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Hemifluorinated surfactants: a non-dissociating environment for handling membrane proteins in aqueous solutions?

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Abstract The instability of membrane proteins in detergent solution can generally be traced to the dissociating character of detergents and often correlates with delipidation. We examine here the possibility of substituting detergents, after membrane proteins have been solubilized, with non-detergent surfactants whose hydrophobic moiety contains a perfluorinated region that makes it lipophobic. In order to improve its affinity for the protein surface, the fluorinated chain is terminated by an ethyl group. Test proteins included bacteriorhodopsin, the cytochrome b_6f complex, and the transmembrane region of the bacterial outer membrane protein OmpA. All three proteins were purified using classical detergents and transferred into solutions of $C_2H_5C_6F_{12}C_2H_4$-*S*-poly-*Tris*-(hydroxymethyl)aminomethane (HF-TAC). Transfer to HF-TAC maintained the native state of the proteins and prevented their precipitation. Provided the concentration of HF-TAC was high enough, HF-TAC/membrane protein complexes ran as single bands upon centrifugation in sucrose gradients. Bacteriorhodopsin and the cytochrome b_6f complex, both of which are detergent-sensitive, exhibited increased biochemical stability upon extended storage in the presence of a high concentration of HF-TAC as compared to detergent micelles. The stabilization of cytochrome b_6f is at least partly due to a better retention of protein-bound lipids.

Key words: Membrane protein; Detergent; Membrane biochemistry; Fluorine

1. Introduction

Self-organization is a key property of living matter. It largely depends on geometrical constraints on the assembly or folding of biological molecules and attraction/repulsion between their parts, both properties being embedded in their chemical structure. In the folding of proteins and the organization of biological membranes, the hydrophobic effect plays a key role [1]. It governs, in particular, the assembly of lipids into bilayers and the way membrane proteins fold and insert into them [2–4]. Stabilizing membrane proteins as discrete, soluble entities in aqueous buffers requires substituting other molecules for the lipids whose acyl chains are normally in contact with the strongly hydrophobic surface of their transmembrane region. This is usually achieved thanks to detergents, i.e. small surfactants with the ability to form lipid-dissolving micelles [5]. A typical protocol for the purification of a membrane protein will start with the solubilization of the membrane containing it with a detergent, followed by several purification steps carried out in the presence of micelles of the same or another detergent. Above their critical micellar concentration (CMC), detergent molecules adsorb onto the hydrophobic transmembrane surface of the protein and form a torus that screens it from contact with water, preventing aggregation [6]. Most detergent molecules are in rapid equilibrium between this layer and the solution. If the concentration of free detergent is brought under the CMC, micelles disappear and proteins generally aggregate and precipitate, presumably a result of the detergent layer around them having disassembled. Membrane proteins therefore are generally handled in the presence of an excess of detergent micelles. Because detergents are, by definition, endowed with dissociating properties, micelles act as sinks for the lipids and other hydrophobic or amphipathic molecules that, very often, are solubilized along with the protein and stabilize it. In addition, the three-dimensional structure of many protein transmembrane regions is determined by the non-covalent association of α-helices whose buried surface is largely covered with methyl groups [2]. Exposing them to detergents creates a competition between detergent binding to these surfaces and the helix–helix interactions that stabilize the native structure, which can end up with subunit dissociation or other structural perturbations. As a result, exposure to detergents is seldom well tolerated by membrane proteins, a recurrent problem in membrane biochemistry [7–9].

Classical approaches to try and limit detergent-induced dissociation include working in the presence of very few micelles (i.e. at concentrations of free detergent as close as possible to the CMC), supplementing those with lipids or cofactors, and/or replacing the detergent used for extraction with a 'milder'

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Abbreviations: BR, bacteriorhodopsin; tOmpA, transmembrane domain of *Escherichia coli* outer membrane protein A; C_{12}-M, dodecyl-β-D-maltoside; HFS, hemifluorinated surfactant; H-TAC, $C_{12}H_{25}$-*S*-poly-*Tris*-(hydroxymethyl)aminomethane; HF-TAC, $C_2H_5C_6F_{12}$-C_2H_4-*S*-poly-*Tris*-(hydroxymethyl)aminomethane; F-TAC, C_8F_{17}-C_2H_4-*S*-poly-*Tris*-(hydroxymethyl)aminomethane; OTG, octylthioglucoside; C_8E_4, *N*-octyl-tetraoxyethylene; HG, Hecameg; PC, egg yolk phosphatidylcholine

