of the chromatographic mobility of the major radioactive products with an internal marker of uracil indicates that the sequential addition of the labeled residues of glucose and N-acetylmannosaminuronic acid yielded products with progressively decreased mobility relative to Component C. The product of Reaction B is presumed to be Glc-(ManNAcUA)-GlcNAc-phosphoundecaprenol (Component D) and that of Reaction C is presumed to be ManNAcUA-Glc-(ManNAcUA)-GlcNAc-phosphoundecaprenol (Component E). The addition of N-acetylmannosaminuronic acid to Component C is dependent upon the prior addition of glucose, since little product of the mobility of Component E was observed in Reaction A which had been incubated with [G-\text{14}C]UDP-ManNAcUA.
with UDP [3H]GlcNAc and UDP ManNAcUA was recovered by sedimentation, washed to remove residual substrates, and heated 3 min at 100° to inactivate all enzymatic activity. Addition of Triton X-100 to a final concentration of 1% followed by a 60-min incubation at 37° solubilized Component C. Essentially all of the radioactivity remained in the supernatant when the residue of inactivated particulate enzyme fraction was sedimented. The soluble fraction displayed the typical pattern of Component C on paper chromatography in solvent System A. When solubilized Component C was added to a reaction mixture consisting of fresh particulate enzyme fraction, UDP-ManNAcUA, and UDP-glucose, the label of Component C was converted to chromatographically immobile material. This conversion was not observed if either UDP-ManNAcUA or UDP-glucose was omitted.

**Solubilization of Transferases Which Catalyze the Second Stage of Teichuronic Acid Biosynthesis** — Preliminary experiments indicated that the second stage of teichuronic acid biosynthesis could be demonstrated even in the presence of concentrations of Triton X-100 which totally solubilized the particulate enzyme fraction, indicating that the glucosyltransferase and the N-acetylmannosaminuryltransferase could be solubilized in an active form. The availability of solubilized Component C permitted the assay for the solubilized transferases. Soluble, unlabeled Component C was incubated with UDP-ManNAcUA, UDP-[14C]glucose, and the soluble enzyme fraction to be tested. These assay mixtures were analyzed by paper chromatography in solvent System A. Radioactivity was found in chromatographically immobile product if all enzymes necessary for the second stage of teichuronic acid synthesis were present (Fig. 5). The glucose phosphate present (Rf of 0.3) was a degradation product of UDP-glucose. Radioactive Component D was observed if the glucosyltransferase was solubilized but not the N-acetylmannosaminuryltransferase or if UDP-ManNAcUA was omitted from the assay. Similar results were obtained if Component C was labeled.

The particulate enzyme fraction was treated for 60 min at 37° with 5% Triton X-100 at various magnesium ion concentrations to solubilize the transferases which were then separated from the particulate residue by centrifugation for 30 min at 48,000 × g. Little solubilization was observed unless the magnesium ion concentration was 10 mm or less. The glucosyltransferase was solubilized by Triton X-100 with magnesium ion concentrations up to 10 mm. The N-acetylmannosaminuryltransferase was most effectively solubilized at magnesium concentrations of 1.0 mm or less. Between 2 and 10 mm magnesium ion, the glucosyltransferase was solubilized with minimal solubilization of the N-acetylmannosaminuryltransferase. Extractions to obtain both transferases were done in the absence of magnesium ion. Neither transferase required magnesium ion for activity.

**Identification of Component D — Solubilized 14C-labeled Component C incubated with UDP-[14C]glucose and solubilized glucosyltransferase was subjected to paper chromatography in solvent System A (Fig. 6). Approximately half of the Component C was converted to Component D as indicated by the peak of tritium at Rf of 0.5 which corresponds to the slower of the incompletely resolved peaks of 14C. Gel filtration on a column of Sephadex G-50 equilibrated with 0.1% Triton X-100, confirmed that the tritium was incorporated into Component D. Component C eluted near the void volume of Sephadex G-50 whereas nucleotides and monosaccharides were included. All of the 14C eluted at the void volume as did an amount of tritium equivalent to that detected in Component D. The doubly labeled material excluded from Sephadex G-50 was treated with 2 N HCl at 100° for 120 min, a 14C-labeled compound was released which had the paper chromatographic mobility of glucose in solvent Systems A and C. Treatment of the doubly labeled material with conditions too mild to hydrolyze glycosidic bonds failed to release any 14C-glucose.

