

Fluorinated and hemifluorinated surfactants as alternatives to detergents for membrane protein cell-free synthesis

Kyu-Ho Park, Catherine Berrier, Florence Lebaupain, Bernard Pucci, Jean-Luc Popot, Alexandre Ghazi, Francesca Zito

► To cite this version:

Kyu-Ho Park, Catherine Berrier, Florence Lebaupain, Bernard Pucci, Jean-Luc Popot, et al.. Fluorinated and hemifluorinated surfactants as alternatives to detergents for membrane protein cell-free synthesis. Biochemical Journal, 2006, 403 (1), pp.183-187. 10.1042/BJ20061473 . hal-00478673

HAL Id: hal-00478673 https://hal.science/hal-00478673

Submitted on 30 Apr 2010 $\,$

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers. L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Fluorinated and hemifluorinated surfactants as alternatives to detergents for membrane protein cell-free synthesis

Kyu-Ho Park^{*}, Catherine Berrier[†], Florence Lebaupain^{*}, Bernard Pucci[‡],

Jean-Luc Popot^{*}, Alexandre Ghazi^{\dagger} and Francesca Zito^{*1}

* Laboratoire de Physicochimie Moléculaire des Membranes Biologiques, UMR 7099, CNRS and Université Paris-7, Institut de Biologie Physico-Chimique, CNRS FRC 550, F-75005 Paris, France.

[†] Institut de Biochimie et de Biophysique Moléculaire et Cellulaire UMR 8619, CNRS and Université Paris-Sud, Bâtiment 430, F-91405 Orsay Cedex, France.

[‡] Laboratoire de Chimie Bioorganique et des Systèmes Moléculaires Vectoriels, Université d'Avignon et des Pays du Vaucluse, F-84000 Avignon, France.

Short title: Membrane proteins cell-free synthesis for functional membrane insertion

Keywords: Membrane protein; Detergent; Fluorinated surfactants; Hemifluorinated surfactants; MscL; *In vitro* protein synthesis; Membrane delivery

Abbreviations: CMC, critical micellar concentration; HFS, hemifluorinated surfactant; FS, fluorinated surfactant; HF-TAC, C₆F-TAC and C₈F-TAC, C₂H₅C₆F₁₂C₂H₄-, C₆F₁₃C₂H₄- and C₈F₁₇C₂H₄-*S*-poly-*Tris*-(hydroxymethyl)aminomethane, respectively; MP, membrane protein; SDS-PAGE, sodium dodecylsulfate-polyacrylamide gel electrophoresis

¹ Corresponding author; Tel. +33 (0)1 58 41 51 09; Fax +33 (0)1 58 4150 24 e-mail: Francesca.Zito@ibpc.fr

Abstract

Hemifluorinated and fluorinated surfactants are lipophobic and, as such, non-detergent. While they do not solubilise biological membranes, they can, after conventional solubilisation, substitute to detergents to keep membrane proteins soluble, which generally improves their stability (Breyton *et al. FEBS Lett.* **564**, 312-318, 2004). Here, we show that (hemi)fluorinated surfactants can be used for *in vitro* synthesis of membrane proteins: they do not interfere with protein synthesis, and they provide a suitable environment for MscL, a pentameric mechanosensitive channel, to fold and oligomerize to its native, functional state. Following synthesis, both types of surfactants can be used to deliver directly MscL to preformed lipid vesicles. The electrophysiological activity of MscL synthesized *in vitro* in the presence of either hemi- or perfluorinated surfactant is similar to that of the protein expressed *in vivo*.

INTRODUCTION

Non-ionic surfactants with fluorocarbon hydrophobic tails (FSs, Fig. 1) are both highly tensioactive and lipophobic: because they do not interact favourably with the alkyl chains of natural lipids, their partition coefficient into biological membranes is low, which renders them non-cytolytic and inefficient at extracting membrane proteins (MPs) (see *e.g.* refs. [1-4]). However, after solubilisation using a classical detergent, they can substitute to the latter to keep MPs water-soluble [5]. To improve interactions with the hydrogenated transmembrane surface of MPs, a hydrogenated tip can be grafted at the end of the fluorinated tail, yielding 'hemifluorinated' surfactants (HFSs) [6]. Previous work has shown that two model MPs, bacteriorhodopsin and the b_6f complex, are stabilized by transfer to a HFS, HF-TAC (Fig. 1), as compared to storage in equivalent concentrations of detergent [7]. This is likely due to the fact that (H)FSs are less dissociating than classical detergents. The non-cytolytic character of HFSs has been exploited to reinsert into preformed lipid vesicles the water-insoluble low-pH form of the pore-forming fragment of diphteria toxin without destabilizing the target vesicles [8].

