Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 1559–1562

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

A New Class of Sulfoxide Surfactants derived from Tris. Synthesis and Preliminary Assessments of their Properties.

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Received 21 January 1998; accepted 18 May 1998

Key Words : *non-ionic surfactant, perfluoroalkyl surfactant, Tris, sulfoxide, protein extraction.*

Abstract : *A new class of non-ionic amphiphilic molecules suitable for biological purposes, especially extraction of membrane proteins, is reported. Such surfactants were prepared in two steps : addition of alkyl or fluoroalkyl mercaptan on Tris(hydroxymethyl)acrylamidomethane (THAM) derivatives, followed by the oxydation of sulfide group in sulfoxide moiety in order to provide water solubility to the molecule. The detergent efficiency of these new surfactants were assayed on rat liver cells.*

This work is dedicated to the memory of our colleague Dr Marc Toussaint.

The study of the various intramembraneous cell components, especially proteins and /or glycoproteins requires their extraction from the bilayer. The key-step in this process, the so-called « solubilization » of the membrane, is achieved by using detergents. In order to allow the selective extraction of different membrane proteins, without any chemical denaturation, many studies have concentrated on potentialities provided by new classes of non-ionic surfactant. The preparation of new non-ionic amphiphilic compounds having both high water solubility and significant detergent efficiency is one of the main problems met in this chemical field.

A few years ago, we showed that telomers obtained by free radical polymerization of Tris(hydroxymethyl)-acrylamidomethane (named trivially THAM) in the presence of alkanethiol as the transfert reagent, encompass these properties[1, 2]. This class of molecules exhibits generally a high water solubility, interesting detergent properties in terms of extraction of proteins and a low CMC depending upon the nature of hydrophobic tail. However, these telomers are polydisperse. The monoadduct (DPn=1) is the only compound of the series to have a precise molecular mass. Unfortunately, it is also the only one to be poorly soluble in water.

In order to keep the basic architecture of the THAM moiety and increase the water solubility of these monoadducts it can be considered either to graft a hydrophilic sugar structure on the hydroxyl groups or to chemically modify the organic sulfide. Works reported herein deal with the synthesis, physico-chemical studies and biological evaluations of new sulfoxide surfactants derived from THAM (scheme 1). Sulfoxide moiety is known to enhance the hydrophilic character of a molecule.

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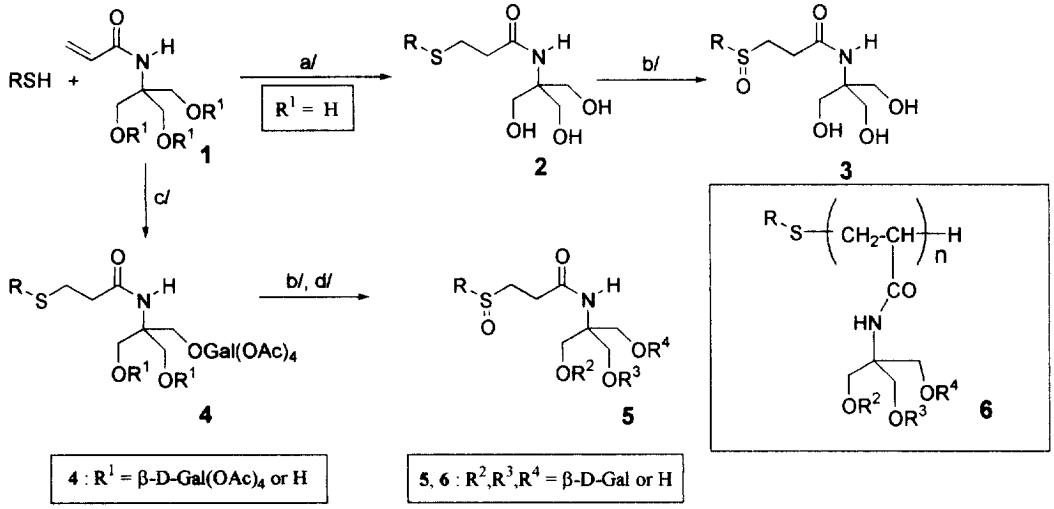
PII: S0960-894X(98)00263-7

A very convenient two step synthesis affords sulfoxide surfactants by using cheap reagents such as sodium methoxide and hydrogen peroxide. The first reaction, realized in methanol in the presence of sodium methoxide as a base, allows to perform the thiolate derivative and leads to a Michael reaction with the THAM monomer. Crystallization in ethyl acetate provides the pure monoadducts **2**.

With galactosylated THAM compounds, sodium methoxide in methanol is prohibited because of the hydrolysis of the acetylprotections. Thus, we synthesized monoadducts **4e** (R^1 = H) , **4h** (R^1 = β-D-Gal(OAc)$_4$, R = $C_6F_{13}(CH_2)_2$), **4j** (R^1 = H, R = $C_8F_{17}(CH_2)_2$) under free radical conditions[3] .



