A DNA Antigen That Reacts With Antisera To Cardiolipin

M. Guarnieri and D. Eisner

Department of Neurology
The Johns Hopkins University School of Medicine
Baltimore, Maryland 21205

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Summary

DNA can replace the cardiolipin hapten in an antigen suspension that precipitates anti-cardiolipin antibody. Structural similarities between cardiolipin and DNA may explain the immunochemical cross reaction between the nucleic acid and the phospholipid molecule.

In earlier work on the location of cardiolipin (Figure 1.1) in the mitochondrial membranes we observed that phosphatidylinositol (Figure 1.2) can react with anti-cardiolipin antisera (1). Subsequently, in work to be reported elsewhere we have found that cardiolipin can cross-react with antisera to phosphatidylinositol (2). A possible explanation for the immunochemical cross reactivity of cardiolipin and phosphatidylinositol (PI) may follow from the known structural requirements for the antigenicity of cardiolipin. The antigenic activity of cardiolipin is associated with its polar head, consisting of the two phosphodiester groups and the interior glycerol moiety (3-5). Since phosphatidylglycerol (Figure 1.3a) and phosphatidylglycerol phosphate (Figure 1.3b) also react with anti-cardiolipin antibody, it appears that the minimum molecular structure recognized by the antibody to cardiolipin is the portion of the

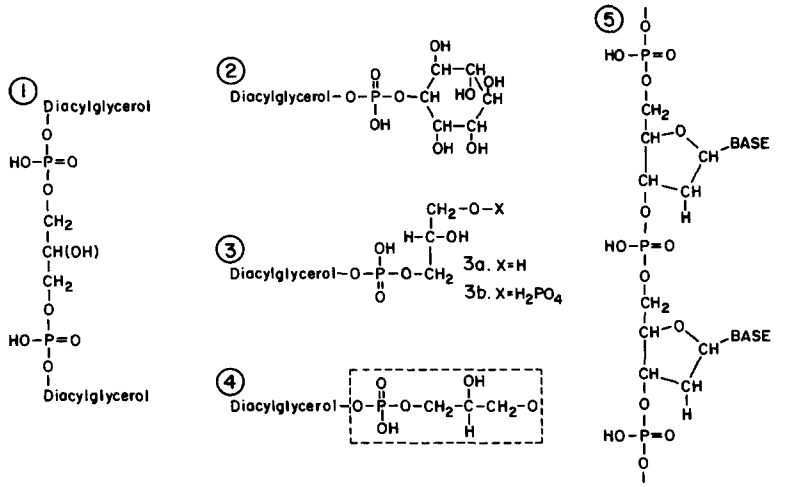

FIGURE 1

The structure of cardiolipin, diphosphatidylglycerol, is illustrated in Fig. 1.1. Phosphatidylinositol, Fig. 1.2; phosphatidylglycerol, Fig. 1.3a; phosphatidylglycerol phosphate, Fig. 1.3b. The minimum antigenic determinants of cardiolipin (cf. Introduction) is outlined in Fig. 1.4. A strand of DNA is drawn in Fig. 1.5 to illustrate the structural similarities between cardiolipin and DNA.

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molecule outlined in Figure 1.4. One of the hydroxyl groups of the inositol moiety of PI may thus be antigenically similar to the free hydroxyl group in cardiolipin. The non-polar hydrophobic lipid portion of cardiolipin and PI apparently has no specific role in the immunogenicity of the phospholipids.

This explanation for immunochemical cross-reactions between these two types of phospholipid molecules suggests that non-lipid molecules having appropriately spaced phosphodiester groups may also react with antisera to these phospholipids. In this report we demonstrate that DNA which fulfills these structural requirements, readily combines with phospholipid antisera.

Experimental

Cardiolipin and phosphatidylinositol were purchased from Supelco Inc (Bellefonte, Pa.). Cholesterol and β-γ-dipalmitoyl-phosphatidylcholine were purchased from Sigma (St. Louis, Mo.) The purity of the phospholipids was verified on both one and two dimensional thin layer chromatography systems (2).

Samples of pure bacteriophage T7 DNA were generously supplied by Paul T. Englund. Sperm DNA (Nutritional Biochemicals Cleveland, Ohio) and yeast RNA (Sigma) were washed with chloroform: methanol (2:1) solution before use.

Antisera to cardiolipin and to PI were obtained as described by Nojima and coworkers (6,7). Antibody activity was measured by a modification of the slide quantitative microflocculation test (VDRL method) as previously described (1). A negative reaction, indicated by the absence of flocculation, was scored as 0. Positive reactions were graded on a 1+ to 4+ scale dependent on the amount of flocculation (8).

The nucleic acid solutions used as antigens in the precipitation reactions contained 0.3 mg of DNA or RNA, 3 mg of phosphatidylcholine, and 9 mg of cholesterol per ml of ethanol. The DNA and RNA solutions were prepared by grinding the auxiliary lipids and the hapten together with ethanol in an all-glass homogenizer with a few strokes of the glass pestle. Antigen suspensions were prepared in 50 ml flasks by adding dropwise 1.0 ml of antigen solution to 1.0 ml of buffered saline while rotating the flask continuously, followed by the addition of 8.0 ml of buffered saline (8).

