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Amphipols Can Support the Activity of a Membrane Enzyme

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A relatively new class of amphipathic polymers referred to as "amphipols" provides a unique approach for solubilizing integral membrane proteins.[1] Amphipols are distinguished from other amphipathic polymers[2] in that they are linear polymers with alternating polar and nonpolar side chains. Like detergent micelles,[3] amphipols are thought to be able to maintain the solubility of integral membrane proteins by providing a toroid which can belt the transmembrane domain of a membrane protein, the inner ring of which is dominated by apolar side chains, the outer surface of which is primarily hydrophilic (Figure 1).[1a] Amphipols and their complexes have several potentially useful properties which are not shared by detergent micelles. For example, membrane protein solubility is fully maintained by stoichiometric amounts of complexed amphipols even in the complete absence of a free amphipol concentration.[1a−c,3] These unique properties suggest amphipols may prove useful as the basis for novel pharmaceutical, structural biological, or biochemical applications.[1,3] For example, it has been shown that amphipols can be used as a vehicle for delivering integral membrane proteins into lipid bilayers in a manner which does not disrupt the integrity of the membrane.[1f]

Despite the appealing characteristics of the best-characterized amphipols, the functionality of a membrane protein when solubilized by an amphipol in the absence of any other detergent or lipid component has not been unambiguously demonstrated.[4] In this work, we introduce a novel amphipol which, by itself, is able to maintain full catalytic functionality of an integral membrane enzyme. The enzyme employed in these studies is *E. coli* diacylglycerol kinase (DAGK), a particularly suitable test case because its catalytic properties have been extensively examined in a variety of different model membrane media including micelles, mixed micelles, lipid vesicles, and bicelles.[5] DAGK is homotrimeric and has three transmembrane segments per 13 kDa subunit.[6−8]

The amphipols employed in this study are summarized in Figure 1. PMAL-B-100[9] represents the first reported amphipol which is completely zwitterionic. DAGK was purified into PMAL-B-100 solutions and into 11 mM decyl maltoside (DM) micellar solutions or was also prepared in solutions where the only amphipol present was the population of PMAL-B-100 which is tightly associated with DAGK.[10] These latter solutions are here referred to as "0% amphipol" solutions because there is no *free* population of amphipol.

When aliquots of the DAGK/PMAL-B-100 mixtures were diluted manyfold into assay mixtures which contained classical DM/cardiolipin mixed micelles, the recovered activities were observed to be 75−80% of the activity observed in control reactions initiated with a micellar stock DAGK solution. From this result, it is clear that if denatured DAGK is present in 0.5 or 0% PMAL-B-100 solutions, it represents a <25% population.

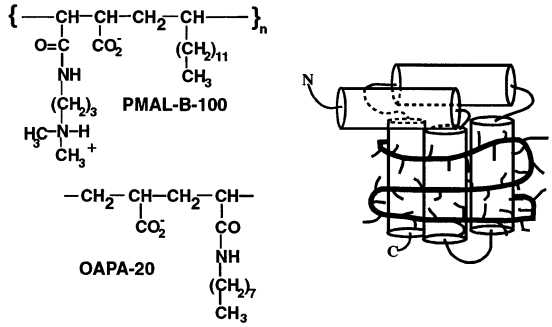

Figure 1. Amphipols used in this work and a model for how amphipols solubilize membrane proteins. Right: A model for an amphipol-membrane protein complex.[1a] The amphipol wraps around the three transmembrane segments of this hypothetical integral membrane protein. While only a single amphipol molecule is depicted, for some amphipol/protein combinations, several amphipols will complex a single protein molecule, although the organizational principles remain the same as shown. Left, Top: For PMAL-B-100, the order between ammoniumamide and carboxylate side chains within each unit is thought to be random from unit to unit. PMAL-B-50 differs from PMAL-B-100 in that 50% of the repeating units are zwitterionic (as shown), while for the other 50% of the units there are two carboxylate side chains and no amide. In PMAL-B-0, all repeating units have two carboxylate side chains, and there are no amides. Left, Bottom: OAPA-20 is derived from poly(acrylic acid) which has been randomly amidated at 20% of its side-chain positions with octylamine.

The kinetic stability of DAGK in PMAL-B-100 was compared to that in 0.5% DM by comparing activities for these solutions (as measured following dilution into a mixed micellar assay mixture) before and after incubation at 37 °C for 48 h. DAGK was observed to retain 78% of its activity upon incubation in DM micelles. In the cases of 0.5 and 0% PMAL-B-100, the enzyme retained 71 and 54% of its activity following incubation, respectively. The kinetic stability of DAGK in PMAL-B-100 is, therefore, only a little lower than that in DM micelles.

