Thermochimica Acta 479 (2008) 28–31

Contents lists available at ScienceDirect

Thermochimica Acta

journal homepage: www.elsevier.com/locate/tca

The effect of quaternary ammonium surfactants on the inhibition of the aerobic metabolism of *Saccharomyces cerevisiae*—A calorimetric study

C.E. Perles*, A. Matheus, P.L.O. Volpe

Instituto de Química, Universidade Estadual de Campinas, C.P. 6154, CEP 13084-970 Campinas, SP, Brazil

ARTICLE INFO

Article history:
Received 15 May 2008
Received in revised form
13 September 2008
Accepted 16 September 2008
Available online 24 September 2008

Keywords:
Saccharomyces cerevisiae
Flow calorimetry
Bioactivity
Quaternary ammonium surfactants

ABSTRACT

The effect of quaternary ammonium surfactants $C_{10}H_{21}N(CH3)_3I$, $C_{12}H_{25}N(CH_3)_3Br$, $C_{14}H_{29}N(CH_3)_3Br$ and $C_{16}H_{33}N(CH_3)_3Br$, and cetylpyridinium chloride $(C_{16}H_{33})PyCl$, on the inhibition of the aerobic metabolism of *Saccharomyces cerevisiae* was studied with a flow calorimeter. All the compounds, except $C_{10}H_{21}N(CH3)_3I$, presented a linear dependence of the biological response (BR) with the logarithm of the concentration (log(dose)). The values of $(dose)_{max}$ has an excellent linear correlation with the number of CH_2 groups. The bioactivity of these compounds follows the order $(C_{16}H_{33})PyCl \gg C_{16}H_{33}N(CH_3)_3Br > C_{14}H_{29}N(CH_3)_3Br > C_{12}H_{25}N(CH_3)_3Br$.

Decyltrimethylammonium iodide did not affect the aerobic metabolism of *S. cerevisiae* under the studied conditions.

1. Introduction

Surfactants are amphiphilic compounds extensively used in products for hygiene and cleanliness. Ionic surfactants are recognized as denaturating agents, able to promote the denaturation of proteins at millimolar concentrations [1]. Several studies have been published on the interaction of ionic surfactants with proteins and with phospholipid systems [2–5]. We recently studied the toxicity of a homologous series of an anionic surfactant (sodium *n*-alkylsulfates) by using an isothermal differential heat conduction flow calorimeter to monitor in real time, the heat output production of the metabolism of *Chromobacterium violaceum* [3].

Vieira and Carmona-Ribeiro [2] studied the action of a homologous series of quaternary ammonium surfactants (QAS) on *Candida Albicans* and Jones et al. [4] and Granjeiro et al. [5] studied the interactions of these surfactants with the enzyme tyrosine phosphatase.

The mechanism of denaturation of biomolecules by ionic surfactants occurs by ionic interactions between the ionic head of the surfactant and charged groups on the side chains of aminoacid residues of the protein and by the interaction of the apolar chain of the surfactant with the hydrophobic regions of the protein, which destabilize the structure of the protein [4–6].

Isothermal differential heat conduction calorimetry has great potential in the evaluation of the effects of addition of drugs on microorganisms since the biological response (BR) can be monitored in real time by the heat output produced by the metabolism of living organisms [7]. Beezer et al. [8,9] demonstrated the application of calorimetry for the study of the effect of homologous series of compounds and their quantitative structure-activity relationship (QSAR).

Data on the effect of surfactants on the metabolism of living organisms are scarce and not quantitatively described in the literature. These data are needed to compare these compounds with other classes of biocides.

In this work we investigate the effect of the quaternary ammonium surfactants decytrimethylammonium iodide, dodecyl (DTAB), tetradecyl (TTAB) and hexadecyl (CTAB) trimethylammonium bromide, and cetylpyridinium chloride (CPyC) on the aerobic metabolism of *Saccharomyces cerevisiae* by using a flow calorimeter.