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detergent or detergent mixture, i.e. a less dissociating surfactant such as digitonin or a member of the Tween family – none of which, however, is chemically well-defined. Over the past 10 years, many attempts have been made to develop specifically designed surfactants that would keep membrane proteins soluble after they have been extracted. Approaches that have met with some success include 'peptitergents' [10], i.e. peptides designed to fold into amphipathic α-helices, 'lipopeptide detergents' [11], based on the same principle but grafted with acyl chains, 'tripod amphiphiles' [12] (surfactants carrying three small hydrophobic chains), 'amphipols' [13,14] (amphipathic polymers), and hemifluorinated surfactants (HFS), which are the subject of the present report (for an overview, see [9]).

Membrane biochemists are used to thinking in a binary world where molecules or chemical groups are either polar or non-polar, members of each class being expected to be soluble either in water or in the hydrocarbon phase that makes up the interior of membranes [4]. Organic chemistry however has produced compounds that are strongly hydrophobic without being lipophilic. A case in point is perfluorocarbons. Because of unfavorable van der Waals interactions, hydrocarbons and perfluorocarbons do not interact well with each other, and these compounds, provided they are long enough, tend to demix [15,16]. As a consequence, a molecule that features a polar headgroup attached to a perfluorinated chain is a very strong surfactant, but it is not a detergent, because of its poor miscibility with lipids. Because they are not cytolytic, fluorinated surfactants are used in medicine in such applications as stabilizing perfluorocarbons in emulsions used as contrast reagents or as oxygen carriers in blood substitutes [17,18]. A membrane protein transferred into a solution of fluorinated surfactant ought to be relatively immune to delipidation and, more generally, to dissociation, since dispersing lipids, cofactors or transmembrane peptides would increase hydrocarbon/fluorocarbon contacts. By the same token, however, fluorinated surfactants can be expected to be poorly efficient at preventing membrane proteins from aggregating. The latter prediction was fulfilled in experiments that we [19,20] and others [21–23] carried out some years ago. In brief, these early attempts in general showed that fluorinated surfactants were unable not only to solubilize membrane proteins, but also to maintain them in solution after they had been extracted from membranes using a classical detergent. In a couple of cases, however, the molecules tested, used at high concentrations, limited or slowed down protein precipitation as compared to surfactant-less controls, suggesting that membrane protein/fluorinated surfactant interactions were weak, but not non-existent [20]. The enzymatic activity of our main test protein, cytochrome b_6f, a complex that is particularly detergent-sensitive [24], was maintained even well above the CMC of one of the two surfactants tested [20,25]. It was clear, however, that the best preparations themselves were on the verge of precipitation even at the highest surfactant/protein ratios that could be reasonably achieved.

Is it conceivable to improve the affinity of such surfactants for the hydrophobic, methyl-covered transmembrane surface of the proteins without increasing their detergency? One possible way out of this dilemma is suggested by a consideration of the ways detergents are thought to interact with lipids, on the one hand, and with membrane proteins, on the other. While, to our knowledge, no molecular dynamics study of lipid/detergent mixed micelles has been published but for the special case of lipid/bile salt complexes [26], such studies have been carried out on micelles of pure surfactants [16,27–30]. They show a high degree of disorder and a rapidly fluctuating structure, quite remote from the well-ordered spheres or ellipsoids by which micelles are ordinarily represented. The acyl chains of a lipid molecule dissolved in such an environment would likely experience contact, at one time or another, with all of the atoms comprising the hydrophobic moiety of the detergent. The amount of detergent bound to membrane proteins has been measured using radiolabeled detergents [6,31] and the structure of the adsorbed detergent layer studied by crystallographic [32,33] (reviewed in [9]) and nuclear magnetic resonance [34] approaches. These data are compatible with the detergent forming a close-packed palisade in which molecules stay more or less parallel to each other, with the tip of their hydrophobic moiety in contact with the protein and their polar head facing the solution [6]. While such a schematic description is bound to be oversimplified, it suggests that surface atoms of the protein are more often in contact with the terminal atoms of the detergent's hydrophobic chain than with those closer to the polar head. Substituting the last few fluorine atoms of a perfluorinated chain with hydrogen, therefore, might be expected to increase the frequency of protein/hydrocarbon contacts at the expense of protein/fluorocarbon ones, thereby improving the affinity of the surfactant for the protein. At the same time, fluorinated groups would still predominate in the core of the micelles, preserving their lipophobic character. Whether correct or not, this view led us to design and synthesize a series of 'hemifluorinated' surfactants [35–37]. We present here a study of the biochemical properties of a member of this family, $C_2H_5C_6F_{12}C_2H_4$-*S*-poly-*Tris*-(hydroxymethyl)aminomethane (HF-TAC, Fig. 1). Our first observations support the view that this novel type of surfactant may indeed hold some potential in membrane biochemistry.