Activity measurements were conducted for DAGK in PMAL-B-100 mixtures under conditions where there was no other detergent or lipid component present.[12] DAGK normally requires the presence of a polar lipid in mixed micelles or vesicles for full catalytic activity. Under optimal mixed micellar conditions using 0.5% DM plus 3 mol % CL, DAGK exhibited a specific activity of 51 units/mg. However, under identical micellar conditions but in the absence of CL, DAGK's activity was only 1 unit/mg. Similar results were obtained when DM was replaced with a variety of other detergents.[13] In assays conducted in the presence of 0.5% PMAL-B-100 and in the absence of other lipid or detergent, DAGK exhibited an activity of 53 units/mg. The corresponding activity in 0% PMAL-B-100 solution was 36 units/mg. DAGK's activity in PMAL-B-100 is therefore very similar to its activity under optimal mixed micellar conditions, with only a modest decrease in activity observed when solutions are depleted of a free population of PMAL-B-100.

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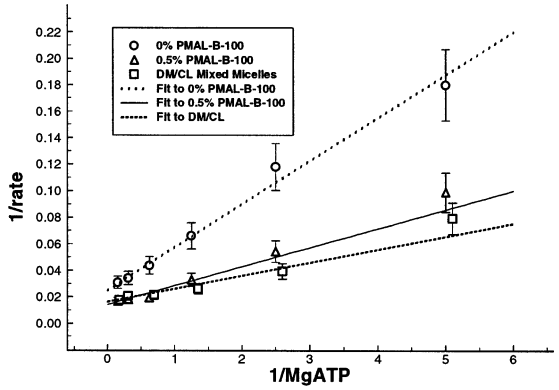

Figure 2. Steady-state kinetic data for DAGK at 30 °C in the presence of DM/CL mixed micelles, 0.5% PMAL-B-100, or 0% PMAL-B-100. The 1/rate units are milligram of DAGK per unit of activity. The 1/(MgATP concentration) units are millimolar^{-1}. [MgATP] versus rate data were directly fit by the Michaelis−Menten equation, with parameters being reported in the text. The data and the fit are plotted in double reciprocal form for the sake of clarity.

The activities of DAGK were also measured in 0 and 0.5% solutions of other amphipols available to our lab: PMAL-B-0, PMAL-B-50, and OAPA-20 (see Figure 1). PMAL-B-0 and PMAL-B-50 are structurally related to PMAL-B-100 but are underamidated, such that PMAL-B-0 is completely polyanionic, while about 50% of PMAL-B-50's repeating units are anionic and the other half are zwitterionic. DAGK's activity in 0 and 0.5% PMAL-B-50 was in both cases observed to be 27 units/mg, somewhat lower than that observed for the fully zwitterionic PMAL-B-100 (see below). That the partially polyanionic nature of PMAL-B-50 is responsible for the reduced activity of DAGK was supported by the fact that DAGK's activity in the completely polyanionic PMAL-B-0 was ca. 1 unit/mg. OAPA-20 is a polyanionic amphipol which is similar to the original amphipols.[1a] DAGK's activity in OAPA-20 was only 1 unit/mg.

Rates were measured as a function of the MgATP concentration in DM micelles, DM/CL mixed micelles, and PMAL-B-100 solutions.[14] In DM micelles, Michaelis−Menten kinetic patterns were not observed (not shown), and the highest measured rate was only 1.5 units/mg. In PMAL-B-100 and DM/CL mixed micellar solutions, Michaelis−Menten kinetics were observed, and V_{max} and $K_{m,MgATP}$ were determined (Figure 2). Kinetic parameters are as follows: DM/CL, $V_{max} = 61 \pm 8$ units/mg, $K_{m,MgATP} = 0.6 \pm 0.1$ mM; 0.5% PMAL-B-100, $V_{max} = 70 \pm 11$, $K_{m,MgATP} = 1 \pm 0.3$; and 0% PMAL-B-100, $V_{max} = 40 \pm 5$, $K_{m,MgATP} = 1.3 \pm 0.2$. Therefore, parameters for mixed micelles and 0.5% PMAL-B-100 were nearly the same, while V_{max} was a little lower in the case of 0% PMAL-B-100.

The catalytic properties of DAGK in micelles and mixed micelles have previously been extensively characterized.[5] While DAGK can be solubilized into many different types of detergents, the presence of polar lipids is absolutely required for the full catalytic activation of the enzyme.[5c,d] Here, we have shown that a novel amphipol, PMAL-B-100, is capable both of solubilizing DAGK and of supporting full catalytic activation of the enzyme in the absence of additional lipids and/or detergents. This was observed to be the case under conditions where there was a significant free population of the amphipol in addition to that complexed to the enzyme. However, even when the *only* PMAL-B-100 present was that which was directly complexed to the enzyme, the apparent V_{max} was fully 65% of the optimum value.

The observation that PMAL-B-100 can supply for DAGK all that would normally be provided by a membrane bilayer to sustain nativelike structure and catalytic properties represents a benchmark in amphipol development.

Of the several amphipols tested in this work, PMAL-B-100 was the only one which was able to support full activity by DAGK in the absence of additional lipid or detergent.[4] The fact that DAGK was almost completely inactive in the fully anionic PMAL-B-0 and OAPA-20 but exhibited intermediate activity in the 50/50 zwitterionic/anionic PMAL-B-50 suggests that the completely zwitterionic nature of PMAL-B-100 is a critical factor. Another contributing factor is the relatively long (dodecyl) nature of PMAL-B-100's apolar side chains. Preliminary studies in which the dodecyl side chain of PMAL-B-100 has been replaced with hexyl or octyl side chains have shown that these analogues support DAGK activities of only 1 unit/mg or less.

