Aggregate formation from 3-alkylindoles: amphiphilic models for interfacial helix anchoring groups

Ernesto Abel, Stephen L. De Wall, W. Barry Edwards, S. Lalitha, Douglas F. Covey and George W. Gokel*

Bioorganic Chemistry Program and Department of Molecular Biology & Pharmacology, Washington University School of Medicine, 660 S. Euclid Ave., Campus Box 8103, St. Louis, MO 63110 USA. E-mail: ggokel@molecool.wustl.edu

Received (in Columbia, MO, USA) 30th November 1999, Accepted 9th December 1999, Published on the Web, 29th February 2000

Indole can function as an amphiphile headgroup, but the counter-intuitive observation that 3-substituted indoles form less stable aggregates than the *N*-substituted isomers has been addressed by use of a Langmuir–Blodgett trough.

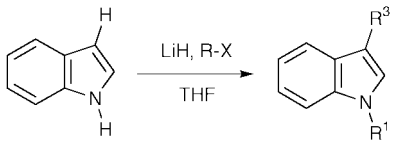
Many proteins that control the passage of cations and small molecules across membrane boundaries are thought to involve multiple α-helical strands that organize into a pore.[1] In most cases, this notion is based on a combination of sequence analysis and hydrophobicity plots[2] but firm structural data has come to hand recently for the K*sc*A channel of *Streptomyces lividans*[3] and the mechanosensitive ion channel homologue from *Mycobacterium tuberculosis.*[4] In many cases, the α-helices are thought to be connected by 'loops' that do not enforce rigid structural arrangements on the transmembrane segments. It has been postulated, for example, that the tryptophan residues of gramicidin serve as anchors at the membrane boundary to stabilize the single-strand, head-to-head dimer structure.[5] The interesting recent controversy over the active channel structure of gramicidin[6] has been argued partly in terms of indole position within the phospholipid bilayer.[7] It has also been noted 'that Trp and Leu are equally hydrophobic in character and able to embed in a bilayer membrane'.[8]

If transmembrane proteins require alignment of multiple α-helical segments for effective function, tryptophan is a reasonable candidate to serve that purpose. Indeed, the known solvatochromism[9] of the indole sidechain and tryptophan-rich[10] and highly conserved periplasmic loops[11] suggest an anchoring function. In the first stage of the present work, the strategy was to determine if vesicles could be formed from single-strand alkanes[12] terminated with a headgroup corresponding to the tryptophan sidechain.

One measure of a group's 'anchoring potential'[13] could be obtained by evaluating amphiphiles in which indole served as headgroup.[14] We felt that the loss of the NH residue resulting from *N*-alkylation of indole constituted a stringent test of the potential for this group to function as an anchor. We thus prepared several *N*-alkylindoles and assessed their ability to form aggregates. When the alkyl chains were of sufficient length, *N*-alkylindoles formed stable aggregates that were characterized by laser light scattering, electron microscopy, and dye entrapment.[15] We report here that the isomeric 3-alkylindoles form aggregates as well but their behavior is quite different.

3-Methylindole **1** was obtained commercially. Compounds **2**–**6** were obtained by treatment of indole with LiH in THF followed by addition of the alkylating agent (Scheme 1). Yields were modest (20–40%); the major by-products were the *N*-substituted isomer and/or the disubstituted compound. The previously unknown materials **2**–**6** were obtained either as oils or low-melting solids.[16]

Aggregates were formed from aqueous suspensions of the amphiphiles reported here by using the lipid hydration[17] vesicle preparation method.[18] Aggregate size was assessed by using a standard laser light scattering instrument[19] and negative stain electron microscopy. Aggregates were not observed for **1** or **2**, which have 3-methyl and 3-hexyl sidechains, respectively.