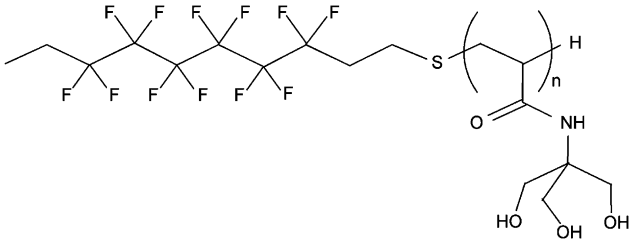

Fig. 1. Chemical structure of HF-TAC. CMC = 0.45 mM. Two batches of HF-TAC were used in the present work, one with $\langle n\rangle = 10$ ($\langle MW\rangle \approx 2140$ g/mol) and one with $\langle n\rangle = 6$–7 ($\langle MW\rangle \approx 1520$ g/mol).

2. Materials and methods

2.1. Chemicals

HF-TAC (Fig. 1) was synthesized as described in [35]. Two batches were used in the present experiments. For batch 1, used for experiments on wild-type cytochrome b_6f complex purified in the presence of mixed micelles (see below), $\langle n\rangle \approx 10$ and $\langle MW\rangle \approx 2140$ g/mol (compound **4e** in [35]); for batch 2, used in all other experiments, $\langle n\rangle \approx 6$–7 and $\langle MW\rangle \approx 1520$ g/mol. The CMC of the two compounds is ~0.45 mM [35]. H-TAC ($C_{12}H_{25}$-*S*-poly-*Tris*-(hydroxymethyl)aminomethane), a non-fluorinated homologue of HF-TAC, was synthesized as described in [38]. The batch used in the present work had an average degree of polymerization $\langle n\rangle \approx 7$. Its CMC is ~0.15 mM [38]. F-TAC ($C_8F_{17}C_2H_4$-*S*-poly-*Tris*-(hydroxymethyl)aminomethane), a perfluorinated homologue ($\langle n\rangle \approx 6$, CMC ≈ 0.03 mM), was synthesized as described in [39].

Classical detergents were purchased from Anatrace except for Hecameg (HG), which was from Vegatec (Villejuif, France). All other chemicals were as in [40].

2.2. Proteins

Cytochrome b_6f was purified either in the presence of HG and extraneous lipids from a *Chlamydomonas reinhardtii* wild-type strain [40] or in the presence of low dodecyl-β-D-maltoside (C_{12}-M) concentrations from a strain in which a histidine tag had been added at the C-terminus of cytochrome *f* [41]. In the first case, the purified protein was in 20 mM HG (CMC = 19.5 mM) supplemented with 0.1 g/l egg phosphatidylcholine ('HG/PC' mixture), and in the second in 0.2 mM C_{12}-M (CMC = 0.17 mM), both in 20 mM Tris–HCl buffer, pH 8.0. The plastoquinol:plastocyanin electron transfer activity of cytochrome b_6f was measured as described in [40]. The b_6f concentration of the different samples used ranged from 0.9 to 0.4 μM. Purple membrane was purified from the overproducing strain S9 of *Halobacterium salinarum* as described in [42]. Bacteriorhodopsin (BR) was solubilized at 4°C with 100 mM octylthioglucoside (OTG; CMC = 9 mM) at a membrane concentration of 15 g/l in 20 mM sodium phosphate buffer, pH 7.4. The transmembrane domain of OmpA (tOmpA) was overexpressed as inclusion bodies in the BL21 strain of *Escherichia coli* using the PET3b-171OmpA vector. Purification and renaturation were performed as in [43]. The purified protein was in 19.6 mM *N*-octyl-tetraoxyethylene (C_8E_4; CMC = 8.5 mM), 20 mM Tris–HCl buffer, pH 8.0.