The availability of at least one amphipol which can sustain the full functional activity of membrane proteins in the complete absence of detergents or lipids extends the range of potential applications of amphipols.

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References

(1) (a) Tribet, C.; Audebert, R.; Popot, J.-L. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 15047−15050. (b) Tribet, C.; Audebert, R.; Popot, J.-L. *Langmuir* **1997**, *13*, 5570−5576. (c) Champeil, P.; Menguy, T.; Tribet, C.; Popot, J.-L.; le Maire, M. *J. Biol. Chem.* **2000**, *275*, 18623−18637. (d) Prata, C.; Giusti, F.; Gohon, Y.; Pucci, B.; Popot, J.-L.; Tribet, C. *Biopolymers* **2001**, *56*, 77−84. (e) Tribet, C.; Millis, D.; Haider, M.; Popot, J.-L. *Biochimie* **1998**, *80*, 475−482. (f) Nagy, J. K.; Hoffman, A. K.; Keyes, M. H.; Gray, D. N.; Oxenoid, K.; Sanders, C. R. *FEBS Lett.* **2001**, *501*, 115−120.

(2) (a) Velichkova, R. S.; Christova, D. C. *Prog. Polym. Sci.* **1995**, *20*, 819−887. (b) Jones, M.-C.; Leroux, J.-C. *Eur. J. Pharm. Biopharm.* **1999**, *48*, 101−111. (c) Tribet, C. *Biochimie* **1998**, *80*, 461−473.

(3) le Maire, M.; Champeil, P.; Moller, J. V. *Biochim. Biophys. Acta* **2000**, *1508*, 86−111.

(4) Prata et al. recently described a new type of nonionic amphipol, referred to as THAM.[1d] This polymer was found to sustain the electron-transfer activity of the cytochrome b_6f. However, all measurements were conducted in the presence of detergent above the critical micelle concentration. It is unclear whether THAM might be able to sustain function in the absence of detergent.

(5) (a) Badola, P.; Sanders, C. R. *J. Biol. Chem.* **1997**, *272*, 24176−24182. (b) Pilot, J. D.; East, J. M.; Lee, A. G. *Biochemistry* **2001**, *40*, 14891−14897. (c) Walsh, J. P.; Bell, R. M. *J. Biol. Chem.* **1986**, *261*, 15062−15069. (d) Walsh, J. P.; Bell, R. M. *J. Biol. Chem.* **1986**, *261*, 6238−6247. (e) Bohnenberger, E.; Sanderman, H. *Eur. J. Biochem.* **1983**, *132*, 645−650. (f) Czerski, L.; Sanders, C. R. *Anal. Biochem.* **2000**, *284*, 327−333.

(6) Smith, R. L.; O'Toole, J. F.; Maguire, M. E.; Sanders, C. R. *J. Bacteriol.* **1994**, *176*, 5459−5465.

(7) Vinogradova, O.; Badola, P.; Czerski, L.; Sonnichsen, F. D.; Sanders, C. R. *Biophys. J.* **1997**, *72*, 2688−2701.

(8) Lau, F. W.; Chen, X.; Bowie, J. U. *Biochemistry* **1999**, *38*, 5521−5527.

(9) The preparation of PMAL-B-50 and OAPA-20 is described in Nagy et al.[1f] PMAL-B-50 is referred to as "PMAL-B" both in the previous work and also by its vendor (Anatrace, Maumee, OH). PMAL-B-100 was prepared by a proprietary procedure and is available from Anatrace as "PMAL-C12" (catalog number P5012). The degree of amidation was determined to be 50% by ^{13}C NMR (meaning that there is one amide side chain and one free carboxyl side chain within every repeating polymer unit). The average molecular weight was 11.5 kDa based on the molecular weight of the precursor polymer and the measured degree of derivatization.

(10) Polyhistidine-tagged DAGK was overexpressed and purified into either detergent or detergent/lipid-free amphipol solutions using metal ion affinity chromatography as described in detail elsewhere.[1f,11]

(11) Lau, F.; Bowie, J. U. *Biochemistry* **1997**, *36*, 5884−5892.

(12) DAGK catalysis was monitored by enzymically coupling its production of MgADP to spectrophotometrically detectable NADH oxidation.[5a] Use of a water-soluble form of diacylglycerol, dibutyrylglycerol, permits use of this assay even in the absence of detergent or free amphipol.

(13) Vinogradova, O.; Sonnichsen, F. D.; Sanders, C. R. *J. Biomol. NMR* **1998**, *4*, 381−386.

(14) Measurements of apparent V_{max} and $K_{m,MgATP}$ were carried out by holding DBG at 10 mM, free Mg(II) at 10 mM, and by varying MgATP from 0.2 to 6.4 mM and measuring the slope of the linear phase of each reaction time trace.

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