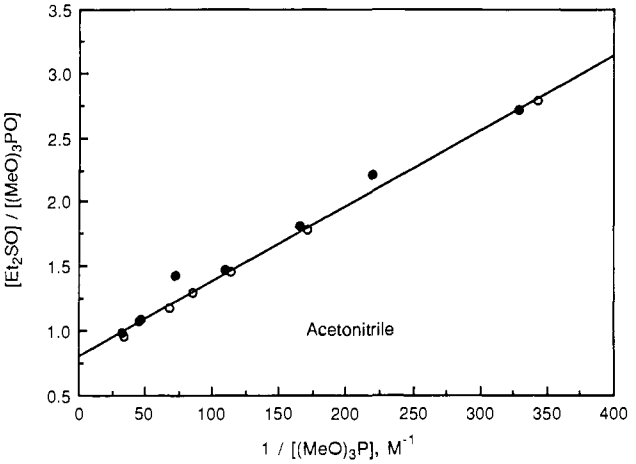

Figure 3. $(MeO)_3P$ trapping of Et_2S intermediate in Ch_3CN: (○) 0.10 M Et_2S, slope = 0.0058 M, (●), 0.20 M Et_2S, slope = 0.0058 M.

quenching of the photooxidation of trimethyl phosphite.[11]

When Et_2S in methanol or acetonitrile was oxidized with $^1O_2$ in the presence of various amounts of $(MeO)_3P$, diethyl sulfoxide and trimethyl phosphate were formed.[12] The concentration of $(MeO)_3P$ was kept lower than that of Et_2S,[13] ensuring that Et_2S was the primary reactant with $^1O_2$. Figures 2 and 3 show plots of $[Et_2SO]/[(MeO)_3PO]$ vs $1/[(MeO)_3P]$ in methanol and acetonitrile, respectively.

In methanol, the slopes depend on the concentration of Et_2S, but not in acetonitrile. These observations are very similar to those in the Et_2S–Ph_2SO system.[2] If the mechanism of trapping by $(MeO)_3P$ in methanol is similar to that reported for Ph_2SO, Scheme I can be derived which gives eq 1 by steady-state kinetics. This equation predicts a sulfide dependence of the slope, as observed with diphenyl sulfide trapping in methanol and as observed in Figure 2.

$$[Et_2SO]/[(MeO)_3PO] = 1 + (2k_s[Et_2S]/k_q)(1/[(MeO)_3P]) \quad (1)$$

Scheme II was derived for sulfide trapping of the intermediate in aprotic solvents, and leads to eq 2, which has no dependence

$$[Et_2SO]/[(MeO)_3PO] = 1 + (2k_x/k_q)(1/[(MeO)_3P]) \quad (2)$$

of the slope on sulfide concentration, as observed with diphenyl sulfide.[2] This scheme is consistent with the results in acetonitrile (Figure 3).

The structures of the intermediates in Schemes I and II are not certain, although intermediate X has been suggested to be a persulfoxide.[2,3,8] However, Schemes I and II are fit reasonably well by the data.[2] The fact that the intercepts of the plots in both Figures 2 and 3 are less than 1 suggests that some process other than intermediate trapping also contributes a small amount (~ 15%) to the production of trimethyl phosphate.[14] This cannot be direct reaction with singlet oxygen, which is negligible under the conditions.

From the slopes of the plots in Figure 2, values for k_s/k_q (Scheme I) were determined (Table II); the rate constant for trapping of the sulfide reaction intermediate by $(MeO)_3P$ in methanol is 420 times that of Ph_2S, 240 times that of Ph_2SO, and 87 times that of Et_2S. Thus trimethyl phosphite also satisfies the second criterion for a trap, reactivity toward the intermediate.

Table II. Relative Quenching Efficiencies

quencher	k_s/k_q	k_x/k_q	k_q (relative)
Ph_2S^a	4.81[c]		1.0
Ph_2SO^a	2.77[c]		1.7 (1)
$(MeO)_3P^a$	0.0115		420 (240)
Et_2S^a	1.0		4.8[d]
Ph_2SO^b		0.082[c]	1
$(MeO)_3P^b$		0.0029	28

[a] Methanol. [b] CH_3CN. [c] Reference 2 (b). [d] k_s.

