Bioorganic & Medicinal Chemistry Letters, Vol.3, No.2, pp. 161-164, 1993
Printed in Great Britain

0960-894X/93 $6.00 + .00

EFFICIENCY OF NON-IONIC TELOMERIC SURFACTANTS FOR THE SOLUBILIZATION OF SUBCELLULAR FRACTIONS PROTEINS

Jean C. Maurizis*[a], André A. Pavia[b] and Bernard Pucci[b]

a) Unité INSERM U 71, BP 184, 63005 Clermont-Ferrand Cedex, France
b) Laboratoire de Chimie Bioorganique, Faculté des Sciences, 33 rue Louis Pasteur, 84000 Avignon, France

(*Received in USA* 18 *September* 1992)

Abstract: We have investigated the behaviour of a new class of non-ionic telomeric surfactants termed H-TAC derived from tris-(hydroxymethyl)-aminomethane, with respect to the solubilization of subcellular proteins of rat hepatocytes and membrane antigens from tumour cells. H-TAC detergents compared favourably with Triton X100 and Nonidet P40, two commonly used commercial surfactants, for both their solubilizing efficiency and the preservation of the tertiary structure and the antigenic specificity of the extracted proteins. In addition, H-TAC surfactants were transparent between 245 and 400 nm.

Non-ionic detergents are extensively used for the solubilization and purification of membrane proteins. These tensioactive agents are known to disrupt membrane phospholipid layers with subsequent protein release. Ideally, these solubilizers should not absorb at 280 nm, the wavelenght used for protein monitoring and titration. In addition, they should be easily removed by dialysis and must be non-denaturant for the solubilized proteins as well as for the chromatographic media used for their purification. Detergents commonly involved in these procedures, e.g Triton X100 or Nonidet P40, belong to the class of arylpolyoxyethylenes that absorb at 280 nm and induce protein aggregation. Consequently their utilization for protein separation by gel-filtration is precluded[1].

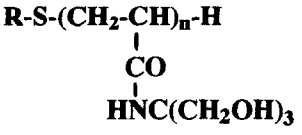
We have recently described[2,3] the synthesis of a new class of non-ionic telomeric surfactants by free radical telomerization of tris-(hydroxymethyl) acrylamidomethane (THAM) in the presence of either an alkanethiol or perfluoroalkanethiol as transfer reagent.

The amphiphilic nature of such molecules arises from the simultaneous presence of the hydrophilic tris-(hydroxymethyl)-aminomethane residues and of the hydrophobic alkanethiol or fluoroalkanethiol used as transfer reagents. For these reasons, compounds were termed H_mTAC_n or F_mTAC_n in which m represents the length of either the hydrocarbon or fluorocarbon chain.

We report here on the solubilizing properties of five of these telomers towards subcellular fractions of rat liver and membrane HLA-DR antigens from tumour cells and compare the results with those obtained with Triton X100 and Nonidet P40.

Telomers assayed in this work were synthesized according to the procedure described in a previous paper[3]. The general formula is given in figure 1.

Figure 1. General formula of the telomers



$3 < n < 15$. H_mTAC_n : R = $-(CH_2)_{m-1}-CH_3$, $8 < m < 16$. F_mTAC_n : R = $-(CH_2)_2-(CF_2)_{m-3}-CF_3$, $8 < n < 12$

The number average degree of polymerization of each telomer (DPn), which is equal to the number of repeating units n, was evaluated by elemental analysis and ^1H NMR spectroscopy (in DMSO-D6) by comparing the area of the signal due to the methyl group of H-TAC ($\delta = 7.2$ ppm) and the hydroxyl groups ($\delta =$ 5 ppm).

Five detergents were tested and compared to Triton X100 as solubilizing agents for membrane proteins: $H_{16}TAC_9$ (Mw 1855), $H_{12}TAC_7$ (Mw 1427), $H_{10}TAC_6$ (Mw 1172), H_8TAC_7 (Mw 1388) and $F_{10}TAC_4$ (Mw 1180).

The solubilizing properties of these products were assayed on subcellular rat liver fractions. Male Wistar rats (average weight 250 g) were killed by decapitation. The liver was immediately removed, washed with 0.05M phosphate buffer (pH 7.2) and homogeneized with a Potter-Evelhjem homogeneizer in 2 volumes of buffer. The suspension was centrifuged for 15 min at 1,000g to give pellet 1, containing membranes and nuclei. The supernatant was centrifuged for 15 mn at 15,000g to give pellet 2, containing mitochondria. The supernatant was centrifuged 1 hr at 100,000g to give pellet 3, containing microsomes. The three pellets were washed with buffer and lyophilized.

