SYNTHESIS OF A D-α-LECITHIN, AN ACTIVE ANTIGEN COMPONENT IN THE SERODIAGNOSIS OF SYPHILIS

BY ERICH BAER AND FRANK MARTIN

(From the Banting and Best Department of Medical Research, Banting Institute, University of Toronto, Toronto, Canada)

WITH A SECTION BY R. H. ALLEN AND D. B. TONKS

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Although the serodiagnosis of syphilis by various tests has been carried out routinely for many years, our knowledge concerning the chemical nature of the specific antibody and antigen is still too limited to allow a satisfactory interpretation of the serological reactions involved. Valuable contributions towards the elucidation of the structure of several constituents of the antigen have been made during the past 10 years by Pangborn, who succeeded in purifying and identifying two of the components, cardiolipin (1) and lecithin (2), and more recently by Rice and Osler (3), who achieved the isolation of a chromatographically homogeneous, immunologically active lecithin from a commercially available beef heart lecithin prepared for serological tests.

Baer and Kates (4, 5) reported 2 years ago the synthesis of several constitutionally and configurationally pure L-α-lecithins. Subsequently Rosenberg (6), Kline (7), and Allen1 demonstrated that these synthetic lecithins, especially the L-α-(dimyristoyl) lecithin (7), can replace advantageously the “purified lecithin of beef heart” as an antigen component in the serodiagnosis of syphilis.2 It was deemed of interest to study the effect of structural variations in the lecithin molecule on its activity as an antigen component. Several relevant facts have recently emerged. We now know, for instance, that the antigen activity of lecithin does not change appreciably (6, 7) if the unsaturated fatty acids are replaced by saturated fatty acids of varying chain length (C_{18} → C_{18}), but that the activity disappears altogether if the phosphoric acid-choline ester linkage is broken. The serological significance of this linkage became apparent when attempts to replace the lecithin component of the antigen with the

1 Private communication from Dr. R. H. Allen, Department of National Health and Welfare, to the author.
2 The synthetic α-lecithins have the advantage of possessing a constant and predictable serological activity. At the instigation of the World Health Organization the synthetic α-lecithins are being subjected, in various serological laboratories, in England and on the continent, to extensive tests in order to estimate their practical value as an antigen component in the serodiagnosis of syphilis.
choline salt of the \( L-\alpha \)-(dimyristoyl) phophatidic acid (8) consistently failed to yield an active antigen. From a practical as well as a theoretical point of view it seemed of interest to ascertain whether or not the serological tests for syphilis possess specificity with regard to the configuration
of the lecithin component of the antigen. The necessary substrate for this investigation, which can be either a D or DL-α-lecithin, had to be obtained by synthesis, since neither the D nor DL form of α-lecithins seems to occur in nature.

In recent publications from this laboratory the synthesis of several L- and DL-α-lecithins (4, 5) has been described, and it was stated that the D isomers are obtainable by the same procedure, provided that L-acetone glycerol is used as starting material. Although the preparation of a D-α-lecithin would have entailed considerably less work (six steps) than that of a D-α-lecithin (twelve steps), a D-lecithin was selected as substrate because it is free from the serologically active L isomer and hence its use should give a more clear cut result. In view of the fact that of the three synthetic α-lecithins reported by us the L-α-(dimyristoyl) lecithin is the most satisfactory antigen component, its D isomer was synthesized for use in the present investigation. The reaction scheme and the following sequence of reactions summarize briefly the synthesis of D-α-(dimyristoyl) lecithin and illustrate the stereochemical relationships of the various intermediate compounds: mesquite gum —(1)→ L-arabinose (9) —(2)→ L-mannonic acid lactone (10) —(3)→ L-mannitol (11) —(4)→ 1,2,5,6-diacetone L-mannitol (11) —(5)→ acetone L-glyceraldehyde (11) —(6)→ L-acetone glycerol3 (11) —(7)→ L-acetone glycerol α-benzyl ether —(8)→ D-α-benzyl glycerol ether —(9)→ L-α,β-dimyristin α-benzyl ether —(10)→ L-α,β-dimyristin —(11a, b)→ dimyristoyl D-α-glycerylphenylphosphorylcholine chloride —(12)→ dimyristoyl D-α-glycerylphosphorylcholine (D-α-(dimyristoyl) lecithin). The D-α-(dimyristoyl) lecithin is the first lecithin of the D series to become known.

