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The Nonequivalence of the Phosphorus Atoms in Cardiolipin

Gary L. Powell and J. Jacobus*

ABSTRACT: Cardiolipin possesses two nonequivalent phosphorus atoms. This conclusion, based on symmetry considerations, is consistent with the available ^{31}P nuclear magnetic resonance (T. O. Henderson, T. Glonek, and T. Myers (1974), *Biochemistry 13,* 623) and phospholipase D hydrolysis data (A. N. Tucker and D. C. White (1971), *J. Bacteriol. 108,* 1058), both sets of data being difficult to rationalize on alternate grounds. Predictions concerning expected chemical reactions of cardiolipin, based on generally applicable symmetry arguments, are advanced.

In a recent application of ^{31}P nuclear magnetic resonance (nmr) spectroscopy two distinct resonances were observed for the two phosphorus atoms in cardiolipin (**1**) (Henderson *et al.,* 1974). The observed resonance doubling was interpreted in terms of an intramolecular hydrogen bond with one of the two "equivalent" phosphorus atoms, this bond undergoing slow exchange (on the nmr time scale) between the two otherwise "equivalent" phosphorus atoms. This explanation was employed to rationalize observed ^{31}P chemical shifts of monophosphorylated derivatives.

In seemingly unrelated experiments (White and Tucker, 1969; Short and White, 1970; Tucker and White, 1971) it has been noted that different metabolic turnover rates exist for the two phosphorus atoms in cardiolipins. Explanations of biphasic metabolic rates have centered on "pools" of materials with differing degrees of availability to metabolic processes.

We should like to pose an explanation for both the nmr results and the metabolic studies based on symmetry arguments. Symmetry considerations demand that the two phosphate moieties in cardiolipins be recognized as intrinsically nonequivalent; the nonequivalence in turn demands that the chemical and physical properties of the two groups be different.

Stereochemical Definitions and Nomenclature

The general interrelations of symmetry, stereochemistry, and nmr spectroscopy have been enunciated previously (Mislow, 1966; Mislow and Raban, 1967; Jacobus *et al.,* 1968; Jacobus and Raban, 1969; Jacobus, 1971). These relationships will be concisely reiterated here since they have bearing on both the previously mentioned nmr and metabolic studies.

Nuclei or groups of nuclei within molecules which can be interconverted by a rotational or rotational-reflectional symmetry operation[1] are equivalent in every sense under all conditions. Such nuclei are termed homotopic.

Nuclei or groups of nuclei within molecules which can only be interconverted by a reflection symmetry operation, *i.e.,* which bear a mirror image relationship one to the other, are termed enantiotopic. The environments of such nuclei or groups of nuclei are enantiomeric to one another.

Those nuclei or groups of nuclei which possess the same connectivity[2] but which cannot be interconverted by any symmetry operation are termed diastereotopic. Such nuclei reside in diastereomeric environments and bear the consequences of these environments, *i.e.,* they are nonidentical in every sense.

Homotopic nuclei are equivalent under all conditions; such nuclei must react with chemical reagents (achiral or chiral) at identical rates as they must also interact with solvent molecules (achiral or chiral) in identical manners. Homotopic nuclei are magnetically equivalent (isochronous) in achiral or chiral solvents, giving rise to single resonances in the absence of spin-spin coupling.

Enantiotopic nuclei or groups of nuclei are chemically and magnetically indistinguishable under achiral conditions; under chiral conditions such nuclei or groups of nuclei must react at different rates and must be chemical shift nonequivalent (anisochronous). Such nuclei or groups under chiral conditions exhibit "resonance doubling," this phenomenon being the basis of optical purity determinations employing chiral solvents (Pirkle and Beare, 1968) or chiral shift reagents (Whitesides and Lewis, 1971).

Diastereotopic nuclei or groups of nuclei are chemically and magnetically nonequivalent under all conditions. Such nuclei or groups of nuclei must react at different rates with any reagent and must be chemical shift nonequivalent in achiral or chiral solvents.

As will be demonstrated for cardiolipin, the application of symmetry considerations to accurately assess nmr spectroscopic and kinetic data on chiral systems is mandatory.