In the same manner, k_x/k_q (Scheme II) was calculated. If k_x is the same in both cases, $(MeO)_3P$ is 28 times more reactive than Ph_2SO in acetonitrile. It is not yet possible to estimate the absolute trapping rate of $(MeO)_3P$, but the high rate and relative unreactivity with $^1O_2$ recommends it as a potential trapping agent for short-lived intermediates formed from other substrates.

The properties of other phosphites are being studied to better understand the trapping process. The application of $(MeO)_3P$ to the trapping of other substrates is also under investigation.

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(12) Methylene blue was used as a sensitizer with a filter solution of 0.1 M $K_2Cr_2O_7$ (cut-off < 460 nm). Control experiments gave no formation of $(MeO)_3PO$ under the same conditions without sensitizer or light.
(13) For example, 0.10 M Et_2S in oxygen-saturated acetonitrile with various amounts of $(MeO)_3P$ was irradiated on a "merry-go-round" for 4-5 min, and then the products were analyzed by GC. $[(MeO)_3P]$, $[Et_2SO]$, and $[(MeO)_3PO]$, mM; 0.0, 5.07, 0.0; 2.9, 3.97, 1.39; 5.8, 3.34, 1.89; 8.8, 3.39, 2.32; 11.7, 3.35, 2.57; 14.6, 3.40, 2.87; 21.9, 3.54, 3.29.
(14) A control experiment with 2,6-di-*tert*-butylphenol (5.8×10^{-3} M) also had an intercept less than 1.

N–H Bond Length Determinations and Implications for the Gramicidin Channel Conformation and Dynamics from ^{15}N–^1H Dipolar Interactions

P. V. LoGrasso, L. K. Nicholson, and T. A. Cross*

Department of Chemistry and Institute of Molecular Biophysics, Florida State University Tallahassee, Florida 32306-3006

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An atomic resolution structure of the transmembrane channel gramicidin A is still undetermined. This pentadecapeptide dimerizes to form monovalent cation selective channels in lipid bilayers and biological membranes and has been the subject of extensive study over the past two decades. Although two crystal structures of dimerized forms of gramicidin A have been recently reported,[1,2] these crystals do not contain lipid and are double-stranded dimers rather than the N-terminal-to-N-terminal single-stranded dimer generally believed to be the channel form. In this report a direct approach for determining structure that avoids the need for crystallization is utilized, and atomic resolution solid-state NMR data of the channel state is presented. It has been shown previously that observations of dipolar interactions of uniformly aligned samples can be interpreted to determine not only the orientation of specific bonds[3,4] but also the orientation of peptide linkage planes in the molecule with respect to a unique axis.[5] Such orientational data can be used to determine three-dimensional structures with atomic resolution[6,7] in a manner analogous to the use of distance constraints in solution NMR and diffraction techniques.

Recent solid-state NMR spectra of gramicidin provide considerable evidence that either the backbone structure or dynamics is variable along the length of the channel,[8-11] although one recent

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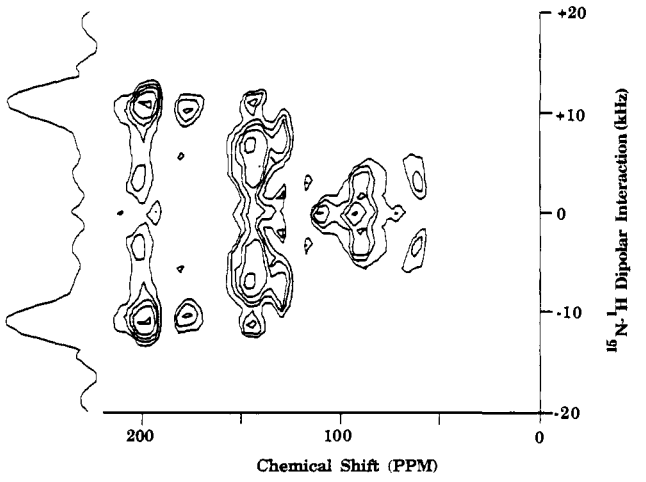