2.3. Surfactant exchange

Purified proteins in their respective detergent were supplemented with 1 mM HF-TAC and incubated 15 min prior to being loaded onto a 10–30% (w/w) sucrose gradient containing 20 mM of their respective buffer and either 1, 2 or 5 mM HF-TAC. bR samples were diluted to reach a final detergent concentration of 30 mM OTG prior to loading on the gradients. Control experiments were performed in gradients containing either HG/PC or 0.2 or 5 mM C_{12}-M for cytochrome b_6f, 19.6 mM C_8E_4 for tOmpA, 15 mM OTG for BR, or no surfactant at all. Gradients were centrifuged for 4–4.5 h at 55 000 rpm (200 000 × *g*) in the TLS 55 rotor of a TL100 ultracentrifuge (Beckman). The bands containing the colored proteins, b_6f and BR, were collected with a syringe, whereas the gradients containing tOmpA were fractionated and the protein distribution determined from its absorbance at 280 nm. Protein samples were kept on ice in the dark.

3. Results

3.1. HF-TAC keeps membrane proteins soluble in the absence of detergent

The ability of HF-TAC to keep membrane proteins soluble was tested on three model proteins representative of different types of structures: (i) bacteriorhodopsin (BR), an archaebacterial protein folded into a bundle of seven α-helices with small extramembrane loops, to which a molecule of retinal is covalently but loosely bound [44]; (ii) tOmpA, an eight-strand β-barrel that forms the transmembrane region of outer membrane protein A from the Gram-negative eubacterium *E. coli* [45]; and (iii) cytochrome b_6f. The b_6f complex, extracted from thylakoid membranes of the chloroplast of the unicellular green alga *C. reinhardtii*, is a superdimer, each monomer being comprised of eight subunits and numerous cofactors such as hemes, an iron–sulfur cluster, a chlorophyll, a carotenoid and lipids. Its transmembrane region comprises 13 α-helices per monomer [41]. These three proteins are good models to validate the use of a new surfactant, for each of them is rather sensitive to the exposure to detergents: the spectrum of the molecule of retinal bound to BR is a sensitive reporter of the conformation of the polypeptide, and the cofactor is readily released upon denaturation [46]; the three-dimensional structure of tOmpA is very rugged, but the protein aggregates easily; and the b_6f complex is extremely sensitive to the delipidation induced by exposure to an excess of detergent, which causes its partial dissociation, including the loss of its associated chlorophyll, monomerization and inactivation [24].

Each protein was supplemented with HF-TAC (see Section 2) and analyzed on sucrose gradients containing either 1, 2 or 5 mM HF-TAC. As a control, samples not supplemented with HF-TAC were applied onto gradients containing the detergent in which the protein was originally purified. In the presence of HF-TAC, the three proteins migrated principally as one main band. This is illustrated for the b_6f complex in Fig. 2A. In the case of tOmpA, however, the position and width of the band depended on the concentration of surfactant (Fig. 2B), indicating that a high concentration of HF-TAC is needed in order to prevent aggregation.

Under all conditions, proteins migrated further into the gradient in the presence of HF-TAC than in that of classical detergents. This is consistent with the higher density expected for fluorinated surfactants. The discrepancy is particularly striking in the case of tOmpA, because the detergent used