2. Experimental

Glucose (Sigma), anhydrous potassium phosphates, K_2HPO_4 and KH_2PO_4 (Synth), methyl iodide (Aldrich), *n*-decylamine (Aldrich), dimethyl sulfoxide (Sigma), Ringer's Solution (Merck), quaternary ammonium sulfactant salts, $C_{12}H_{25}N(CH_3)_3Br$, $C_{14}H_{29}N(CH_3)_3Br$, $C_{16}H_{33}N(CH_3)_3Br$ and $(C_{16}H_{33})PyCl$ (Aldrich) and tetrahydrofuran (THF) (Sigma) were of analytical grade and used as received. Lyophylized *Saccharomyces cerevisiae* (Fermix) was purchased from DSM Bakery.

$C_{10}H_{21}N(CH_3)_3I$ was synthesized following the procedure know as exhaustive methylation [10]. 50 mL of a methyl iodide solution

* Corresponding author. Tel.: +55 19 3521 3091; fax: +55 19 3521 3023.
E-mail address: ceperles@iqm.unicamp.br (C.E. Perles).

0040-6031/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.tca.2008.09.011

in tetrahydrofuran with concentration 3.64 mol L^{-1} were slowly added into 50 mL of a THF solution of *n*-decylamine with concentration of 1.10 mol L^{-1} cooled in an ice water bath. After 5 h stirring, the solvent was partially removed under reduced pressure and the remaining solution was maintained in the refrigerator overnight to promote the product crystalization. The solution was filtered and the solid purified by recrystalization in acetone.

2.1. Preparation of cells

Phosphate buffer solution (PBS) was prepared by dissolving 17.00 g of KH_2PO_4 in 250.00 mL of distilled water. The pH was adjusted to 7.2 using 1.00 mol L^{-1} sodium hydroxide solution and the volume was completed to 500.00 mL with distilled water. The PBS solution was autoclaved at 121 °C at 15 psi for 15 min. The composition of the culture media used for the aerobic metabolism of *S. cerevisiae* was (g L^{-1}); glucose, 0.50; K_2HPO_4, 8.75; KH_2PO_4, 3.75; pH 7.0.

Lyophylized *Saccharomyces cerevisiae* (5.40 g) was suspended in 30.0 mL of sterilized PBS solution and stirred. After 10 min, the cells were separated by centrifugation at 4000 rpm during 15 min. The cells were washed three times by suspending in PBS solution and then centrifuged. Then the cells were suspended in 50 mL of PBS solution containing 10% dimethylsulfoxide and stored in 2 cm^3 polypropylene ampoules (Corning). The ampoules were inserted into a thin perforated styrofoam plate which was placed 8 cm above liquid nitrogen in a an appropriate container. When the temperature in the control ampoule reached 200 K, the ampoules were immersed in liquid nitrogen and stored in the cryogenic cylinder [11].

2.2. Calorimetric experiments

The calorimetric study of the effect of the surfactants on the inhibition of the metabolism of *S. cerevisiae* is based on the heat flow rate produced during the process, which is monitored in real time using an isothermal differential twin heat conduction flow microcalorimeter (Thermometric model TAM 2277) at 303.15 ± 0.02 K. This instrument contains a sample and a reference flow-through vessels. The reaction mixture, e.g., buffered glucose aqueous solution, microorganism plus surfactant is pumped through the vessel by a peristaltic pump from a micro-reactor vessel, outside the calorimeter and returned to the micro-reactor, to form a closed loop system. The calorimeter measures the power generation by the reaction process within the flow-through vessel, relative to power changes in the empty reference flow-through vessel. The output of the microcalorimeter (heat flow d*q*/d*t* *vs.* time) provides a continuous record of the progress of the metabolism. Thus, in contrast to many classical procedures, the calorimeter responds to the total metabolic activity of only the active microbes present and thus displays details of the dynamic and complex process of the aerobic metabolism of *S. cerevisiae*. Any substance that can modify the metabolic process in the cell, will change the biological response obtained from the calorimeter and so it becomes possible to study by a direct method and in real time, the effect of compounds on the metabolism of living cells in both a qualitative and quantitative way. This calorimetric method requires only an observable difference between the biological response in the treated and untreated (control) incubation. Flow microcalorimetry is most commonly used for studies of the effect of compounds on the inhibition of the metabolism of microorganisms and the microcalorimeter scheme has been described elsewhere [12].