Biosynthesis of Cardiolipin

Two distinct pathways for the biosynthesis of cardiolipins

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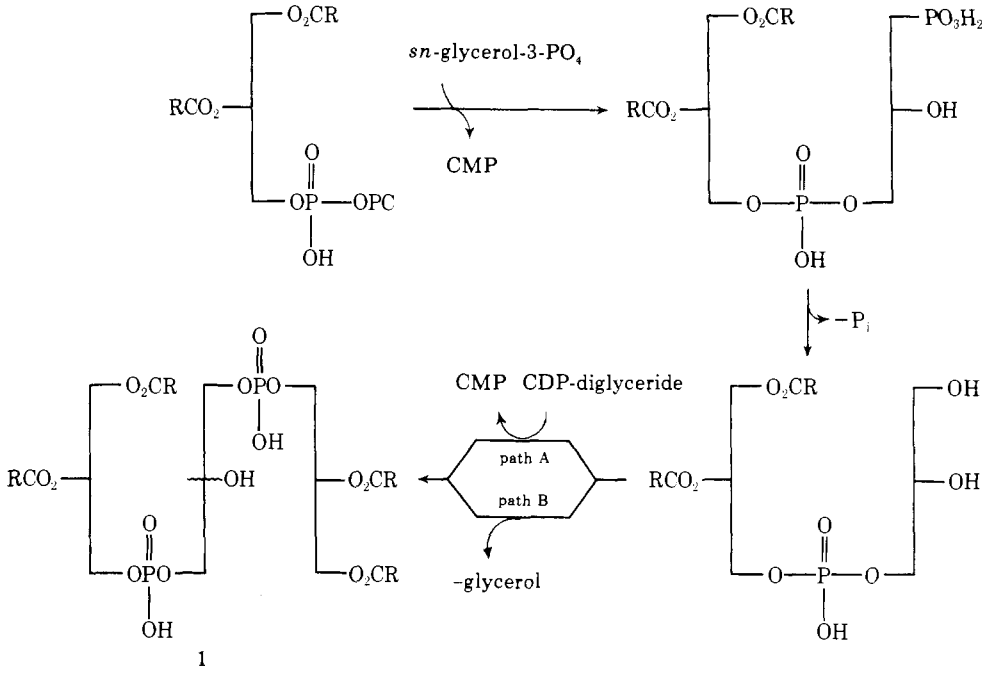
[1] A symmetry operation is a manipulation (either real or imaginary) of an object such that the object after the manipulation is indistinguishable from the original object. Symmetry operations are of three types: rotation, reflection, and reflection rotation (Mislow, 1966).

[2] Like nuclei or like groups of nuclei bonded to the same atom or to identical groups of intervening nuclei possess the same connectivity.

CHART I: Biosynthesis of Cardiolipin.



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have been recognized. Cardiolipin (**1**) has been demonstrated to arise from CDP diglyceride and *sn*-glycerol 3-phosphate in liver mitochondria (path A) (Hostetler *et al.*, 1972) and from phosphatidylglycerol alone in *Escherichia coli* (path B) (Hirschberg and Kennedy, 1972). The two pathways are stereoconvergent, *i.e.*, the stereochemistry of the product, cardiolipin, possessing two terminal *sn*-3-phosphatidylglycerol moieties, is the same by either pathway. As has been previously stated (LeCocq and Ballou, 1964), only one of the three possible cardiolipin diastereomers is capable of being optically active and thus the absolute configuration is established as that depicted in Chart I (Fischer projection).

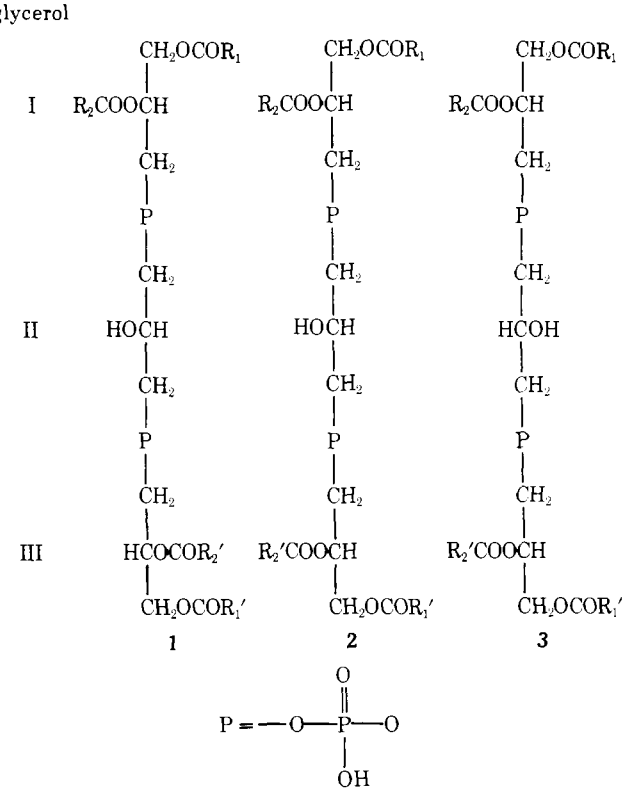
Stereochemistry of Cardiolipin

Cardiolipin is composed of three glycerol molecules linked *via* the primary hydroxyl groups through two phosphodiester linkages. The primary and secondary hydroxyl groups of the two terminal glycerol moieties are both acylated, hence the alternate name: diphosphatidylglycerol.

We shall represent the three possible diastereomers of cardiolipin (in Fischer projection) in a manner similar to that employed by LeCocq and Ballou (1964), who designated the forms lyxo (**1**), ribo (**2**), and xylo (**3**), respectively. As represented, we shall designate the three glycerol moieties as I, II, and III. For simplicity we shall assume that the two phosphatidylglycerol moieties are substituted in like manner ($R_1 = R_1'$; $R_2 = R_2'$). Although this assumption is required for the symmetry arguments, it is probably not required in practice.