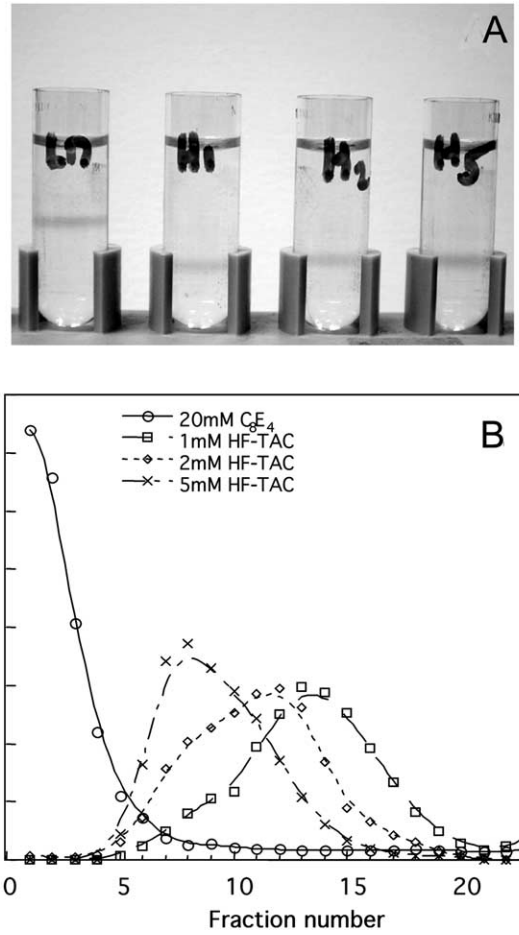

Fig. 2. Migration of membrane proteins upon centrifugation in sucrose gradients in the presence of either a detergent or a HFS. A: Distribution of histidine-tagged cytochrome b_6f in gradients containing, from left to right, either 0.2 mM C_{12}-M or 1, 2 or 5 mM HF-TAC. Gradients were centrifuged for 4 h at 200 000 × *g*. B: Distribution of tOmpA in gradients containing either 19.6 mM C_8E_4 or 1, 2 or 5 mM HF-TAC. Gradients were fractionated after 4.5 h of centrifugation at 200 000 × *g* and protein distribution was determined by light absorption at 280 nm.

for the purification, C_8E_4, has a density close to 1 g/ml [47] and is present in a 1.3:1 C_8E_4/tOmpA mass ratio (M. Zoonens, personal communication): after 4.5 h of centrifugation, the tOmpA/C_8E_4 complex had barely started to enter the gradient, while the tOmpA/HF-TAC one had migrated almost half-way down it.

In keeping with previous observations [24], after 4 h of migration in a gradient containing 5 mM C_{12}-M the b_6f complex had already started to monomerize, as shown by the presence of two bands in the gradient (not shown). No such phenomenon was observed in the presence of HF-TAC, whatever its concentration (Fig. 2A).

3.2. *Bacteriorhodopsin is more stable in HF-TAC than in OTG solutions*

After 4 h of centrifugation in the presence of 15 mM OTG, the spectrum of BR featured a peak at 390 nm, characteristic of free retinal (Fig. 3A). Free retinal was present at a much lower concentration in the sample centrifuged in the presence of 5 mM HF-TAC (Fig. 3A). After 14 days of storage, the difference was even more pronounced: while a further 36% of the BR had denatured in the OTG sample, BR in HF-TAC remained largely intact (drop of ~15%) (Fig. 3B). The visible absorbance spectrum of BR peaked at the same wavelength (~555 nm) in the two surfactants, indicating that transfer to HF-TAC had not reversed the typical detergent-induced blue shift [48].

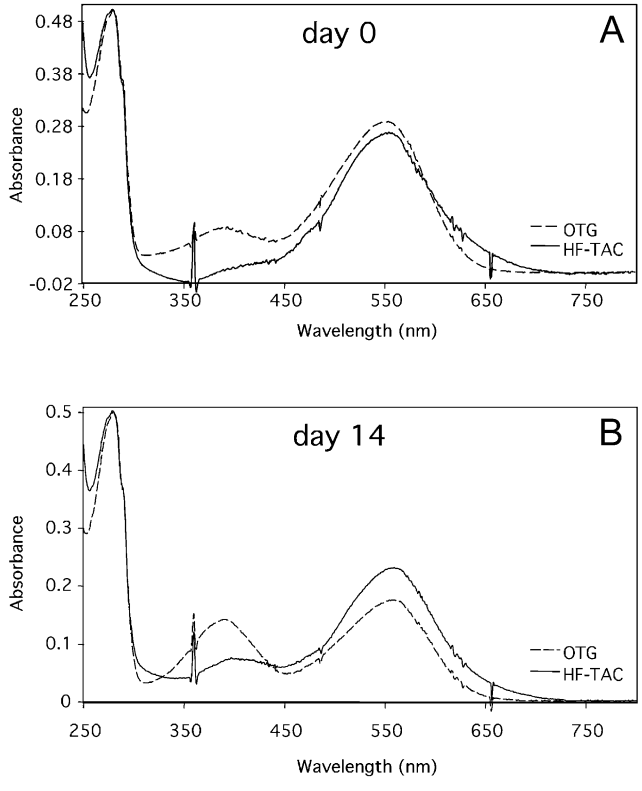

Fig. 3. Spectrum of BR incubated either in 15 mM OTG (dashed line) or in 5 mM HF-TAC (solid line). A: Immediately after 4 h of centrifugation in sucrose gradients. B: After 14 days of incubation on ice in the dark.