Figure 1. ^{15}N–^{1}H separated local field spectrum[22] of oriented uniformly ^{15}N labeled gramicidin in a hydrated lipid bilayer of dimyristoylphosphatidylcholine. The gramicidin was isotopically labeled by a biosynthetic approach from cultures of *Bacillus brevis*.[23] Gramicidin and lipid were codissolved in an organic solvent and dried on glass coverslips. These coverslips were stacked in a short segment of square glass tubing and then hydrated at 40 °C for at least 48 h. Samples were oriented with the normal to the plane of the coverslip (and lipid bilayers) parallel with the magnetic field of the spectrometer. Present studies indicate a mosaic spread of the gramicidin channel orientation of less than ±3°. The spectrum was obtained on a heavily modified IBM 200SY spectrometer equipped for solids and by using a homebuilt probe with a 5 μs ^{15}N 90° pulse length and a 2.0 mT ^{1}H decoupling field during acquisition. The pulse sequence[5] was composed of a cross polarization sequence with a 1 ms mixing time, and a 180° refocusing pulse was applied to the ^{15}N spins preceeded and followed by a fixed delay equal to the dipolar dwell, 20 μs, times the total number of experiments. For this data set 16 t_2 experiments were performed. Heteronuclear decoupling was applied following the dipolar evolution period (dwell times experiment no.). No ^{1}H homonuclear decoupling was applied during the evolution period. A slice through the chemical shift dimension at 198 PPM is also shown.

^{13}C solid-state NMR study concludes differently.[12] Figure 1 shows a two-dimensional spectrum of uniformly ^{15}N labeled gramicidin in oriented lipid bilayers with the bilayer normal aligned parallel with the magnetic field. Each ^{15}N site gives rise to a single sharp resonance in the chemical shift dimension and a doublet in the ^{15}N–^{1}H dipolar dimension. This data shows that ^{15}N chemical shifts are spread over a wide frequency range and that the dipolar couplings are also highly variable. Figure 2 presents the ^{15}N–^{1}H dipolar spectrum of oriented ^{15}N Ala_3 gramicidin A. The single sharp resonance in the chemical shift dimension and the doublet in the dipolar dimension clearly demonstrate that the Ala_3 site has a unique conformation throughout the sample. The magnitude of the chemical shift and the dipolar splitting both suggest that these spin interactions are not extensively averaged by molecular motions for this site. These magnitudes further suggest that σ_{33} of the static chemical shift tensor and $\nu_{\parallel}$ of the static dipolar tensor lie close to the magnetic field and bilayer normal directions. Since the $\nu_{\parallel}$ orientation is coincident with the N–H bond, this bond is approximately parallel with the bilayer normal.

The averaging of a nuclear spin interaction will depend on the orientation of a specific nuclear site with respect to the motional axis and on the orientation of the motional axis relative to the magnetic field. It is well accepted that, above the gel-to-liquid crystalline phase transition of the lipids, the channel will rotate about an axis parallel with the bilayer normal. Consequently, the variable averaging along the backbone may be caused by different

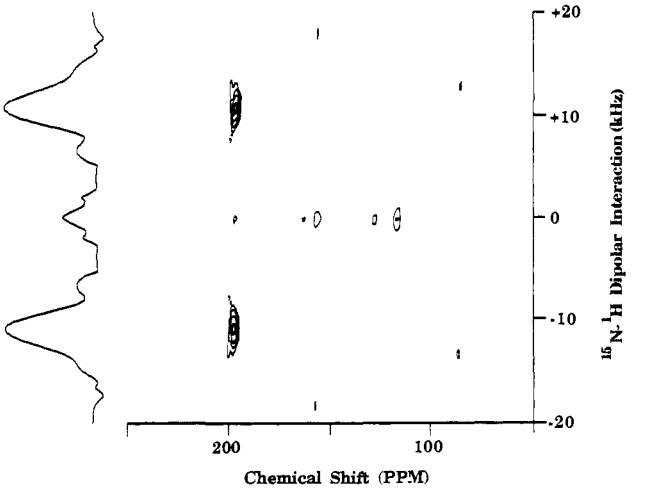

Figure 2. ^{15}N–^{1}H separated local field spectrum of oriented ^{15}N Ala_3 gramicidin A in a hydrated lipid bilayer. The gramicidin was isotopically labeled by solid-phase peptide synthesis as described elsewhere.[24] The hydrated bilayers were prepared as described for Figure 1 as were the spectroscopic conditions. A slice through the chemical shift dimension at 198 PPM is also shown.

orientations of the peptide linkages with respect to the axis of this global motion. Alternatively, it is possible to explain the variability in terms of local dynamics. The amplitude of librational motions of the individual peptide linkages which result from transitions between local conformational substates in the backbone about the C_α–C_α axis may differ along the length of the channel.[13] While a recent computational study[14] indicates that the librational motions are nearly uniform in amplitude along the backbone, another study[15] indicated considerable heterogeneity. From the limited data available it is not possible to determine whether the variability in the averaging of the nuclear spin interactions along the channel axis is a result of dynamic or structural heterogeneity.