Initially, a power-time plot baseline was obtained by pumping the culture media without the microorganisms from a micro-reactor into the flow-through cell (35 mL h^{-1}) using a LKB-2132 peristaltic pump in a closed loop system [12]. When a stable baseline was established, an electrical calibration was performed to calibrate the heat production output scale. After the calibration, 2.0 mL of the cell suspension were inoculated into 50 mL of the culture media in the micro-reactor. This procedure was carried out immediately after the thawing the cryogenic ampoule containing the suspension of cells for 3 min in a water bath at 37 °C, followed by manual stirring for 20 s. The calorimetric curve without the addition of the surfactant solution was used as a control curve [12]. The calorimetric curves with the addition of the surfactants were performed following the same procedure, using 50 mL of the culture media in the micro-reactor with aliquots of different volumes of the surfactant stock solution before the inoculation of 2.0 mL of the cell suspension.

The control curve corresponds to the maximum heat flow rate generated by the metabolism of the microorganisms under the experimental conditions in the absence of the bioactive compound. From the control curves, we obtain the maximum biological response, which is the difference between the maximum heat output value of the calorimetric curve and the baseline [3]. During the course of this work, calorimetric control curves were obtained periodically with an average value of 89 ± 1 μW.

Calorimetric curves (power-curves plots) were obtained for each surfactant at various concentrations. BR, which is the percentage of calorimetric response in relation to the control response, was calculated for different concentrations of the surfactant.

3. Results and discussion

Calorimetric curves of the aerobic metabolism of *S. cerevisieae* and the effect of the addition of the surfactant DTAB at various concentrations are shown in Fig. 1.

The values of BR as a function of the concentration (dose), obtained from the calorimetric curves for each surfactant are shown in Fig. 2. The value of the concentration needed for complete suppression of biological activity was obtained by extrapolation of the curve to BR = 0. The concentration of a compound that completely suppresses the calorimetric signal is called $dose_{max}$. The surfactant decyltrimethylammonium iodide did not show any toxicity in the concentration range from 0.20 to 1.68 mmol L^{-1}.

A plot of $\log(dose)_{max}$ *vs.* the number of CH_2 groups in the quaternary ammonium surfactant yields a straight line for the homologous compounds from C_{12} to C_{16} and is shown in Fig. 3. The toxicity of DTAB, TTAB and CTAB is correlated with the number of CH_2 groups in the hydrocarbon chain of the surfactant. The pyridinium derivative, CPyC, showed the highest activity when compared with DTAB, TTAB and CTAB.

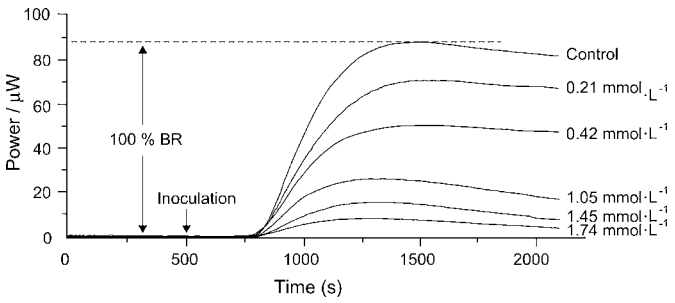

Fig. 1. Calorimetric curve of the aerobic metabolism of *S. cerevisiae* and the effect of the addition of the surfactant DTAB. The biological response, BR, was calculated as the percentage of the maximum heat flow rate compared with that of the control.