Scheme 1 : Synthesis of sulfoxide surfactants.

				Sulfide[a)]			Sulfoxide			
R	R^2	R^3	R^4	n°	DPn[b)]	cmc (mM)[b)]	n°	cmc (mM)[b)]	Ws. (g/l)	Yield %[c)]
C_8H_{17}-	H	H	H	-	-	-	**3a**	2.67	>10	73
$C_{10}H_{21}$-	H	H	H	**6b**[a/]	5.7	0.59	**3b**	1.1	>10	86
$C_{10}H_{21}$-	Gal	Gal	Gal	**6c**	1	1.0	-	-	-	-
$C_{12}H_{25}$-	H	H	H	**2d**[a/]	4	0.15	**3d**	0.165	>6	85
$C_{12}H_{25}$-	Gal	H	H	**6e**	1	Ins.	**5e**	0.222	>10	81
$C_{12}H_{25}$-	Gal	H	H	**6e**[a)]	5.6	0.26	-	-	-	-
$C_{16}H_{33}$-	H	H	H	**6f**[a)]	9.3	0.015	**3f**	0.011	0.085	74
C_6F_{13}-$(CH_2)_2$	H	H	H	**6g**[a)]	5.4	0.33	**3g**	0.42	1.2	75
C_6F_{13}-$(CH_2)_2$	Gal	Gal	Gal	**6h**	1	0.2	**5h**	0.24	>10	84
C_8F_{17}-$(CH_2)_2$	H	H	H	**6i**[a)]	5.8	0.03	**3i**	ND	0.025	60
C_8F_{17}-$(CH_2)_2$	Gal	H	H	**6j**	1	Ins.	**5j**	0.033	>1.5	88
C_8F_{17}-$(CH_2)_2$	Gal	Gal	H	**6k**[a)]	1	0.04	-	-	-	-

a) see ref. 3 - Ws = water solubility. - Ins : Insoluble - ND = No determination-
Gal = β-D-Galactopyranosyl ; DPn = average degree of polymerization = n
b) cmc = critical micellar concentration ; Each data is the average of three experiments ± 5%.
c) Yield of sulfide oxidation reaction.

Table 1 : Physico-chemical data of sulfide and sulfoxide THAM derivatives.

In order to convert sulfides to sulfoxides, the oxydation reaction was performed with hydrogen peroxide in methanol[4]. Usually the major difficulty encountered in the preparation of sulfoxides by this method is a facile over-oxidation to the corresponding sulfones[5]. In the case of THAM monoadducts this drawback is avoided simply by choosing a stoechiometric amount of hydrogen peroxide. Here again, the pure sulfoxides **3** were isolated after crystallization. With galactosylated monoadducts, the deprotected compounds **5e, 5h, 5j** were obtained after the reaction from mixing methanol and a catalytic amount of sodium methoxide.

As expected, the sulfoxide function strongly enhances the hydrophilic character of monoadducts and improves their water solubility (Table 1). For example, sulfide **2d** is almost insoluble in water (< 2 mg/L), but the solubility of its sulfoxide analogue **3d** is higher than 6 g/L.

Concerning the amphiphilic properties, as we observed previously[3], the critical micellar concentration (CMC) of THAM or galactosylated THAM sulfide derivatives (monomers or telomers) depends almost exclusively upon the nature and the length of the hydrophobic tail. For instance, the trigalactosylated sulfide monoadduct **6c** exhibits the same CMC as that observed with sulfoxide **3b** bearing ten carbons itself. The sulfoxide monoadducts **3f, 5e** or **5j** exhibit the same CMC as their telomer sulfide analogue **6f, 6e** or **6i** respectively. In the same way, **6h** and **5h** have a CMC close to 0.2-0.25 mM. Likewise, **6i, 6k** and **5j** which bear the same fluorocarbon tail exhibit a CMC close to 0.03-0.04 mM whatever the number of galactose moieties grafted on the Tris groups. Thus, values of CMC observed are by no means correlated with the size of the polar head.

The oxidation of sulfide moiety increases the overall water solubility of the THAM surfactants but does not seem to modify their free energy of micellisation[6]. Indeed, it is noteworthy that CMC data of the THAM derived sulfoxides **3** can be linearly correlated to the tail length. The equation where nC is the number of carbon, being :

$$Ln(CMC) = -0.71\ nC + 6.87 \qquad \text{(with a correlation coefficient : } R^2=0.991\text{)}.$$

It could be postulated that the sulfoxide group enhances the hydration ratio of the surfactant head, like THAM telomer, without any alteration of the micellar equilibrium.