The magnitude of the static dipolar interaction can be calculated[16] and is dependent on the N–H bond length. The large multiresonance dipolar splittings observed in Figure 1 are 22.6, 22.1, and 21.1 kHz, and in Figure 2 for the Ala_3 site the splitting is 21.8 kHz. These values are not consistent with the long bond length determined in previous ^{15}N NMR efforts for crystalline dipeptides[17,18] and hydrated protein[19] but is completely consistent with the neutron diffraction result obtained from crystalline glycyl-glycine of 1.024 Å as a maximum bond length.[20] The ^{15}N NMR approach utilized here is different from that used before in that this study is not dependent upon assessing the dipolar scaling factor caused by homonuclear ^{1}H decoupling. Measurement of this scaling factor is fraught with complications, and, despite very extensive efforts, the most recent bond length determination of 1.061 Å[17] implies a maximum dipolar splitting of only 20.4 kHz. For these hydrated samples it is apparent that the homonuclear broadening of the heteronuclear dipolar resonances is not substantial. Therefore, homonuclear decoupling was not applied during the evolution period, greatly simplifying the spectral interpretation of the data reported here in comparison with that of previous studies. By avoiding homonuclear decoupling, both the dipolar and J interactions are expressed in the observed

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spectra. As in previous efforts,[21,5] the relatively small contribution of the J coupling is ignored in this study. The possibility also exists that in hydrated samples of polypeptides the bond lengths will be different than the bond lengths obtained from crystalline dipeptides. Structural models of the gramicidin channel show that the alternating L and D amino acids fold into a β-helix in which the Ala_3 amide proton is hydrogen bonded to the carbonyl of Val_8. Therefore, it is anticipated that, like the crystalline dipeptides, the Ala_3 amide proton will be hydrogen bonded. However, crystal packing forces as well as the packing of this polypeptide into a β-helix in a model membrane may result in certain constraints that affect either the bond length directly or the accurate determination of the bond length.

The observations reported here provide for a direct determination of the maximum value for the N-H bond lengths in the gramicidin channel without resorting to crystallization, dehydration, model compounds, or scaling of the dipolar interaction. Furthermore, by assuming that the N-H bond length is 1.024 Å the Ala_3 N-H bond makes an angle with the bilayer normal of less than 10°. The gramicidin channel is typically modeled as a left-handed β-helix. Such a model has the Ala_3 N-H bond orientation tipped by approximately 25° with respect to the bilayer normal. Alternatively, a right-handed β-helix would allow for this experimentally determined N-H bond orientation. Therefore, our results reopen the possibility that the helix is right-handed and not left-handed as generally assumed.

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Cyclodextrin-Sandwiched Porphyrin†

Yasuhisa Kuroda,* Takashi Hiroshige, Takashi Sera, Yasushi Shiroiwa, Hiroyuki Tanaka, and Hisanobu Ogoshi

Department of Synthetic Chemistry Kyoto University, Sakyo-ku, Kyoto 606, Japan

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One of the most characteristic features of hemoproteins is that the porphyrin molecules are usually bound in the hydrophobic pocket formed in the apoprotein structure. Such hydrophobic microencapsulation of porphyrins may significantly affect their properties such as reactivity and substrate interactions.[1] We report here the syntheses of the new type of porphyrins, β-cyclodextrin-sandwiched porphyrin (**I**), which mimics these characteristics of holohemoproteins.