1 R^1 = H, R^3 = CH_3
2 R^1 = H, R^3 = $(CH_2)_5CH_3$
3 R^1 = H, R^3 = $(CH_2)_9CH_3$
4 R^1 = H, R^3 = $(CH_2)_{17}CH_3$
5 R^1 = $(CH_2)_{17}CH_3$, R^3 = H
6 R^1 = R^3 = $(CH_2)_{17}CH_3$

Scheme 1

Large aggregates (340 ± 93 nm) were observed for **3** (R′ = *n*-decyl) and when the 3-sidechain was *n*-octadecyl (**4**), the aggregate diameter was 142 ± 34 nm. The latter value compared with 138 nm (narrow size distribution) observed when the octadecyl chain was attached to indole at the 1-position. When both the 1- and 3-positions of indole were substituted by *n*-octadecyl groups, light scattering showed that the liposomes had an average diameter of 210 nm (size distribution was broad). Aggregates were further characterized by electron microscopy. Aggregates formed from **3** are shown in the accompanying photomicrograph (Fig. 1). These aggregates proved to be too fragile to stain with 0.2% uranyl acetate, converting into large, ill-defined structures. Previous studies with the corresponding *N*-alkylindoles suggested that the aggregates formed from those isomers were robust. Moreover, when an aqueous suspension of *N*-*n*-decylindole was maintained at ambient temperature in the dark for one week, the unimodal diameter increased from 272 ± 43 to 373 ± 130 nm. No other alteration in the system could be detected. In contrast, an aqueous suspension of the isomer **3** was monitored weekly

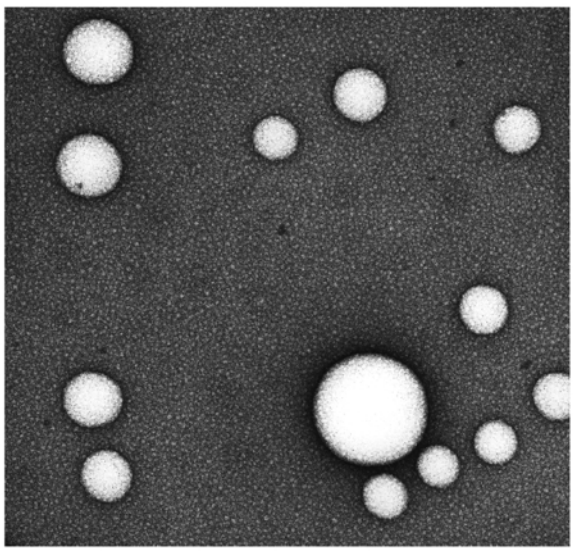


Fig. 1 Photomicrograph of **3** (field of view = 2000 × 2000 nm).

DOI: 10.1039/a909527f

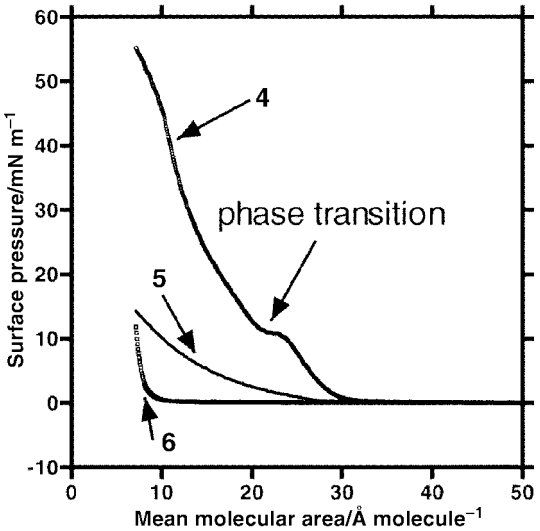


Fig. 2 Pressure–area isotherms for **4**–**6**.

during one month. The size observed for the aggregates, 340 ± 93 nm, remained constant. However, a foamy residue was observed to deposit on the walls of the vessel during this period and the amount of very large particles[20] detected by the laser light scattering instrument increased from 0% to 7%. It thus appears that some fusion[21] and precipitation have occurred.