Over-expressing MPs in vivo is notoriously difficult because, unlike soluble proteins, MPs will not properly fold unless targeted to a membrane. If insertion in the host membrane is a limiting factor, an alternative strategy is to synthesize the protein in a cell-free system, in the absence of membrane but in the presence of surfactant. Cell-free synthesis of MPs under their functional form has recently been reported for EmrE [9,10], MscL [11], a bacterial lightharvesting complex [12], several G-protein coupled receptors [13,14], and Tsx, a MP of the β barrel type [14]. Most MPs are unstable in detergent solutions, however, which indicates that their native fold does not, in this environment, lie at their free energy minimum. It is therefore of great interest to examine whether milder surfactants such as (H)FSs are compatible with cell-free synthesis and folding. As a model MP, we have used the mechanosensitive channel MscL from Escherichia coli, a pentameric protein whose protomer (14 kDa) comprises two transmembrane helices [15]. We have shown previously that MscL can be produced *in vitro* in the presence of various detergents [11]. After purification and reconstitution, the functionality of the protein can be assessed at the single-molecule level by patch-clamp measurements. In this report, we establish that (H)FSs are compatible with in vitro synthesis, folding and oligomerisation of MscL. In addition, we show that they allow direct and rapid incorporation of functional MscL into preformed lipid bilayers.

2. EXPERIMENTAL

2.1. Organic synthesis

Two forms of F-TAC, bearing either a C_6F_{13} - (C_6F -TAC) or a C_8F_{17} - (C_8F -TAC) fluorinated chain, were synthesized by radical polymerisation in the presence of fluoroalkyl mercaptan as transfer reagent [18]. The synthesis of HF-TAC followed the same chemical pathway, using a hemifluorinated mercaptan as transfer reagent [6]. Stock solutions (100 mM in water) were kept at 4°C. Chemical structures, molecular masses and CMCs are given in Fig. 1 and its legend.

2.2. In vitro protein synthesis.

Cell-free expression was carried out using the Roche system [16], in which an *E. coli* lysate provides the machinery to drive coupled transcription and translation in the presence of a DNA template. For large-scale production, a semipermeable membrane allows for a continuous supply of substrates and the removal of inhibitory by-products, thus extending the duration of expression and the protein synthesis yield [17].

For small-scale test (50 μ l), 10 μ g/ml of plasmid carrying the *MscL* coding region [11] were incubated for 6 hours in the Roche RTS 100 HY (High Yield) lysate with either HF-TAC, C₆F-TAC or C₈F-TAC at the concentrations indicated on the figure 2A.

When the RTS 500 HY lysate (1 ml) was used for preparative experiments, 15 μ g/ml of plasmid were incubated for 20 hours in the Roche RTS 500 HY lysate. The reaction system was supplemented either with either 2 mM HF-TAC or 4 mM of either F-TAC. Surfactants were added at the same concentration in the feeding chamber. Radiolabelling of MscL was achieved by addition of 0.4 and 2.2 MBq (2.8 TBq/mmole) of L-[methyl-³H]methionine (Amersham Biosciences) in the reaction and feeding chamber, respectively.

All syntheses were carried out according to the manufacturer's protocols.

2.3. Biochemical analysis and protein purification

At the end of the small-scale tests, 10μ L samples were diluted with the same volume of 5mM Tris-HCl pH 8.0 buffer, then centrifuged for 10 min at room temperature and 16000 × g to check for protein solubility. For western blot proteins were separated on a 15% 8M urea SDS-PAGE. The tagged protein was detected by immuno-blotting, using an antibody against the His₆ tag (Sigma). Following large-scale synthesis, in *vitro* produced MscL was purified as described in ref. [11], in the presence of 2 mM (H)FSs. The purified protein samples were separated on a 15% SDS-PAGE. Concentrations were determined using the Bicinchoninic acid method (Pierce Chemicals). Purified samples were kept at 4°C until reconstitution into liposomes

2.4. Integration of MscL into liposomes

Purified radiolabelled MscL was added to a suspension of sonicated liposomes (1 mg of asolectin from soybean, type IV-S, Sigma) in 10 mM HEPES-KOH, pH 7.4, 100 mM KCl (liposome suspension buffer), to achieve a lipid to protein ratio of 40:1 (w/w). The suspension of liposomes contained (H)FSs at the same concentration as the MscL solution. Either immediately or after 30-min incubation at room temperature, the mixture was centrifuged at $300,000 \times g$ for 20 min and the radioactivity associated to the pellet and the supernatant counted. Control experiments were performed in the same way but in the absence of liposomes.