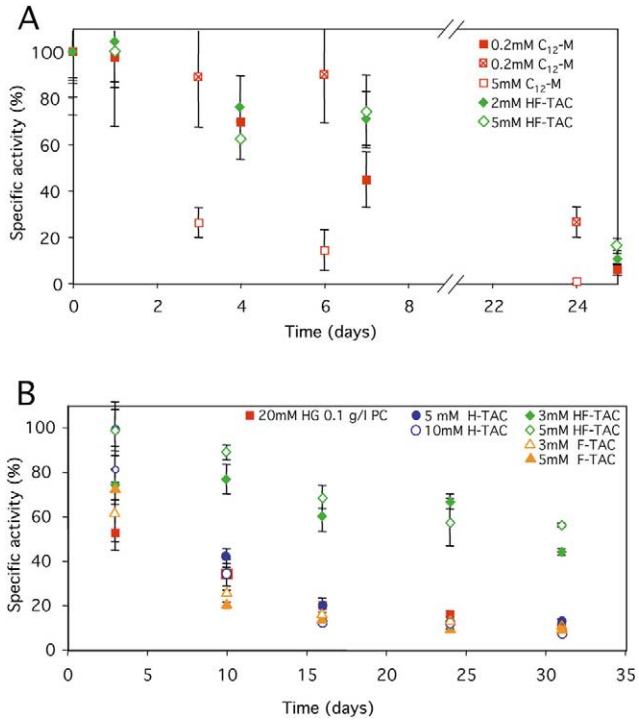

Fig. 4. Evolution over time of the enzymatic activity of two preparations of cytochrome b_6f incubated in different surfactants. A: Histidine-tagged b_6f purified in C_{12}-M and incubated in either 0.2 mM C_{12}-M (two distinct samples, initial specific activities 359 and 278 e^-/s per mol of cytochrome *f*), 5 mM C_{12}-M (514 e^-/s), or 2 or 5 mM HF-TAC (320 and 338 e^-/s, respectively). Specific activities were normalized to the initial activity of each sample, measured on day 0. B: Wild-type b_6f purified in HG/PC mixed micelles and incubated either in 20 mM HG, 0.1 g/l PC (specific activity on day 3, 79 e^-/s), in 3 or 5 mM HF-TAC (111 and 148 e^-/s, respectively), in 5 or 10 mM H-TAC (149 and 122 e^-/s, respectively), or in 3 or 5 mM F-TAC (93 and 110 e^-/s, respectively). Specific activities were normalized to 100% = 150 e^-/s. Samples were incubated in the dark on ice.

3.3. *An excess of HF-TAC micelles is well tolerated by cytochrome* b_6f

The spectrum of the chlorophyll associated to cytochrome b_6f is very sensitive to its environment, shifting from 666–667 nm in the native state to 669–670 nm when the complex is perturbed [24,49]. In HF-TAC solutions, the chlorophyll spectrum peaked at ~666 nm, witnessing the native state of the complex (not shown). This was confirmed by the high specific activity measured immediately after surfactant exchange, which was comparable to that of a control sample centrifuged on a gradient containing 0.2 mM C_{12}-M, the detergent used for the purification (Fig. 4A).

Upon prolonged incubation of the b_6f complex in either surfactant solution, the specific activity decreased, rapidly in 5 mM C_{12}-M, much more slowly in 0.2 mM C_{12}-M or 5 mM HF-TAC (Fig. 4A). Whereas the stability of cytochrome b_6f in HF-TAC is equivalent to that in C_{12}-M for low surfactant concentrations, it becomes much higher in more concentrated solutions: after a week in 5 mM C_{12}-M, the complex had lost ~85% of its activity, whereas the drop was only ~25% in 5 mM HF-TAC (Fig. 4A).