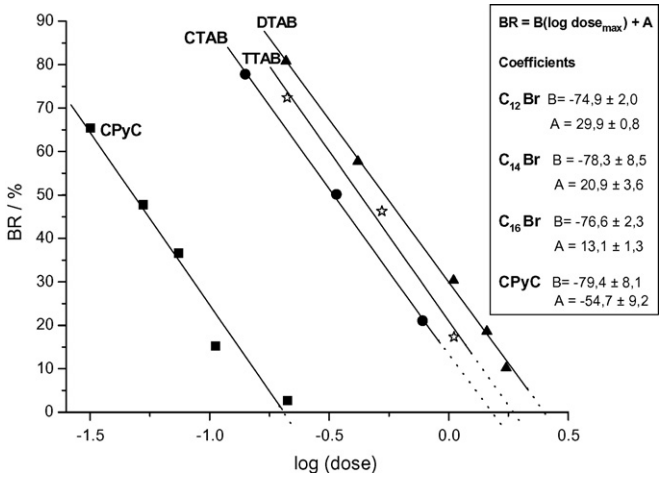

Fig. 2. BR *vs.* $\log(\text{dose})_{max}$ values for the compounds DTAB, TTAB, CTAB and CPyC. The concentrations needed for complete suppression of biological activity, $(\text{dose})_{max}$ were, 2.51, 1.86, 1.48 and 0.20 mmol L^{-1} for DTAB, TTAB, CTAB and CpyC, respectively.

Fig. 2 shows that the concentration of CTAB necessary to inhibit the metabolism of *S. cerevisiae* is smaller than those of DTAB and TTAB, respectively. In a recent study [3], we showed that the toxicity of *n*-alkylsulfates on the aerobic metabolism of the bacteria *C. violaceum* increases with the hydrophobicity (number of CH_2 groups).

We have recently studied the effect of a homologous series of sodium *n*-alkylsulfate (C_8–C_{14}) and *n*-alkyltrimethylammonium bromide surfactants (C_{12}–C_{16}) on the inhibition of the protein tyrosine phosphatase (PTP) [5]. The enzyme was deactivated in the presence of the surfactants and the deactivation was dependent on the chain length of the surfactants and independent of the pH. In the present study with living cells, we verified that the toxicity of the cationic surfactants shows the same tendency, i.e., the inhibition of the metabolic process increases with the hydrophobicity of the compounds following the order CTAB > TTAB > DTAB. However, for the trimethylalkylammonium bromides surfactants, the effect of the increment of CH_2 groups on the inhibition of the metabolism of the living cell is smaller in comparison with the inhibition of PTP.

The data obtained in this study are in agreement with the data on the inactivation of the enzyme PTP by quaternary ammonium surfactants obtained by Granjeiro et al. [5]. The increase of hydrophobicity has a same effect in both cases, on the aerobic metabolism of *S. cerevisiae* and on the inhibition of PTP. An increment of one methylene group has a small effect on the toxicity and on the inhibition of PTP [5]. For the homologous series of the anionic surfactants sodium *n*-alkyl sulfates [3], the effect of an increment of one CH_2 group on the inhibition of the metabolism of living cells and on the inhibition of PTP was greater when compared with the cationic surfactants. These correlations between the inhibition of an enzymatic reaction and toxicity on living cell metabolism are an strong indication that the main action of ionic surfactants on living cells is the inactivation of the enzymatic activities of the membrane and cytossol proteins. The inhibition of these enzymes disturbes the metabolic pathways and the metabolite transport through the cell membrane.

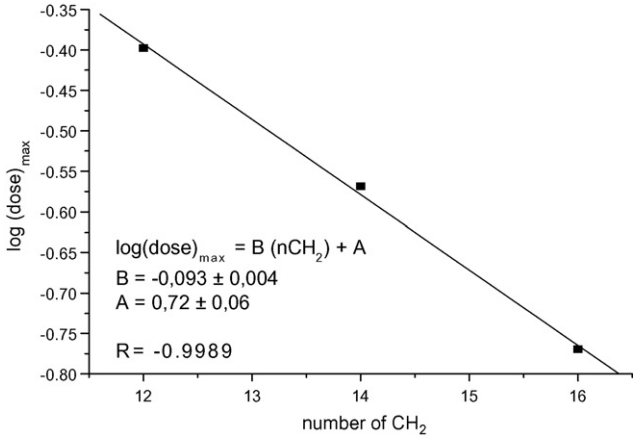

Fig. 3. Effect of methylene groups on $\log(\text{dose})_{max}$ values for the homologous series of alkyltrimetylammonium bromides DTAB, TTAB and CTAB.