For flotation experiments, liposomes were incubated with MscL in HF-TAC. The mixture was adjusted to 1.5 M sucrose (final volume 400 μ l), overlaid with 330 μ l of 0.3 M sucrose in suspension buffer and topped with 100 μ l of sucrose-free buffer. Following centrifugation at 300,000 × g for 150 min in the TLA-100.2 rotor of a TL100 centrifuge (Beckmann), five fractions of 100 μ l, 350 μ l and 3 × ~120 μ l, respectively, were collected from the top, the last fraction being used to resuspend any eventual pellet, and the radioactivity present in each fraction counted.

2.5. Formation of giant proteoliposomes

Proteoliposomes were prepared as described in the previous section, except that the suspension was diluted in 3 ml of liposome suspension buffer before ultracentrifugation. The pellet was washed in 3 ml of the same buffer, and then resuspended in 20 μ l of 10 mM HEPES-KOH, pH 7.4. To obtain giant proteoliposomes amenable to patch-clamp measurements, this suspension was subjected to a dehydration/rehydration cycle as previously described [18].

2.6. Patch-clamp measurements

Single-channel activity was recorded using standard patch-clamp methods [19]. Patch electrodes were pulled from Pyrex capillaries (Corning code 7740) using a P-2000 laser

pipette-puller (Sutter Instruments Co.); they were not fire-polished before use. Micropipettes were filled with a buffer similar to that of the patch-clamp chamber, supplemented with 2 mM MgCl₂ and 5 mM CaCl₂. Negative pressure (suction) was applied to the patch pipette by a syringe and monitored with a piezo-electric pressure transducer (Bioblock Scientific). Unitary currents were recorded using a Biologic RK-300 patch-clamp amplifier with a 10-gigaOhm feedback resistance. Records were subsequently filtered at 1 kHz (-3 dB point) through a four-pole Bessel low pass filter, digitized off-line at a rate of 2 kHz, and analyzed on a personal computer using Pclamp (Axon). Data were plotted using Sigmaplot software (Jandel).

3. RESULTS

3.1 Cell-free synthesis of MscL in hemifluorinated and fluorinated surfactants

Previous experiments have shown that it is possible to express MscL in a cell-free system in the presence of various detergents, in the absence of membranes [11]. Here, we performed *in vitro* synthesis of MscL in the presence of (H)FSs [7, 20]. Small scale syntheses were carried out in presence of either C₆F-TAC, C₈F-TAC or HF-TAC at either 2, 5 or 18 mM, which corresponds respectively to ~6, ~15 and ~60× the CMC of C₆F-TAC, ~60, ~150 and ~600× that of C₈F-TAC, and ~2, ~10 and ~40× that of HF-TAC (Fig. 2A and B). For the two fluorinated surfactants, we additionally performed syntheses at 50 mM FS, *i.e.* ~150× (C₆F-TAC) and ~1500× (C₈F-TAC) their CMC (data not shown). All conditions were found to be compatible with *in vitro* synthesis. In all cases, the protein produced was soluble (Fig. 2A and B). In contrast, when 1 mM HF-TAC, was used part of the protein was found in the pellet after centrifugation, indicating that this concentration of surfactant was not sufficient to maintain the protein in a soluble form (data not shown).

To obtain large amounts of purified protein we scaled up the synthesis using the RTS 500 HY lysate. Large-scale synthesis of MscL was carried out in the presence of either 2 mM HF-TAC (Fig. 2C), 4 mM C₆F-TAC, or 4 mM C₈F-TAC. The protein was radiolabelled using L-[methyl-³H]methionine in order to facilitate monitoring of MscL incorporation into lipid vesicles. Syntheses were performed for 20 h at 30° C and the protein purified on a Ni-NTA column (Fig 2C). The yield of all three large-scale syntheses was ~1 mg of pure protein per ml of lysate, a value comparable to that obtained in the presence of 0.2% Triton X-100 [11]. The oligomeric state of MscL synthesized in the presence of (H)FSs was examined as described in ref. [11]. The protein was found to form native-like oligomers (data not shown).