100 mg of lyophilized pellet 1 were suspended in 5 mL of detergent solution and 10 mg of lyophilized pellets 2 or 3 were suspended in 2 mL of detergent solution. The telomers and Triton X100 were used at concentrations of 0.1mg/mL, 0.5mg/mL and 1mg/mL respectively in 0.05 M phosphate buffer (pH 7.2). The suspensions were stirred under magnetic agitation at room temperature for 30 min, then centrifuged 15 min at 5,000g. Since H-TAC was transparent between 245 and 280 nm, the supernatant could be assayed for protein concentration by absorbance measurement at 280 nm (Tyrosine absorbance), using bovine serum-albumin as the protein reference. Indeed, methods using Coomassie blue[4] or Folin reagent[5] are subject to interferences with non-ionic detergents such as Triton X100. The sensitivity of this method is 50 µg/ml.

Table 1 shows the percentage of proteins solubilized by H_mTAC_n or F_mTAC_n surfactants and Triton X100 respectively at 1, 0.5 and 0.1 mg/mL concentrations.

These results show that at 1mg/mL concentration, no significant differences were found between the telomers and Triton X100 with the exception of $F_{10}TAC_4$ which proved to be a poor detergent. On the other hand, for 0.1mg/mL concentration, the solubilizing potency varied according to the telomer. For the three subcellular fractions, the best yield occured with $H_{12}TAC_7$ followed by $H_{10}TAC_6$, H_8TAC_7 and $H_{16}TAC_9$. To this concentration, $H_{12}TAC_7$ showed a protein solubilization potency identical or slightly superior to the one of Triton X100. Thus, detergent efficiency is related to the length of the hydrophobic thioalkyl chain and does not depend on the critical micellar concentration (CMC). The latter decreases from 4.5 mM (H_8TAC_7) to 0.015 mM ($H_{16}TAC_9$), with $H_{12}TAC_7$ exhibiting an intermediate value of 0.15 mM. Fluorination of the

thioalkyl chain results in a considerable decrease of the solubilizing capacity of TAC compounds. This observation is confirmed by the remarkable inertness of F_mTAC_n compounds with respect to the hemolysis of human erythrocytes[6].

Table I. Percentage of proteins solubilized in the lyophilized subcellular fractions. Each value is the average of 3 experiments ± SD.

Fraction	C (mg/mL)	$H_{16}TAC_9$	$H_{12}TAC_7$	$H_{10}TAC_6$	$F_{10}TAC_4$	H_8TAC_7	TritonX100
Pellet 1	1	50.50±3.26	58.15±4.12	n.d	26.28±2.42	56.40±3.20	53.30±4.54
	0.5	48.75±1.70	57.80±3.85		23.47±3.04	51.60±4.60	50.16±4.80
	0.1	30.10±2.85	47.75±3.26		14.36±1.45	40.50±1.60	43.81±3.56
Pellet 2	1	67.50±2.75	72.56±4.98	71.05±2.69	30.54±2.47	70.45±3.36	68.56±7.10
	0.5	60.42±3.78	68.42±5.05	64.62±4.29	25.32±1.86	65.56±1.47	62.97±4.16
	0.1	38.56±2.75	46.75±3.75	42.65±3.75	17.15±1.41	40.54±3.42	50.32±4.20
Pellet 3	1	78.50±4.12	83.56±5.12	86.83±3.75	35.56±3.01	80.12±6.05	72.44±5.46
	0.5	76.42±3.87	78.63±3.10	78.63±2.66	30.82±2.56	74.16±3.18	71.48±5.76
	0.1	44.56±8.02	64.35±4.15	57.09±9.37	19.27±1.45	56.42±4.12	61.61±8.01

The rate of dialysis was evaluated as follows : 2 mL of telomer solution in 0.05 M phosphate buffer (pH 7.2) (5 mg/ml) were poured into a dialysis tube (Visking, Poly-Labo, France), diameter 6.3 mm. The tube was immersed in a vessel containing 500 ml of 0.05 M phosphate buffer (pH 7.2), with stirring at room temperature. Aliquot fractions were taken after 2, 4, 6, 10, 16 and 24 hr and assayed for telomer concentration by measuring at 616 nm the absorbance of the complex between these molecules and Coomassie blue reagent (Pierce, Rockford, USA). The detection limit averaged 5×10^{-6} M for both telomers. 1 ml of dialyzed solution was added to 1 mL of Coomassie reagent and the absorbance measured at 616 nm. A standard curve was established for telomer concentration ranging from 10^{-4} to 5×10^{-6} M. The results are given in table 2.