Quaternary ammonium surfactant salts have a strong electrostatic interaction with small charged molecules and also with proteins of the cell membrane, which induces cellular lyses [2,13]. These salts are also recognized as efficient denaturing agents [4,13]. The combination of these effects may be the main cause of the inhibition effect caused by the surfactants on the metabolism of the microorganisms. However, the relative importance of each effect may vary with the specie microorganism. QAS are also extensively used as fabric softeners in households and antistatic agents in industry. This has resulted in concentrations of up to 25 mg/L in river water. A study on bacterial and plankton activities in the Rhine river revealed an inhibition starting at a concentration of 1 mg/L [14]. Comparisons with uncharged surfactants indicate that the electrical charge of QAS is necessary for it activity against microorganisms [15].

For the surfactant cetylpyridinium chloride, the high activity may be due to the small steric hinderance of the polar pyridinium head group in comparison with the trimethylammonium group, which allows a stronger interaction of its positive head groups with negative charged molecules of the microorganism. Subsequently, the formation of ionic pair compounds interferes with the cell metabolic processes [16–18]. However, effects on plasmatic membrane are expected, but in small proportion. Some authors observed lyse of cellular membrane of spheroplasts and protoplasts, but *S. cerevisae* and *C. violaceum* have cell wall which assure mechanical stability, making difficult the cellular lysis.

The high antimicrobial effect of CPyC has been shown to in some works on *Salmonella Typhimurium* and *E. coli*, being highly effective at concentrations lower 1% [15–18]. Nakagawa et al. [15] shown that the electrical charge is necessary for it to be active against microorganisms.

Acknowledgments

The authors thanks the *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (*CNPq*) for financial support and professor Fred Yukio Fujiwara for critical comments.

References

[1] M.N. Jones, Int. J. Pharm. 177 (1999) 137–159.
[2] DB. Vieira, A.M. Carmona-Ribeiro, J. Antimicrob. Chemother. 58 (2006) 760–767.
[3] C.E. Perles, P.L.O. Volpe, J. Braz. Chem. Soc. 5 (2005) 1085–1088.
[4] M.N. Jones, G. Prieto, J.M. del Rio, F. Sarmiento, J. Chem. Soc. Farad. Trans. 91 (1995) 2805–2809.
[5] J.M. Granjeiro, A.A. Miranda, M.G.S.T. Maia, C.V. Ferreira, E.M. Taga, H. Aoyama, P.L.O. Volpe, Mol. Cell. Biochem. 265 (2004) 133–140.
[6] M.J. Schwuger, F.G. Bartnik, in: C. Gloxhuber (Ed.), Surfactant Science Series, Anionic Surfactants: Biochemistry, Toxicology, Dermatology, vol. 10, Marcel-Dekker, New York, 1980.
[7] A.E. Beezer, Biological Microcalorimetry, Academic Press, London, 1980, pp. 195–246.
[8] A.E. Beezer, P.L.O. Volpe, C.A. Gooch, W.H. Hunter, Anal. Proceed. 23 (1986) 399–400.
[9] A.E. Beezer, P.L.O. Volpe, C.A. Gooch, W.H. Hunter, R.J. Miles, Int. J. Pharm. 29 (1986) 237–242.
[10] A.C. Cope, R.D. Bach, Org. Synth. 5 (1973) 315–320.

[11] B.E. Kirsop, J.J. Snell, Maintenance of Microorganisms, Academic Press, New York, 1991, pp. 21–24, 175.
[12] P.L.O. Volpe, J. Braz. Chem. Soc. 8 (1997) 343–348.
[13] H. Schott, C.Y. Young, J. Pharm. Sci. 61 (1972) 762–765.
[14] D.M.J. Tubbing, W. Admiraal, Appl. Environ. Microbiol. 57 (1991) 3616–3622.
[15] Y. Nakagawa, T. Tawaratani, H. Kourai, T. Horie, I. Shibasaki, Appl. Environ. Microbiol. 47 (1984) 88–93.
[16] J.W. Kim, M.F. Slavic, J. Food Prot. 59 (1996) 322–326.
[17] C.N. Cutter, W.J. Dorsa, A. Handie, S. Rodriguez-Morales, X. Zhou, P.J. Breen, C.M. Compadre, J. Food Prot. 63 (2000) 593–600.
[18] J.M. Bosilevac, T.M. Arthur, T.L. Wheeler, S.D. Shackelford, M. Rossman, J.O. Reagan, M. Koohmaraie, J. Food Prot. 67 (2004) 646–650.