A dye entrapment study conducted on vesicles formed from *N*-decylindole using the fluorescent dye carboxyfluorescein (1 and 100 mM) found inclusion to be 8%.[15] When such a study was undertaken with **3**, the observed aggregate size tripled from 340 to ~1000 nm. The inclusion was calculated to be 3.5% but the variation in aggregate size makes the meaning of the inclusion volume unclear. Alternative attempts to entrap other dyes, methylumbellyferyl-D-glucopyranose, or Na^{125}I also proved to be equivocal. The fragility of vesicles formed from 3-alkylindoles, which retain a free NH, compared to the stability of the *N*-substituted indoles, was remarkable. This seemed counter-intuitive as the free NH was expected to make the indole more capable of hydrogen bonding and thus interacting with water.

When the alkyl group is attached at indole's 3-position, the indolyl NH remains free and available to interact with proximate water. Hydrogen bonding could, in principle, compete with the organizational force[22] available from π-stacking.[23] When vesicles form, association of the lipid tails help to stabilize the overall structure. When there is no NH residue available for H-bonding, the hydrophobic forces are expected to dominate vesicular organization. The experimentally-determined pressure–area isotherm π–A curves (π = surface pressure, Langmuir–Blodgett trough) for compounds **4**–**6** at 20 °C are shown in Fig. 2. The *N*-alkylated indoles **5** and **6** gave no significant isotherm. 3-Octadecylindole **4** revealed a transition (20 °C) from expanded to condensed phase (surface pressure of the condensed phase, π_c, of ~12 mN m^{-1}) typical of many known amphiphiles when the monolayer is below its critical temperature.[24] The mean molecular area of the expanded phase at this pressure was 22.5 Å^2 molecule^{-1}. The area of the condensed phase was obtained by extrapolating the condensed isotherm **($\pi = \pi_c$).** The corresponding value is ~20.5 Å^2 molecule^{-1}. Unlike various other monolayer systems known in the literature,[25] the monolayers formed from **4**, which has an accessible NH functional group, did not collapse. Instead, the surface pressure rose until the trough barriers could not further compress the layer.

An important conclusion of the present work is that indole is capable of serving as a head group in the formation of stable aggregates, whether the alkyl chain is attached at the 1-(*N*) or 3-position. As expected for a typical amphiphile, aggregates are observed only when the alkyl chains are sufficiently long. These aggregates were characterized by standard methods. The difference in behavior for the isomeric indole amphiphiles may be understood in terms of hydrogen bonding. The Langmuir–Blodgett trough work suggests that lipid–lipid and headgroup stacking interactions control the formation of aggregates. When the NH residue is free (3-alkylindoles), the interaction with water becomes dominant, altering the stability of the aggregates. The multiple indolyl (Trp) headgroups in membrane-inserted gramicidin are focused to the aqueous boundary and stabilize the position of the structure. The present results demonstrate that indole may function generally as an organizing element in membrane-bound peptide[26] and protein structures.

We thank the NIH for grants (GM-36262 to G. W. G.) and (GM-47969 to D. F. C.) that supported this work.