3.2 Insertion of MscL into liposomes

Insertion of MscL into preformed liposomes was performed in the presence of either 1 mM HF-TAC, 3 mM C₆F-TAC or 3 mM C₈F-TAC. In keeping with previous observations [8], light diffusion measurements at 430 nm indicate that liposomes are not solubilised under these conditions, even though all three surfactants are present above their CMC. Purified radiolabelled MscL, synthesized in the presence of either HF-TAC or C₆F-TAC, was added to a 10 mM HEPES-KOH, pH 7.4, 100 mM KCl buffer containing asolectin liposomes, and either surfactant at the above concentration. Aliquots of the suspension were centrifuged either immediately or 30 min after addition of the protein, and the amount of $[^{3}H]$ methionine present in the pellet and supernatant determined. Most of it (~95% for HF-TAC, ~90% for C₆F-TAC) was found in the liposome pellet of the aliquot taken immediately after addition of the protein. The distribution observed after 30 min of incubation was not significantly different. Incorporation of MscL into liposomes therefore is faster than the time (20 min) required for centrifugation. All of the protein remained in the supernatant when the centrifugation was performed under similar conditions but in the absence of liposomes, showing the ability of all tested surfactants to maintain the protein in solution at the concentrations used.

To rule out the possibility that, during the insertion experiment, part of the radiolabelled proteins aggregated and subsequently pelleted with the liposome fraction, we performed flotation experiments with liposomes incubated with MscL in HF-TAC (see *Experimental*). Liposomes floated to the top of the 1.5 M-sucrose fraction. Fractions were collected and counted for radioactivity. In two separate experiments, 90% and 85% of the labelled MscL were recovered with the 0.3 M-sucrose fraction (data not shown). The radioactivity present in the last fraction, including any eventual pellet, was less then 4 %.

3.3 MscL synthesized *in vitro* in the presence of HFS or FS forms active channels once integrated into giant lipid vesicles

For functional assays, MscL was integrated into asolectin vesicles at a lipid-to-protein ratio of 40:1 (w/w) and the suspension washed free of surfactants by two rounds of ultracentrifugation. Giant proteoliposomes were generated by vesicle fusion, induced by a cycle of dehydration/rehydration. The resulting structures were assayed by patch-clamp. After seal formation in the micropipette, suction was applied to the membrane patch to induce opening

of mechanosensitive channels, at a membrane voltage of +10 mV. Mechanosensitive channels similar to the native ones were detected in all three preparations, opening under the applied pressure and closing upon its release (Fig. 3A).

The mechanosensitive activity was studied in detail so as to determine whether the use of (H)FSs is compatible with functional in vitro expression of MscL. The average conductance of the channels was found to be 1.52 ± 0.06 nS (HF-TAC), 1.44 ± 0.06 nS (C₆F-TAC) or 1.55 ± 0.05 nS (C₈F-TAC), the number of active channels per patch 6.0 \pm 1.4, 4.3 ± 1.3 and 5.2 \pm 1.2, respectively (Table 1, N_{obs}). These values are close to those observed for the same protein expressed in vivo and analysed under identical buffer and voltage conditions (1.5 nS of unitary conductance and 9.3 \pm 5.5 channels per patch, n = 26; ref. [11]). For each surfactant, up to 20 cumulated openings were observed in a single membrane patch. MscL channel is activated by membrane tension (T) rather than pressure (p). The channel dependence on pressure, $P_0(p)$, was therefore converted to dependence on tension, $P_0(T)$, according to a method previously used by Sukharev and co-workers [22]. Seven $P_0(p)$ curves were obtained for HF-TAC, five for C₆F-TAC and five for C₈F-TAC. For each (H)FS, $P_0(T)$ data sets were either fitted individually (Table 1), or cumulated (Fig. 3B), with a fixed half-maximal tension of 11.8 dyn/cm. Average values of $N_{\rm max}$, calculated from individual $P_0(T)$ curve fittings, are 7.1 ± 2.4 (*n* = 7), 10.0 ± 4.4 (*n* = 5), and 8.0 ± 1.3 (*n* = 5) channels per patch for channels synthesized in HF-TAC, C₆F-TAC, and C₈F-TAC, respectively. Their respective sensitivity to tension $(1/\beta)$ was 0.63 ± 0.13, 0.53 ± 0.20, and 0.68 ± 0.06 dyn/cm (see Table 1), which is similar to that of native MscL channel (0.63 dyn/cm; ref. [22]).