3.4. The stability of the b_6f complex in HF-TAC solutions improves in the presence of lipids

Interestingly, the stability of the b_6f complex in HF-TAC solutions depends on its mode of purification prior to the transfer. Indeed, the b_6f complex purified in mixed HG/PC micelles was much more stable following trapping with HF-TAC than when purified in C_{12}-M solutions devoid of extraneous lipids: whereas the stability of the complex is similar in 0.2 mM C_{12}-M and HG/PC micelles (Fig. 4), the HG/PC complex transferred to HF-TAC retained ~60% activity after a month of storage (Fig. 4B), by which time the complex transferred from C_{12}-M was largely inactivated (Fig. 4A). While there are other differences between the two sets of experiments (one of the two complexes is histidine-tagged, the other is not, and the two batches of HF-TAC have polar heads of slightly different sizes – see Section 2), the most probable explanation for this discrepancy is the stabilizing effect of lipids. Previous experiments indeed have shown that 36 ± 22 molecules of egg PC are retained by each b_6f dimer after they have been transferred from HG/PC to 0.2 mM C_{12}-M [24]. Given the non-detergent character of HFS, it is reasonable to expect that at least this amount will remain associated following transfer from HG/PC to HF-TAC.

In the same series of experiments, we noted that the b_6f complex resisted about as well being transferred to HF-TAC, to H-TAC, a non-fluorinated homologue, or to F-TAC, a perfluorinated one (see also [20]), but that its long-term stability was much higher in HF-TAC than in either its non-fluorinated or its perfluorinated version (Fig. 4B).

4. Discussion

The present work establishes that HF-TAC, a HFS, can be used to keep water-soluble membrane proteins that have been initially solubilized using a classical detergent. The three test proteins used here, cytochrome b_6f, BR, and tOmpA, cover a wide range of origins (eukaryotic, archaebacterial, eubacterial), secondary and quaternary structures (from one to 16 subunits, α-helix bundle vs. β-barrel), sizes (from 19 to ~200 kDa), functions, and compositions. In particular, BR, solubilized under the conditions used in this work, retains most of the purple membrane lipids [50], the b_6f complex is associated, depending on its mode of purification, either with natural [41] or with extraneous lipids [24], while preparations of tOmpA (which is purified from inclusion bodies) do not contain any (P. Hervé and M. Zoonens, unpublished data). Examination of the composition and enzymatic activity of cytochrome b_6f solubilized and purified in HG/PC or C_{12}-M and transferred to HF-TAC confirmed the very mild character of this surfactant: the b_6f complex, which is rapidly inactivated if exposed to millimolar concentrations of C_{12}-M, stood exposure to 5 mM HF-TAC very well. Its stability under these conditions, where micelles represent ~4.5 mM of surfactant and ~0.5% by volume of the solvent phase, was comparable to that in 0.2 mM C_{12}-M, where the volume of the micellar phase, and therefore of the sink available for dissociation, is at least two orders of magnitude less. BR, similarly, was found to be more stable in HF-TAC than in OTG. By comparison, the perfluorinated (F-TAC) or unfluorinated (H-TAC) versions of HF-TAC were much less satisfactory: in F-TAC, the b_6f was initially kept soluble, but was on the verge of aggregation [20,25] and, probably for this reason, inactivated rapidly upon storage ([25] and Fig. 4B). In H-TAC, the complex was initially soluble and active, but its stability was much inferior, under comparable conditions, to that in HF-TAC ([25] and Fig. 4B). These observations are consistent with our initial expectation that adding an ethyl tip at the extremity of the fluorinated moiety of HF-TAC would increase its affinity for membrane proteins without making it dissociating.

Our observations indicate that the mildness of HF-TAC is due to two of its molecular features. On the one hand, H-TAC, the fully hydrogenated homologue of HF-TAC, is by itself a mild detergent (Fig. 4B), a behavior that we have tentatively attributed to the bulkiness of its polar moiety [20,25]. On the other, (i) the long-term stability of the b_6f complex is much higher in HF-TAC than in H-TAC and (ii) several analogues of HF-TAC with the same hemifluorinated tail and different polar heads show a comparable mildness ([25]; E. Rousselet and C. Breyton, unpublished observations). The latter two observations point to the non-dissociating character of the hemifluorinated tail.