Table 2. Dialysis of 2ml of telomer solution (5 mg/mL) against 500ml of 0.05 M phosphate buffer (pH 7.2). Results are given in g/100 mL of solution.

t (hr)	$H_{16}TAC_9$	$H_{12}TAC_7$	H_8TAC_7	$F_{10}TAC_4$
0	0.5	0.5	0.5	0.5
2	0.41	0.36	0.30	0.30
4	0.22	0.16	0.12	0.10
6	0.07	0.042	0.025	0.015
10	0.008	0.003	<0.002	<0.002
16	<0.002	<0.002	<0.002	<0.002
24	<0.002	<0.002	<0.002	<0.002

These results show that $F_{10}TAC_4$ was the most rapidly removed, followed by H_8TAC_7, $H_{12}TAC_7$ and $H_{16}TAC_9$. In contrast with the protein solubilization potency, the dialysis rate correlates well with the CMC, H_8TAC_7 being the most rapidly dialyzed among the hydrocarbons telomers.

$H_{12}TAC_7$ was used and compared to Nonidet P40 for the solubilization of a HLA-DR histocompatibility antigen from breast tumour cells[7]. Cultured MCF7 cells were treated with 0.5% (w/v) $H_{12}TAC_7$ or Nonidet P40 after metabolic labelling with ^{35}S-methionine[8]. The glycoproteins were isolated by affinity chromatography on Lentil-lectin-Sepharose 4B (Pharmacia, Uppsala, Sweden), then purified by chromatofocusing on PBE 9-4 gel (Pharmacia). The yield of each purification step was measured by radioactivity counting in a Packard liquid scintillation counter after dissolution of an aliquot in Beckman Ready-Solv[8].

The results are given in table 3.

Table 3. Comparison of HLA-DR antigen solubilization from 10^7 MCF7 cells between $H_{12}TAC_7$ and Nonidet P40. Each value, given in mg of proteins, is the average of 5 measurements ± SD.

Purification step	H12TAC7	Nonidet P40
Membrane solubilization	2700 ± 450	2540 ± 400
Lentil-lectine Sepharose 4B	109 ± 9	124 ± 11
Protein A-Sepharose	15 ± 2	14 ± 2
Chromatofocusing	4.0 ± 0.5	4.5 ± 0.5

At 0.5% concentration, $H_{12}TAC_7$ showed similar efficiency than that of Nonidet P40 for both protein solubilization, immune complex formation and affinity chromatography. The affinity of solubilized membrane glycoproteins for lectins was not changed upon treatment with $H_{12}TAC_7$, and their ability to bind monoclonal antibodies was maintained.

The results reported above show the ability of H_mTAC_n derivatives to disrupt phospholipid membranes. Molecules with a 12 carbon-thioalkyl chain are as efficient as Triton X100 for the solubilization of membrane proteins and as Nonidet P40 for the conservation of the tertiary structure and the antigenic properties of the solubilized molecules.

Work is in progress in the view to determine if this category of new detergents exhibits selective solubilization of certain membrane proteins and whether the enzymatic properties of the latter are altered by this treatment.

REFERENCES

1. Helenius, A. and Simons, K., *Biochim. Biophys. Acta*, **1975**, 415, 29.
2. Faurie, M.; Pavia, A. A.and Pucci, B. French Patent Appl. N°: 14 358, **1990.**
3. a : Pucci, B ; Maurizis, J.C., and Pavia,A. A., *Eur. J. Polymer*, **1991**, 27 1101.
   b : Pavia, A. A.; Pucci, B.; Riess, J G. and Zarif, L., *Makromol. Chem*, **1992**, 193, 2505.
4. Bradford, M. M., *Anal. Biochem.*, **1976**, 72, 248.
5. Lowry, O. H. ; Rosebrough, N. J.; Farr, A. L., and Randall, R. J., *J. Biol. Chem.*, **1951**, 193, 265.
6. Pavia, A. A ; Pucci, B.; Riess, J. G. , and Zarif, L., *Bioorg. Med. Chem. Letters*, **1991**, 1, 103.
7. Bernard, D ; Maurizis, J. C.; Chassagne, J.; Chollet, .P., and Plagne, R., *Anticancer Res.*, **1986**, 6, 78.
8. Bernard, D.; Maurizis, J. C.; Chollet, P ; Chassagne, J., and Plagne, R., *J. Chromatography*, **1984**, 308, 322.