**Component D Is Acceptor for Teichuronic Acid Synthesis** — The application of permethylation procedures to 14C-glucose-labeled Component D and to teichuronic acid prepared from 14C-glucose-labeled Component D permitted determination of the degree of substitution of the glucose residue in each. 14C-glucose-labeled Component D was prepared from unlabeled, three-times washed Component C-enzyme complex by incubation with UDP 14C-glucose. A portion of the 14C-glucose-labeled Component D was converted to teichuronic acid by incubation with UDP-ManNAcUA and UDP-glucose after removal of the residual labeled substrate by sedimentation and washing of the Component D-enzyme complex. Following exhaustive methylation and hydrolysis, the methylated monosaccharides were separated by thin layer chromatography in benzene:absolute ethanol:water:NH4OH (200:47:15:1, v/v) which separates the tri-O-methyl-substituted monosaccharides from the tetra-O-methyl derivatives (Fig. 7). Of the labeled derivatives obtained from 14C-glucose-labeled

2 Substantial quantities of glucose phosphate have always been formed from UDP-glucose in incubation mixtures independently of the presence of any enzyme preparation. Glucose phosphate is generated by alkaline degradation of UDP-glucose with the elimination of UMP and the production of glucose 1,2-cyclic phosphate (2). The extent of degradation is decreased in the presence of EDTA.
Component D, 80% had the mobility of tetra-O-methyl glucose, whereas of those obtained from the \(^{14}C\)glucose-labeled teichuronic acid, 85% corresponded to tri-O-methyl glucose. These results indicate that the glucose residue of Component D is nonreducing terminal and that incorporation of Component D into teichuronic acid converts the glucosyl residue of Component D to an internal residue.

UDP-glucose and UDP-ManNAcUA Release UDP—In order to identify the reaction products containing the uridine moieties of UDP-glucose and UDP-ManNAcUA, substrates were utilized which were radioactively labeled in both the uridine and glucose moieties. The reaction products together with suitable reference compounds were resolved and identified by two-dimensional paper chromatography in solvent Systems A and B. \(^{14}C\)UDP-[\(^{3}H\)]glucose yielded \(^{3}H\)-labeled teichuronic acid and \(^{14}C\)UDP. The formation of UDP (3.0 nmol) was dependent upon conditions which permitted teichuronic acid synthesis and was stoichiometric with the quantity of glucose incorporated (4.2 nmol). Although 40% as much UMP was detected (1.6 nmol), its formation was not dependent upon teichuronic acid synthesis but was due to the nonenzymatic alkaline degradation of UDP-glucose to UMP and glucose cyclic phosphate. UDP was also the major uridine-containing product from \(^{14}C\)glucose-labeled Component D (H) were permethylated, hydrolyzed, and chromatographed on thin layer plates. Tracings of radiochromatograms scans are shown. The reference compounds are 2,3,4-tri-O-methyl glucose and 2,3,4,6-tetra-O-methyl glucose. The latter has the faster mobility.
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The reactions of the second stage of teichuronic acid synthesis require two substrates, UDP-glucose and UDP-ManNAcUA, as well as the final intermediate of the initial stage of synthesis, (ManNAcUA-Glc)-(ManNAcUA)-GlcNAc carrier lipid (Component C). The reactions of the second stage are summarized in Scheme 1. A comparison of the stoichiometry of incorporation of N-acetylglucosamine to N-acetylmannosaminuronic acid and glucose residues indicates that the value for n is about 40 (cf. Figs. 1 and 2). This value is the same as that reported for teichuronic acid isolated from cell walls (10).