An interesting observation, very much in line with the reasoning that initially led us to design HFS, is that the stability of b_6f/HF-TAC complexes was found to depend on the way the b_6f complex was initially prepared: complexes purified in the presence of mixed lipid/detergent micelles were found to be, following transfer to HF-TAC, more stable than complexes that had retained some endogenous lipids but had not been supplemented with extraneous ones. This suggests that they had retained, after transfer to HF-TAC, at least some of the extraneous lipids initially bound to them, despite the surfactant being in vast excess (~250 mol of micellar HFS per mol of egg PC in 5 mM HF-TAC solutions for a b_6f concentration of 1 μM). This is consistent with one of the probable mechanisms by which HF-TAC provides a mild environment being its non-delipidating character. Both BR and b_6f complexes purified without addition of egg PC retain endogenous lipids [41,50]. It is not possible, on the basis of the present data, to determine whether their better stability in HF-TAC as compared to detergent solutions is entirely due to the non-delipidating character of the HFS, or whether its expected less intrusive character (due to poor van der Waals interactions, but also to the bulkiness and rigidity of the fluorinated region) may also play a role.

While these results are promising, the validation of HFS as a useful new tool for membrane biochemistry calls for significant investments in the fields of chemistry, physical chemistry and biochemistry. For one thing, the polymeric nature of the polar head of HF-TAC, while very efficient to ensure a good solubility, is not a desirable feature from the point of view of the biochemist. Batches of HF-TAC are mixtures of molecules with slightly different characteristics. One consequence thereof is that the surfactant layer associated with a membrane protein can have a composition different from the average one. Another is that different batches with, inevitably, different chemical compositions may feature slightly different physicochemical and biochemical properties, never a desirable feature when working with temperamental proteins. For this reason, we have started developing HFS with chemically monodisperse polar heads ([25,36,37]; E. Rousselet and C. Breyton, unpublished observations). Optimization may also be sought as regards the relative length of the hydrocarbon and fluorocarbon sections of the hydrophobic tail. It should not be underestimated, however, that the chemistry of hemifluori-

nated compounds is difficult and that these developments represent a time-consuming and costly endeavor.

The chemical and physico-chemical properties of those molecules that appear the most promising from the point of view of biological applications will have to be studied in detail. For one thing, it is essential to establish their chemical stability over extended periods of time under the usual conditions of biochemical work. Synthesis protocols will have to be scaled up to the stage where the gram amounts required by biologists can be produced at an acceptable cost. Another point worthy of interest is the special properties of those hydrogen atoms of the hydrophobic tail that are vicinal to the fluorinated section. Because electrons are attracted by the latter, vicinal H atoms are thought to bear partial positive charges (α-β elimination reactions commonly observed with functionalized 1H,1H,2H, 2H-perfluoroalkyl compounds are accounted for by the acidity of the F-vicinal methylene). They may therefore well be able to form hydrogen bonds with water or with the protein.

The physical chemistry of aqueous solutions of HFS forms a fascinating new field. Micellar properties, for instance, reflect the fact that the contributions of each section of the tail to the free enthalpy of transfer between water and the micellar core do not add linearly, as they do for fully hydrogenated or fluorinated surfactants [51,52]. The CMC of HFS is, indeed, higher than additivity would lead one to expect [35,53]. This could be explained (i) by the unfavorable enthalpy contribution of mixing fluorinated and hydrogenated groups in the core of the micelles (hydrogenated and fluorinated surfactants mix non-ideally, to the point of forming separate micelles; see [16,54,55], and references therein), and/or (ii) by the polar character of F-vicinal methylenes, which should work against transfer of the hydrophobic moiety from the aqueous solution to the core. It is interesting to note that, while both H-TAC and F-TAC form stable Newton black films, HF-TAC does not [53], which may again betray the less favorable free enthalpy of transfer of the hemifluorinated moiety from water to a hydrophobic phase. Validation of HFS for biochemical and biophysical work will call for a careful study of their micellar properties and phase diagrams.

HFS were initially designed with the view of providing a novel solution to the old problem of stabilizing membrane proteins in aqueous solutions. They have, however, other interesting putative applications. To mention two, one of them would be to deliver membrane proteins to preformed lipid bilayers, whether those of live cells, of lipid vesicles or of black films. This is difficult to control with classical detergents, which are lytic above their CMC and usually unable to prevent membrane protein aggregation under it. Another type of application that may deserve to be investigated is the formation of monolayers at the air–water interface for the purpose of membrane protein two-dimensional crystallization. HFS ought to retain many of the advantages of hemifluorinated lipids [56], while being (relatively) easier to synthesize. Alternatively, they could be used to stabilize membrane proteins to be crystallized under monolayers of unfluorinated lipids. Other interesting applications are likely in the fields of membrane protein renaturation and of galenics.

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