Since considerations of CPK space-filling models of **I** suggested that there may be five possible isomers (**Ia-e**) according to their connection pattern of porphyrin with two cyclodextrins, both of the two coupling reactions of porphyrin with cyclodextrins, diagonal and side coupling, were attempted (Scheme I). The atropisomers of porphyrin, tetrakis(*o*-thioacetoxyphenyl)-porphyrins, prepared from *o*-thioacetoxybenzaldehyde and pyrrole by the method of Lindsey et al.[2] (20% yield), were separated by the preparative HPLC (YMC Co., SIL, benzene/$CHCl_3$ = 90/10), and the coupling reactions of $\alpha\beta\alpha\beta$ and $\alpha\alpha\beta\beta$ atropisomers with A,D-diiodo-β-cyclodextrin[3] were carried out under the basic condition shown in Scheme I. Interestingly, the two types of coupling reactions, diagonal and side coupling reactions, were confirmed by HPLC analyses of the products (YMC Co., ODS-AQ, CH_3CN/H_2O = 14/86 - 17/83 linear gradient) to be completely specific; i.e., two diagonal coupling products and three side coupling products were obtained starting from $\alpha\beta\alpha\beta$ and $\alpha\alpha\beta\beta$ atropisomers of porphyrin, respectively, in the expected statistical yield of each isomer (**Ia/Ib** = 1/1, **Ic/Id/Ie** = 1/2/1 and 10-15% total yield). The FAB mass spectra of these five isomeric products show molecular peaks at 2940 (mw 2940.96 as $C_{128}H_{162}O_{66}N_4S_4$).[4] Further confirmation of structures of **Ia-e** was obtained from analyses of their 400 MHz NMR spectra based on their molecular symmetries. The NMR spectra of **Ia(b)**, **Id**, and **Ie** were shown in Figure 1 as the typical example. Since **Ie** have the C_2 symmetry as shown in Scheme I, two cyclodextrin molecules in **Ie** are expected to be identical. Thus, six C1-H doublets of cyclodextrin containing one overlapped signal were observed at δ 3.9 - δ 5.1 as 14 C1-H protons. On the other hand, 12 C1-H doublets containing two overlapped signals were observed at the same range for **Id** which have no symmetric element. Similar situations were

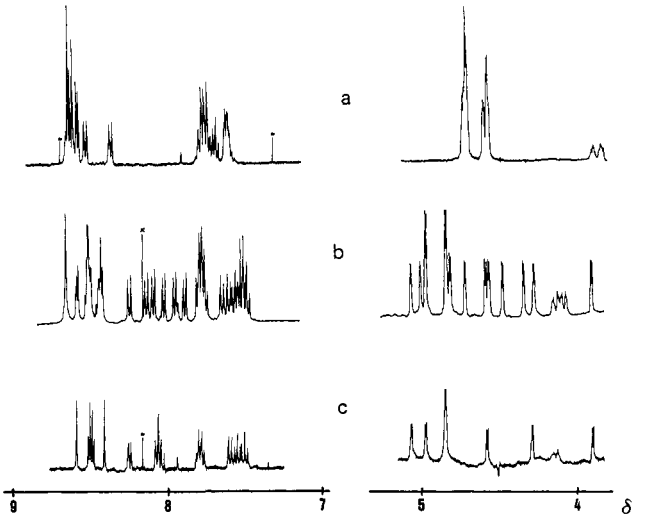
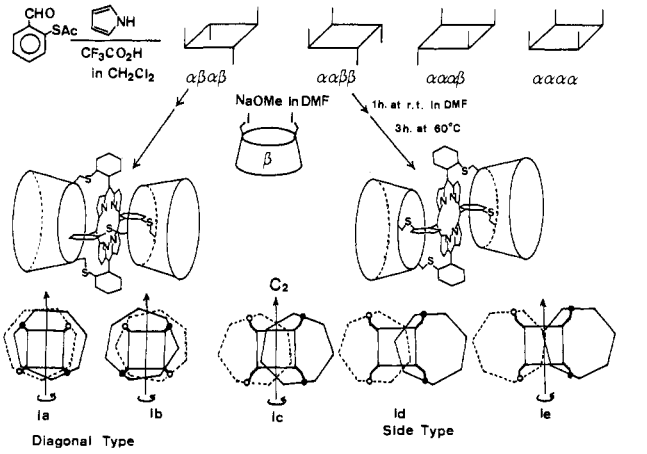


Figure 1. Aromatic and C1 proton regions of 400 MHz ^1H NMR spectra of cyclodextrin-sandwiched porphyrins in 5% D_2O-DMSO-d_6 at 80 °C: (a) isomer **Ia(b)**, (b) isomer **Id** and (c) isomer **Ie**.

Scheme I



† This paper is dedicated to the late Professor Iwao Tabushi.

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