Results reported here show that Component C labeled with either N-acetylglucosamine or N-acetylmannosaminuronic acid is incorporated into teichuronic acid under conditions appropriate for the second stage of teichuronic acid synthesis. Neither paper chromatography nor gel filtration in the presence of detergent separated the label introduced as Component C. The sequential addition of glucose and N-acetylmannosaminuronic acid residues suggests that each saccharide is incorporated 1 residue at a time alternately by two transferases. Each transferase has a specificity for the donor nucleoside and for the acceptor which must have the alternate saccharide as the nonreducing terminal residue. The solubilization of the glucosyltransferase and the N-acetylmannosaminuronyltransferase supports the concept of alternate addition of single residues. Complete verification must await purification of both transferases and demonstration that the two purified enzymes act sequentially. The experimental results eliminate an alternate mechanism by which a mixed disaccharide might form, possibly as a derivative of a carrier lipid, and subsequently polymerize to yield a heteropolysaccharide having a sequence of alternating saccharide residues.

The data presented here do not indicate whether the carrier lipid moiety of Component C (undecaprenol monophosphate) is incorporated into the final in vitro reaction product. If still attached to the carrier lipid, the in vitro product might be the precursor for the attachment of fully assembled polysaccharide onto the peptidoglycan to form cell wall which is the ultimate product of the biosynthetic pathway. Such a transfer reaction might not occur in the in vitro system catalyzed by the particulate enzyme fraction for lack of an appropriate final acceptor.

If the teichuronic acid formed in vitro is ultimately transferred from carrier lipid to peptidoglycan, the trisaccharide portion formed by the reactions of the initial stage of teichuronic acid synthesis must become part of a linkage region by which the polysaccharide of glucose and N-acetylmannosaminuronic acid is covalently attached to peptidoglycan. The structure of the linkage between teichuronic acid and peptidoglycan in native cell walls is not yet fully established. Nasiruddin and Jeanloz (10) have proposed attachment of teichuronic acid from a glucose residue through phosphate to C-6 of an N-acetylmuramic acid residue of peptidoglycan. In contrast, our results suggest that the teichuronic acid is attached through the saccharide residues of Component C. Two groups have reported an excess of hexosamine residues in the teichuronic acid isolated from cell walls following enzymatic digestion of the peptidoglycan (11, 12) which is consistent with the linkage region suggested by our biosynthetic studies.

REFERENCES

1. Rohr, T. E., Levy, G. N., Stark, N. J., and Anderson, J. S. (1977) J. Biol. Chem. 252, 3460-3465
2. Page, R. L., and Anderson, J. S. (1972) J. Biol. Chem. 247, 2471-2478
3. Anderson, J. S., Page, R. L., and Salo, W. L. (1972) J. Biol. Chem. 247, 2480-2485
4. Anderson, J. S., Matsushashi, M., Haskin, M. A., and Strominger, J. L. (1967) J. Biol. Chem. 242, 3180-3190
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5. Hakomori, S. I. (1964) *J. Biochem. (Tokyo)* 55, 205-208
6. Sandford, P. A., and Conrad, H. E. (1966) *Biochemistry* 5, 1508-1517
7. Garegg, P. J., and Lindberg, B. (1960) *Acta Chem. Scand.* 14, 871-876
8. Hay, G. W., Lewis, B. A., and Smith, F. (1963) *J. Chromatog.* 11, 479-486
9. Patterson, M. S., and Greene, R. C. (1965) *Anal. Chem.* 37, 854-857
10. Nasir-ud-Din, and Jeanloz, R. W. (1976) *Carbohydr. Res.* 47, 787-791
11. Campbell, J. N., Leyh-Bouille, M., and Ghuysen, J. M. (1969) *Biochemistry* 8, 193-200
12. Imanaga, Y., and Park, J. T. (1972) *Biochemistry* 11, 4006-4012
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