4. DISCUSSION

Perfluorocarbons are strongly hydrophobic without being lipophilic. As a result, compounds carrying a polar headgroup attached to a fluorinated chain, while they are very strong surfactants, are not detergents, due to their poor miscibility with lipids (see *e.g.* refs [2,3]). Presumably because they do not compete effectively with the protein/protein and protein/lipid interactions that stabilise the native fold of MPs, FSs and HFSs can keep MPs water-soluble under particularly mild conditions [5,7].

Here, we show that FSs and HFSs *i*) are compatible with *in vitro* protein synthesis and *ii*) provide an environment that allows efficient solubilisation, folding and assembly of a pentameric MscL. In parallel experiments, we have observed that, in addition to MscL, other MPs can also be synthesised *in vitro* in the presence of FSs or HFSs (unpublished results). At variance with detergents, (H)FSs do not interfere with protein synthesis up to 50× their CMC, suggesting that these surfactants interact weakly if at all with the transcription-translation machineries. Following integration into lipid vesicles, MscL synthesized *in vitro* in the presence of (H)FSs presented similar properties as native MscL, in terms of mechanosensitivity and number of channels formed per patch: this implies correct folding and oligomerisation, in keeping with biochemical observations. (H)FSs therefore appear as a very interesting alternative for the cell-free production of those MPs that will not achieve their native fold when synthesized in the presence of classical detergents.

Another interesting result of this study, which is relevant to membrane biochemistry and cell biology applications of (H)FSs, is the demonstration that MscL, in the presence of (H)FS concentrations above the CMC, can insert rapidly into preformed liposomes, without the latter being solubilised by the surfactants. This behaviour, which is in keeping with previous observations with the pore-forming fragment of diphtheria toxin [8], is consistent with the hydrophobic regions of MPs having a higher affinity for the lipids than for (H)FSs. Insertion without lysis of the target membrane is difficult to control using classical detergents. In this case, indeed, if the concentration of free detergent falls below the CMC, the layer of MP-associated detergent disassembles, which leads to protein aggregation, whereas detergent concentrations above the CMC, which keep MPs soluble, are lytic. F- and HF-TAC can be applied, above their CMC, to cultures of mouse embryonic stem cells or fibroblasts without killing the cells (unpublished data obtained in collaboration with K. Mesbah and J. Barra). Using (H)FSs, it should therefore be possible to directly deliver MPs not only to artificial lipid bilayers, but also to the plasma membrane of living cells.

Copyright 2006 Biochemical Society

9

5. ACKNOWLEDGEMENTS

We thank Cécile Breyton for critical reading the manuscript and fruitful discussion. F.L. and K.-H. P. were recipients of fellowships from the Ministère de l'Education Nationale, de la Recherche et de la Technologie and from the Fondation Rothschild, respectively. This work was supported by the CNRS, by the Universities of Paris-7, Paris-11, and Avignon et Pays du Vaucluse, and by the European Community Specific Targeted Research Project grant "Innovative tools for membrane protein structural proteomics".