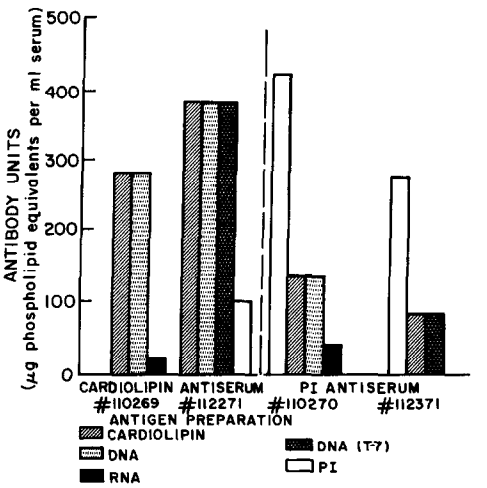

FIGURE 2

REACTIONS OF CARDIOLIPIN AND PI ANTISERA
WITH SPERM DNA, T7 DNA AND RNA

The bar heights represent the average value for the concentration of antibody units, cardiolipin, DNA, RNA, or PI, in each antiserum. The range of values deviated 15-20 percent from the average. The average value was determined from 5-6 tests with each serum with antigen suspensions prepared from 2-3 different stock solutions of the various antigens.

The concentration of the antibodies in the antiserum was graded in antibody units by determining the maximum dilution of antisera, 50 ul of which produced a 4+ flocculation reaction with 20 ul of antigen suspension (1). The stock antigen suspension contained 0.6 ug of cardiolipin or DNA per 20 ul. Thus, if 50 ul of 1:16 dilution of cardiolipin antiserum was just sufficient to produce a 4+ reaction, that antiserum contained 16 x 0.6 antibody units per 50 ul of serum or 192 antibody units per ml, and the concentration of the antiserum in antibody units.

Results and Discussion

The reactivity of a cardiolipin antisera with sperm DNA,

TABLE I

REACTION OF DNA WITH ANTI-CARDIOLIPIN ANTISERUM

Composition of Antigen Solution VS Microflocculation Test Score

% DNA	% Cholesterol	% PC			
	0.9	0.12	0.25	0.50	1.00
0.003		0	0	0	0
0.01		1	2	3	0
0.03		4	4	0	0
0.10		1	1	0	0
0.30		0	0	0	0

bacteriophage T7 DNA, and cardiolipin prepared in solutions containing the same adjuvants used in the preparation of the lipid antigens are compared in Figure 2. For reference, the reaction of DNA with a PI antiserum which contains anti-cardiolipin activity is also shown. The data show that cardiolipin and DNA have the same capacity to precipitate either PI or cardiolipin antisera. RNA specimens were only 10-20 percent as effective in producing flocculation of the anti-cardiolipin antisera. Over a three year period, similar tests with anti-cardiolipin antisera from 20 rabbits and anti-phosphatidylinositol antisera from 10 rabbits produced the same results.

The 5'-mono-, di-, and triphosphates of adenosine, guanosine, cytidine, uridine, and thymidine, glucose-1-phosphate, -6-phosphate, -1,6-diphosphate, NAD and NADPH when mixed with PC and cholesterol had no capacity to precipitate antiserum,

demonstrating that the antigenicity of DNA requires the phosphate bridge-groups which link the nucleoside molecules in the nucleic acid polymer.

The reaction of DNA with anti-cardiolipin antisera requires that the DNA be prepared with the same phosphatidylcholine-cholesterol adjuvants as cardiolipin antigen preparations (Table 1). For both cardiolipin (9) and sperm DNA, the most reactive antigen suspension as measured by the VDRL microflocculation assay contains 0.9 percent cholesterol, 0.25 percent PC, and 0.03 percent hapten. Treatment of anti-cardiolipin antiserum with the T7 or sperm DNA, or cardiolipin quantitatively removed cardiolipin or DNA antibody activity respectively.

The results of our experiments demonstrate that native DNA molecules may react with anti-cardiolipin antiserum, and that to demonstrate this phenomenon it is necessary to mix the DNA with auxilary lipids. Because precipitation reactions lack the sensitivity of other immunochemical test systems, we measured the reaction of DNA with anti-cardiolipin antisera by the Kolmer (complement fixation), Test(8). Serial dilutions of antisera (from 0.2 ml to 0.2 μl) were mixed with antigen dilutions containing 0.15 to 0.01 μg of DNA and cardiolipin antigen suspensions. Such "box titrations" revealed that DNA mixed with phosphatidylcholine and cholesterol had essentially the same capacity as cardiolipin to react with the anti-cardiolipin antiserum.

The finding that DNA reacts with anti-cardiolipin antiserum suggests that DNA and cardiolipin have similar if not

the same antigenic determinants. As shown in Figure 1.5, segments of the backbone of DNA can be seen to resemble the structure of cardiolipin: the two phosphodiester groups are separated by three carbon atoms, namely carbons 3, 4, and 5 of the deoxyribose ring. The hemiacetal oxygen which connects carbon 4 and carbon 1 of the deoxyribose ring therefore must be antigenically equivalent to the hydroxyl group on the interior glycerol molecule of cardiolipin.

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