The ribo and xylo forms possess planes of symmetry bisecting the atoms H-O-C-H of glycerol II which interconvert all corresponding groups within the molecule (a symmetry operation). Since two identical groups are attached to C-2 of glycerol II of **2** and **3**, this atom is nonasymmetric. For **2** and **3**, C-2 of glycerol I possesses the *R* configuration and C-2 of glycerol III possesses the *S* configuration. Thus, **2** and **3**, by virtue of their planes of symmetry, are achiral. The two groups attached to H-C-O-H of glycerol II are enantiotopic and corresponding groups within these groups are isochronous in achiral circumstances.

For **1**, C-2 of glycerol I possesses the *R* configuration as does C-2 of glycerol III. These two identical groups render C-2 of glycerol II nonasymmetric. Identical configurations at the two "ends" rule out a plane of symmetry (*R* would convert to *S* across a mirror plane) and rotation by 180° about C-2 of glycerol II is not a symmetry operation (HO converts to H at C-2 of glycerol II). Thus, *the two apparently "identical" groups* attached to C-2 of glycerol II *are not identical;* these groups are diastereotopic and each of the corresponding sets of atoms in the two groups possess diastereomeric relationships relative to each other. Further, the lack of any element of symmetry



(other than C_1) renders the molecule chiral. Natural cardiolipin is optically active (LeCocq and Ballou, 1964).

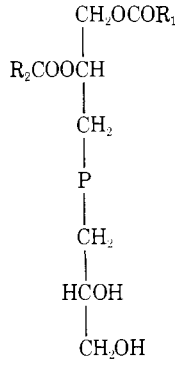
Consequences of the Chirality of Cardiolipin

The consequences of the diastereotopicity of corresponding groups in cardiolipin are twofold: (1) the nmr resonances of any corresponding atoms or groups of atoms in this molecule must be anisochronous, and (2) the rates of reactions for any corresponding atoms or groups of atoms within this molecule under achiral or chiral conditions must be different.

Thus, the observation of Henderson *et al.* (1974) that two phosphorus resonances are observed for cardiolipin is *a priori* predicted on the basis of symmetry arguments and is not related to static hydrogen bonding. Although the chemical shift data for the monophosphorylated derivatives may be related to either hydrogen bonding or dipolar interactions, the doubled resonance (^{31}P) of cardiolipin cannot be construed as evidence in favor of this rationalization. To the contrary, the observed resonance doubling is an elegant, albeit previously unrecognized, demonstration of the stereochemical conclusions reached earlier by LeCocq and Ballou (1964). The only cardiolipin capable of exhibiting doubled phosphorus resonances is the lyxo form 1 or its enantiomer. The meso (ribo and xylo) forms are incapable of giving rise to doubled phosphorus resonances.

Similarly, the observation that "identical" groups in cardiolipin exhibit different turnover rates in metabolic processes is *a priori* predicted on symmetry grounds. It could be reliably predicted that phospholipases must attack the two phosphorus linkages at different rates, as must any achiral reagent, *e.g.*, hydrochloric acid. The concept of "pools" in light of symmetry considerations will be deferred for discussion elsewhere.

The consequences of phospholipase cleavage of cardiolipin appear to have been largely unrecognized. Cleavage of glycerol I from cardiolipin yields 2-(*R*)-phosphatidyl-2′-(*R*)-glycerol



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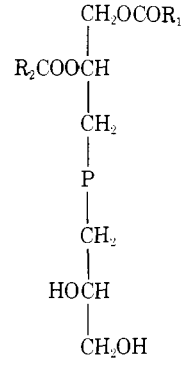

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(**4**), whereas cleavage of glycerol III from cardiolipin yields 2-(*R*)-phosphatidyl-2′-(*S*)-glycerol (**5**), these two products being diastereomers. To our knowledge, only a single phosphatidylglycerol has been recognized in nature. Since the "natural" phosphatidylglycerol has configuration 2-(*R*)-2′-(*S*)-(**5**) (the result of cleavage of glycerol III) (Kiyasu *et al.*, 1963), the failure to observe two phosphatidylglycerols in metabolic studies of cardiolipin is explicable in any of the following manners: the cardiolipin specific phospholipase D (Tucker and White, 1971) specifically attacks **1** to yield **5** to the virtual exclusion of **4**; it attacks **1** to yield **4** to the virtual exclusion of **5** which has been unrecognized; it attacks the two phosphorus groupings of **1** at commensurate, yet different, rates to yield both **4** and **5** which has simply been unrecognized; or, less likely, two enzymes of similar activity for the two "ends" of cardiolipin are actually involved. The correct alternative, the specific attack on **1** to yield **5**, has recently been recognized (Astrachan, 1973).

Conclusion

The application of symmetry principles to cardiolipin, as to molecules in general, is a simple, yet powerful, predictive tool of both spectroscopic and kinetic behavior. Failure to employ such principles can, as in the case of cardiolipin, lead to unjustifiable conclusions.

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