6. REFERENCES

- Pavia, A.A., Pucci, B., Riess, J.G. and Zarif, L. (1991) New fluorinated biocompatible non-ionic telomeric amphiphiles bearing trishydroxymethyl groups. Bioorg. Med. Chem. Letters 1, 103-106
- [2] Myrtil, E., Zarif, L., Greiner, J., Riess, J.G., Pucci, B. and Pavia, A.A. (1995) Perfluoroalkylated telomers derived from tris(hydroxymethyl)acrylamidomethane as surfactants and co-surfactants in fluorocarbon emulsions. J. Fluorine Chem. 71, 101-105
- [3] Krafft, M.-P. and Riess, J.G. (1998) Highly fluorinated amphiphiles and colloidal systems. Biochimie **80**, 489-514
- Barthélémy, P., Tomao, V., Selb, J., Chaudier, Y. and Pucci, B. (2002) Fluorocarbonhydrocarbon non-ionic surfactant mixtures: a study of their miscibility. Langmuir 18, 2557-2563
- [5] Chabaud, E., Barthélémy, P., Mora, N., Popot, J.-L. and Pucci, B. (1998) Stabilization of integral membrane proteins in aqueous solution using fluorinated surfactants. Biochimie 80, 515-30
- [6] Barthélémy, P., Améduri, B., Chabaud, E., Popot, J.-L. and Pucci, B. (1999) Synthesis and preliminary assessment of ethyl-terminated perfluoroalkyl non-ionic surfactants derived from *Tris*(hydroxymethyl)acrylamidomethane. Org. Lett. 1, 1689-1692
- [7] Breyton, C., Chabaud, E., Chaudier, Y., Pucci, B. and Popot, J.-L. (2004) Hemifluorinated surfactants: a non-dissociating environment for handling membrane proteins in aqueous solutions? FEBS Lett. 564, 312-318
- [8] Palchevskyy, S.S., Posokhov, Y.O., Olivier, B., Popot, J.-L., Pucci, B. and Ladokhin,
 A.S. (2006) Chaperoning of membrane protein insertion into lipid bilayers by hemifluorinated surfactants: application to diphtheria toxin. Biochemistry 45, 2629-2635
- [9] Elbaz, Y., Steiner-Mordoch, S., Danieli, T. and Schuldiner, S. (2004) *In vitro* synthesis of fully functional EmrE, a multidrug transporter, and study of its oligomeric state.
 Proc. Natl. Acad. Sci. USA 101, 1519-1524
- [10] Klammt, C., Löhr, F., Schäfer, B., Haase, W., Dötsch, V., Rüterjans, H., Glaubitz, C. and Bernhard, F. (2004) High level cell-free expression and specific labeling of integral membrane proteins. Eur. J. Biochem. 271, 568-580

- [11] Berrier, C., Park, K.-H., Abes, S., Bibonne, A., Betton, J.-M. and Ghazi, A. (2004) Cell-free synthesis of a functional ion channel in the absence of a membrane and in the presence of detergent. Biochemistry 43, 12585-12591
- [12] Shimada, Y., Wang, Z.Y., Mochizuki, Y., Kobayashi, M., Nozawa, T. (2004) Functional expression and characterization of a bacterial light-harvesting membrane protein in Escherichia coli and cell-free synthesis systems. Biosci. Biotechnol. Biochem. 68, 1942-1948
- [13] Ishihara, G, Goto, M., Saeki, M., Ito, K., Hori T., Kigawa, T., Shirouzu, M., Yokoyama, S. (2005) Expression of G protein coupled receptors in a cell-free translational system using detergents and thioredoxin-fusion vectors. Protein. Expr. Purif. 41, 27-37
- [14] Klammt, C., Schwarz, D., Fendler, K., Haase, W., Dötsch, V., Bernhard, F. (2005) Evaluation of detergents for the soluble expression of alpha-helical and beta-barreltype integral membrane proteins by a preparative scale individual cell-free expression system. FEBS J. 272, 6024-6038
- [15] Chang, G., Spencer, R.H., Lee, A.T., Barclay, M.T., Rees, D.C. (1998) Structure of the MscL homolog from *Mycobacterium tuberculosis*: a gated mechanosensitive ion channel. Science 282, 2220-2226
- [16] Betton, J.-M. (2003) Rapid Translation System (RTS): A promising alternative for recombinant protein production. Curr. Protein Pept. Sci. 4, 73-80.
- [17] Spirin, A.S., Baranov, V.I., Ryabova, L.A, Ovodov, S.Y., and Alakho, Y.B. (1988) A continuous cell-free translation system capable of producing polypeptides in high yield. Science 242, 1162-1164.
- [18] Berrier, C., Coulombe, A., Houssin, C., Ghazi, A. (1989) A patch-clamp study of ion channels of inner and outer membranes and of contact zones of E. coli, fused into giant liposomes. Pressure-activated channels are localized in the inner membrane. FEBS Lett. 259, 27-32
- [19] Hamill, O.P., Marty, A., Neher, E., Sakmann, B., Sigworth, F.J. (1981) Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. Pflügers Arch. 391, 85-100.