The serological evaluation of the D-α-(dimyristoyl) lecithin was carried out by Dr. R. H. Allen and Dr. D. B. Tonks of the Department of National Health and Welfare, Ottawa, both of whom graciously gave the author permission to report their results in this communication (see the experimental part). These investigators found that the two enantiomeric forms of the α-(dimyristoyl) lecithin possess virtually identical activity as antigen components in the Venereal Disease Research Laboratory (V. D. R. L.) slide flocculation test. The serological reaction thus is unspecific with regard to the configuration of the lecithin and for this reason it should be possible to use the more readily accessible synthetic DL-α-lecithins as antigen components.

EXPERIMENTAL

The synthesis of L-acetone glycerol (Steps 1 to 6) was carried out as described by Baer and Fischer (11). The L-α,β-dimyristin (Steps 7 to 10)
10) was obtained by the following procedure reported for the preparation of d-\(\alpha\),\(\beta\)-dimyristin (5) but with L-acetone glycerol as starting material. Compounds VII to XII are reported for the first time.

**VII. L-Acetone Glycerol \(\alpha\)-Benzyl Ether**—Yield 78 per cent; b.p. (0.5 mm.) 90–93°; \([\alpha]_D = -18.3°\) in substance.

**VIII. d-\(\alpha\)-Benzyl Glycerol Ether**—Yield 68 per cent; b.p. (0.2 mm.) 121–123°; \([\alpha]_D = -5.36°\) in substance.

**IX. L-\(\alpha\),\(\beta\)-Dimyristin \(\alpha\)-Benzyl Ether**—Yield 65 per cent; m.p. 33–34°; \([\alpha]_D = -5.6°\) in anhydrous and ethanol-free chloroform (c, 7 per cent).

\(\text{C}_{22}\text{H}_{49}\text{O}_4\) (602.9). Calculated. C 75.70, H 11.03

Found. C 75.81, H 11.01

**X. L-\(\alpha\),\(\beta\)-Dimyristin**—Yield 90 per cent; m.p. 58–59°; \([\alpha]_D = +3.4°\) in anhydrous and ethanol-free chloroform (c, 7 per cent).

\(\text{C}_{22}\text{H}_{48}\text{O}_4\) (512.8). Calculated. C 72.60, H 11.79

Found. C 72.80, H 11.70

The phosphorylation of the L-\(\alpha\),\(\beta\)-dimyristin (Step 11a), the esterification of the resulting dimyristoyl d-\(\alpha\)-glycerylphenylphosphoryl chloride with choline chloride (Step 11b), and the removal of the phenyl group (Step 12) were carried out as described for the optical isomer in the abbreviated procedure for the synthesis of \(\alpha\)-lecithins by Baer and Maurukas (18).

**XI. Dimyristoyl d-\(\alpha\)-Glycerylphenylphosphorylcholine Chloride**—Yield 35.5 per cent; \([\alpha]_D = -1.5°\) in anhydrous and ethanol-free chloroform (c, 10 per cent); starts to sinter at 65°, forming gradually a semitransparent mass which melts with the formation of a meniscus from 126–127° (rate of heating approximately 3° per minute).

\(\text{C}_{44}\text{H}_{97}\text{O}_7\text{PNCl}\) (790.6). Calculated. C 63.81, H 9.82, P 3.92, N 1.77

Found. C 63.90, H 10.09, N 3.91, P 1.73

**XII. d-\(\alpha\)-(Dimyristoyl) Lecithin**—Over-all yield, based on L-\(\alpha\),\(\beta\)-dimyristin, 32.6 per cent. The crude lecithin was already sufficiently pure for most purposes. For analysis it was recrystallized by warming its suspension in diisobutyl ketone (20 ml. per gm.) to 80° (water bath temperature), centrifuging the solution while still hot, placing the decanted supernatant solution in a water bath at 50°, and allowing the lecithin solution to attain room temperature gradually. The lecithin was collected with suction on a Büchner funnel, washed with a small portion of diisobutyl ketone, and freed from solvent by being kept for several days in a high vacuum (0.01 mm.) over paraffin shavings until constant weight was reached; recovery 90 per cent.\(^4\) The dimyristoyl lecithin started to

\(^4\) It is advisable to avoid the recrystallization of the lecithins at higher temperatures. It has been observed recently that lecithins which were recrystallized from
sinter at approximately 90–95° and on further heating shrank to a translucent mass which melted suddenly with the formation of a meniscus at 238–239° (5) (rate of heating 20° per minute up to 210°; from there on 10° per minute). Alternatively, if the capillary was immersed in a preheated bath of 200° and the temperature from there on was raised at the rate of 3° per minute, the lecithin melted with meniscus formation from 226–227° (5). \([\alpha]_D = -7.1^\circ\) in methanol-chloroform (1:1) \((c, 6\text{ per cent})\). Both solvents were anhydrous.