Notes and references

1 G. Von Heijne, *Annu. Rev. Biophys. Biomol. Struct.*, 1994, **23**, 167.
2 T. E. Creighton, *Proteins: Structures and Molecular Properties*, 2nd edn., Freeman, New York, 1993.
3 D. A. Doyle, J. M. Cabral, R. A. Pfuetzner, A. Kuo, J. M. Gulbis, S. L. Cohen, B. T. Chait and R. MacKinnon, *Science*, 1998, **280**, 69.
4 G. Chang, R. H. Spencer, A. T. Lee, M. T. Barclay and D. C. Rees, *Science*, 1998, **282**, 2220.
5 W. Hu, K.-C. Lee and T. A. Cross, *Biochemistry*, 1993, **32**, 7035; R. R. Ketchem, W. Hu and T. A. Cross, *Science*, 1993, **26**, 1457.
6 B. M. Burkhart, N. Li, D. A. Langs, W. A. Pangborn and W. L. Duax, *Proc. Natl. Acad. Sci. USA*, 1998, **95**, 12 950; B. M. Burkhart, R. M. Gassman, D. A. Langs, W. A. Pangborn, W. L. Duax and V. Pletnev, *Biopolymers*, 1999, **51**, 129.
7 R. E. Koeppe II, F. J. Sigworth, G. Szabo, D. W. Urry and A. Woolley, *Nat. Struct. Biol.*, 1999, **6**, 609; T. A. Cross, A. Arseniev, B. A. Cornell, J. H. Davis, J. A. Killian, R. E. Koeppe II, L. K. Nicholson, F. Separovic and B. A. Wallace, *Nat. Struct. Biol.*, 1999, **6**, 610.
8 B. M. Burkhart and W. L. Duax, *Nat. Struct. Biol.*, 1999, **6**, 611; W.-M. Yau, W. C. Wimley, K. Gawrisch and S. H. White, *Biochemistry*, 1998, **37**, 14713.
9 T. B. Truong, *J. Phys. Chem.*, 1980, **84**, 964; S. R. Meech, D. Phillips and A. G. Lee, *Chem. Phys.*, 1983, **80**, 317; H. Lami and N. Glasser, *J. Chem. Phys.*, 1986, **96**, 4768.
10 M. Schiffer, C.-H. Chang and F. J. Stevens, *Protein Eng.*, 1992, **5**, 213.
11 R. E. Jacobs and S. H. White, *Biochemistry*, 1989, **28**, 3421.
12 T. Kunitake, Y. Okahata, M. Shimomura, S. Yasunami and K. Takarabe, *J. Am. Chem. Soc.*, 1981, **103**, 5401.
13 R. E. Jacobs and S. H. White, *Biochemistry*, 1986, **25**, 2605; 1989, **28**, 3421.
14 W. C. Wimley and S. H. White, *Biochemistry*, 1992, **31**, 12 813; A. S. Ladokhin and P. W. Holloway, *Biophys. J.*, 1995, **69**, 506.
15 E. Abel, M. F. Fedders and G. W. Gokel, *J. Am. Chem. Soc.*, 1995, **117**, 1265.
16 The spectral properties of all new compounds were as expected and combustion analytical data within ±0.4% for each element were obtained.
17 L. Saunders, J. Perrin and D. B. Gammock, *J. Pharm. Pharmacol.*, 1962, **14**, 567.
18 The *N*-alkylindole suspensions were sonicated using either a bath or tip sonicator (40 MHz) at ice-bath temperature and the 3-alkylindoles were irradiated at 40 °C. Sonication was maintained for 20–45 min, shorter times being required for the 3-alkylindoles.
19 The dynamic light scattering measurements were carried out by using a Coulter Model N4MD spectrophotometer equipped with a 4 mW helium–neon laser source, operated at 632.8 nm. The detection angle was fixed at 90°.
20 The light scattering instrument's internal calculation program reports large particles as 'dust'.
21 F. M. Menger and M. I. Angelova, *Acc. Chem. Res.*, 1998, **31**, 789.
22 E. E Tucker and S. D. Christian, *J. Phys. Chem.*, 1979, **83**, 426.
23 C. A. Hunter and J. K Sanders, *J. Am. Chem. Soc.*, 1990, **112**, 5525.
24 F. MacRitchie, *Chemistry at Interfaces*, Academic Press, New York, 1990; M. C. Phillips and D. Chapman, *Biochim. Biophys. Acta*, 1968, **163**, 301; M. W. Kim and D. S. Cannell, *Phys. Rev. A*, 1976, **13**, 411; O. Albrecht, H. Gruler and E. Sackmann, *J. Phys. Fr.*, 1978, **39**, 127; K. Motomura, T. Terazono, H. Matuo and R. Matuura, *J. Colloid. Interface. Sci.*, 1976, **57**, 52; D. A. Cadenhead, F. Muller-Landau and B. M. J. Kellner, *Ordering in Two Dimensions*, ed. S. K. Sinha, Elsevier, Amsterdam, 1980, p. 73.
25 J. P. Slorte, *Biochim. Biophys. Acta*, 1992, 326.
26 A. S. Ladokhin, M. E. Selsted and S. H. White, *Biophys. J.*, 1997, **72**,794.

Communication a909527f