- [20] Pavia, A.A., Pucci, B., Riess, J.G. and Zarif, L. (1992) New perfluoroalkyl telomeric non-ionic surfactants: synthesis, physicochemical and biological properties. Makromol. Chem. 193, 2505-2517
- [21] Barthélémy, P., Ameduri, B., Chabaud, E., Popot, J.-L., Pucci, B. (1999) Synthesis and preliminary assessments of ethyl-terminated perfluoroalkyl nonionic surfactants derived from tris(hydroxymethyl)acrylamidomethane. Org. Lett. 1., 1689-92
- [22] Sukharev, S.I., Sigurdson, W.J., Kung, C., Sachs, F. (1999) Energetic and spatial parameters for gating of the bacterial large conductance mechanosensitive channel, MscL. J. Gen. Physiol. 113, 525-40

7. LEGENDS TO FIGURES

Figure 1. Chemical structures of HF-TAC, $(n \approx 8, \langle MM \rangle \approx 1790 \text{ Da}, \text{ CMC} = 0.45 \text{ mM})$, C₆F-TAC $(n \approx 7, \langle MM \rangle \approx 1605 \text{ Da}, \text{ CMC} = 0.3 \text{ mM})$ and C₈F-TAC $(n \approx 7, \langle MM \rangle \approx 1705 \text{ Da}, \text{ CMC} = 0.03 \text{ mM})$.

Figure 2. *In vitro* synthesis and purification of histidine-tagged MscL. **A and B.** Smallscale synthesis was carried out in the absence of surfactants (C: control) and in the presence of C₆F-TAC, C₈F-TAC or HF-TAC at the concentrations indicated. At the end of the synthesis, samples were centrifuged 16,000 \times *g* for 10 min and the pellet (P) and supernatant (S) analysed by 15% 8M urea SDS-PAGE. MscL was detected by immunoblotting using an anti-histidine tag antiserum. **C.** One-step purification of radiolabelled MscL synthesized *in vitro* in the presence of 2 mM HF-TAC. BS: total extract before synthesis; AS: total extract after synthesis; PP: purified protein after purification on a Ni-NTA column in the presence of 2 mM HF-TAC. Samples were analysed on a 15% SDS-PAGE gel and stained with Coomassie blue.

Figure 3. Functional properties of *in vitro* synthesized MscL. Patch-clamp experiments were performed on giant proteoliposomes in the inside-out excised configuration. (**A**) The upper trace shows the current and the lower trace the pressure applied to a membrane patch containing MscL synthesized *in vitro* in the presence of HF-TAC. Time and current scales are indicated by small bars; arrows indicate the cumulative fully opened levels of channels. (**B**) Sensitivity to tension of MscL synthesized *in vitro*. Channel activity was recorded at different levels of suction. The open probability was calculated for each level, on a 20-s recording segment, and plotted *vs*. the applied pressure. Data were first fitted with a Boltzman distribution of the form $P_0 = (1 + \exp \alpha (p_{1/2} - p))^{-1}$, where P_0 is the open probability, *p* the applied pressure, $p_{1/2}$ the pressure at which P_0 is 0.5, and $1/\alpha$ the sensitivity to pressure. Each $P_0(p)$ curve was converted to a $P_0(T)$ curve by rescaling to a midpoint $T_{1/2}$ of 11.81 dyn/cm, and fitted to the equation $P_0 = (1 + \exp \beta (T_{1/2}-T))^{-1}$, where $1/\beta$ is the sensitivity to tension and *T* the tension. *Left* to *right*, $P_0(T)$ curves were fitted by a single fitting curve from 7, 5 and 5 recordings for HF-TAC (Δ), for C₆F-TAC (\bullet), and for C₈F-TAC (\bigcirc). The corresponding sensitivity factor $1/\beta$ is 0.59, 0.59 and 0.65 dyn/cm for HF-TAC, C₆F-TAC, and C₈F-TAC, respectively.

8. TABLES

Surfactant	N_{obs}	$1/\beta$ (dyn/cm)
HF-TAC	$6.0 \pm 1.4 \ (n = 24)$	$0.63 \pm 0.13 \ (n = 7)$
C ₆ F-TAC	$4.3 \pm 1.3 \ (n = 20)$	$0.53 \pm 0.20 \ (n = 5)$
C ₈ F-TAC	$5.2 \pm 1.2 \ (n = 10)$	$0.68 \pm 0.06 \ (n = 5)$

Table 1. Properties of reconstituted MscL after synthesis in the presence of each surfactant. The number of active channels per membrane patch, N_{obs} , and the slope factor or sensitivity to tension, $1/\beta$, are presented as mean \pm S.E.M. The values of $1/\beta$ were calculated from individual fitting curves, as indicated in the legend to Fig. 3. For data regarding MscL synthesized *in vivo* see ref 11.



Figure 1



Figure 2



Figure 3