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\text{C}_{18}\text{H}_{40}\text{O}_{2}\text{NP} \quad (695.94).
\]

Calculated. \(C 62.12, H 10.72, N 2.01, P 4.45\)

Found. \(\text{C} 62.29, \text{H} 10.82, \text{N} 2.00, \text{P} 4.60\)

Slide Flocculation Tests with Both Enantiomers of \(\alpha\)-(Dimyristoyl) Lecithin

BY R. H. ALLEN AND D. B. TONKS

(From the Laboratory of Hygiene, Department of National Health and Welfare, Ottawa, Canada)

Parallel tests with synthetic L-\(\alpha\)-(dimyristoyl) lecithin and D-\(\alpha\)-(dimyristoyl) lecithin were carried out. In previous work with L-\(\alpha\)-(dimyristoyl) lecithin it was found that an antigen of the following composition has an activity close to that of the standard V. D. R. L. antigen: cardiolipin 0.03 per cent, synthetic lecithin 0.30 per cent, cholesterol 0.9 per cent.

The antigen stock solutions were prepared by mixing the given amounts of the following alcohol solutions (absolute ethyl alcohol was used); cardiolipin (3.21 mg. per ml.) 0.234 ml., lecithin (15 mg. per ml.) 0.500 ml., cholesterol (15 mg. per ml.) 1.500 ml., with sufficient alcohol to make 2.5 ml.

The antigen suspensions were prepared from these stock solutions as directed for the V. D. R. L. test and were used \(\frac{1}{2}\) hour after preparation. The tests were carried out on several days with negative, weakly positive, and strongly positive sera. The same results were obtained with both antigens in 71 cases. The \(L\) form was more reactive than the \(D\) form in eleven cases, and similarly the \(D\) form was more reactive than the \(L\) form in eight cases.

The synthetic lecithin antigens gave slightly larger particles with negative sera than are usually obtained with the V. D. R. L. antigen, the \(D\) form giving slightly better negatives than the \(L\) form. Therefore, in this respect the V. D. R. L. antigen is better than the synthetic antigens tested.

dibisobutyl ketone at approximately 80° decompose more readily, as evidenced by the liberation of trimethylamine, than lecithins reprecipitated from chloroform with acetone at room temperature.

\(^{6}\) Short stem thermometers with a range of 50° were used.

\(^{6}\) Average of readings by three observers.
With positive sera the synthetic lecithin antigens gave very similar results. The clumping found with them was different in appearance from that obtained with the V. D. R. L. antigen. With strongly positive sera the latter gave large black clumps, while the synthetic lecithin antigens gave smaller clumps but many more of them. The difference makes comparative reading difficult, but does not detract from the usefulness of the latter antigens. The results with the synthetic lecithin antigens were often easier to read, since there was a more gradual increase in size from negatives to strong positives and less background material (particles not clumped) was present.

To summarize, it can be definitely stated that the d-α-(dimyristoyl) lecithin has an activity which is very close to that of its L isomer, and that antigens can be prepared from both which parallel quite closely results obtained with V. D. R. L. antigen.

No attempts were made to prepare antigens of different composition in order to find one having a sensitivity equal to that of the V. D. R. L. antigen, since this would have required considerably more work, time, and material.

SUMMARY

1. The synthesis of d-α-(dimyristoyl) lecithin, the first lecithin of the D series to become known, is described.
2. Its serological activity as an antigen component in conjunction with cardiolipin in the serodiagnosis of syphilis (V. D. R. L. slide flocculation test) was investigated and was found to resemble closely that of the L isomer.
3. The fact that the serological reaction is not specific with regard to the optical configuration of the lecithin should make it possible to use the more readily accessible synthetic DL-α-lecithins as substitutes for the beef heart lecithin in the serodiagnosis of syphilis.

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