The solubilizing properties of these products were assayed on various liver subcellular fractions : cell membrane and nuclei, mitochondria, microsomes. Table 2 shows the amount of protein solubilized, expressed as a percentage of the weight of freeze-dried tissue. The results are compared with those obtained with conventional surfactants such as Triton X100. They deserve the following remarks :

- First, as already observed with the THAM derived telomers, the introduction of a perfluoroalkyl tail on the sulfoxide surfactant strongly decreases the detergent efficiency. Probably, this kind of hydrophobic-lipophilic chain could not penetrate into the phospholipidic bilayer.

- Second, it is well known that the detergent efficiency reaches a maximum when the surfactant concentration is at least equal to its CMC. Among the sulfoxide surfactants, compounds **3a** and **3b** bearing

respectively a C8 or C10 hydrocarbon tail show the best efficacy. However, their concentrations (0.1mg/ml) are much lower than their CMC (1.1 mM ; 0.4 mg/ml in the case of the surfactant **3b**). One can reasonably assume that these detergent efficiencies should increase with the concentration until the CMC is reached. Unfortunately, the strong detergent activities already observed with concentration of 0.1mg/ml prohibit new extraction experiments with higher amount of such surfactants. With the other hydrocarbon sulfoxides (**3d** and **3f**), extraction experiments were carried out at a concentration higher than the CMC and their solubilizing potency is in each case lower than that observed with **3a** or **3b**. Moreover, it can be observed on table 2 that the extraction of proteins from membranes by the sulfoxides surfactants depends upon the length of the hydrophobic chain and seems to reach a maximum efficacy with a C10 hydrocarbon chain.

Comp.	R	R^2	R^3	R^4	Nuclear/ Membrane[a)]	Mitochondria[a)]	Microsomes[a)]
3a	C_8H_{17}	H	H	H	55.45 ± 4.78	69.78 ± 8.28	70.98 ± 9.10
3b	$C_{10}H_{21}$	H	H	H	57.41 ± 2.87	70.95 ± 7.24	68.90 ± 5.67
3d	$C_{12}H_{25}$	H	H	H	52.41 ± 4.25	65.62 ± 4.68	62.47 ± 4.10
3f	$C_{15}H_{31}$	H	H	H	45.71 ± 2.87	58.64 ± 5.78	58.41 ± 3.14
3g	$C_6F_{13}(CH_2)_2$	H	H	H	25.47 ± 4.41	30.28 ± 5.56	32.64 ± 6.22
	Triton X100	-	-	-	62.21 ± 7.74	74.87 ± 5.25	76.24 ± 7.72

100 mg of freeze-dried membrane/nuclei or 20 mg of freeze-dried mitochondria or microsomes obtained from rat liver[1] suspended into 5 mL of phosphate buffer (0.05 M, pH = 7.4), containing concentration of detergents of 0.1mg/mL. The suspension was stirred 30 mn at room temperature, then centrifugated on additional 30 mn at 15000g. Proteins titration was achieved by optical density measurements of solutions at 280 nm.
a) The amount of protein solubilized, expressed as a percentage of the weight of freeze-dried tissue Each data is the average of 3 experiments ± SD.

Table 2 : Efficiency of sulfoxide surfactants for the extraction of proteins from plasma membrane and intracellular organelles compared with that of Triton X-100.

One of the main interest of the synthesis reported herein, *i.e* the oxidation of the sulfide and/or THAM galactosylation, is to allow the easy biological use of low CMC surfactant by enhancing their overall water solubility. Work is in progress in order to determine if this category of new detergents exhibits selective solubilization of certain membrane proteins and maintains them in solution. Furthermore, we have shown that these surfactants could be used in the agrochemical field to increase the efficiency of herbicide or another biocide[7].

Acknowledgements :. We thank the Monsanto Company for its financial support and Professor A. Laschewsky of Université Catholique de Louvain for his valuable discussion about sulfoxide properties.

Références

1/ Pucci, B., Maurizis, J.C., Pavia, A.A., *Eur. Polym. J.*, **1991**, 27, 1101-1106.
2/ Pavia, A.A., Pucci, B., Riess, J .G., Zarif, L., *Makromol. Chem.*, **1992**, 193, 2505-2517.
3/ Polidori, A., Pucci, B., Maurizis, J.C., Pavia, A.A., *New. J. Chem.*, **1994**, 18, 839-848.
4/ Madesclaire, M. , *Tetrahedron*, **1986**, 42, 5459-5495.
5/ Drabowicz, J., Mikolajczyk, M., *Org. Prep. Procedures Int.*, **1982**, 14, 45-82.
6/ Shaw, J. D. , *Introduction to Colloid and Surface Chemistry, Butterworths, London*, **1980.**
7/ Pucci B. , Barthelemy P. , Polidori A., Lacombe J.M., Bonnet M., Toussaint, M. ; *Eur. Patent 97 870 130.8 du 03.09.97*, Monsanto Company.