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Kinetics of ABTS derived radical cation scavenging by bucillamine, cysteine, and glutathione. Catalytic effect of Cu^{2+} ions

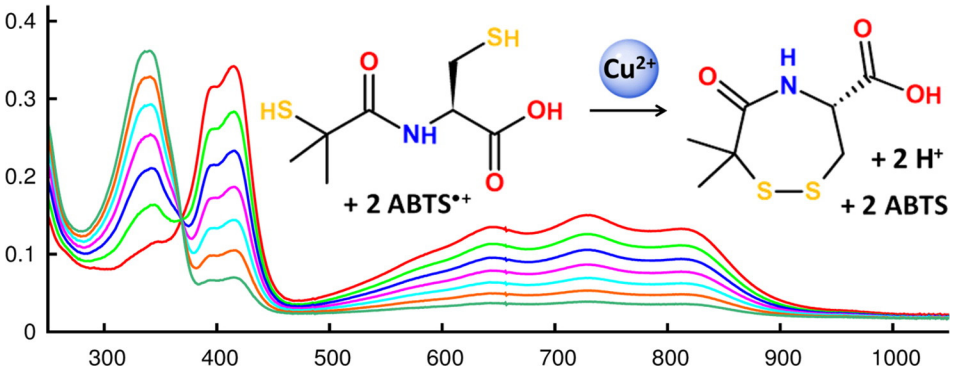
Ivan Valent [a,*], Dominika Topoľská [b], Katarína Valachová [b], Juraj Bujdák [a], Ladislav Šoltés [b]

[a] Department of Physical and Theoretical Chemistry, Faculty of Natural Sciences, Comenius University, Mlynská dolina, Ilkovičova 6, 842 15 Bratislava, Slovak Republic
[b] Institute of Experimental Pharmacology and Toxicology, Slovak Academy of Sciences, Dúbravská cesta 9, 841 04 Bratislava, Slovak Republic

HIGHLIGHTS

- Decay of $ABTS^{\bullet+}$ is governed by pseudo-first order kinetics
- H^+ ions display second order inhibition with all the studied thiols
- BUC exhibits zero order kinetics to $ABTS^{\bullet+}$ with H^+-catalysis at pH > 2.7
- Cu^{2+} ions show strong catalysis for all the species in the order BUC > Cys > GSH
- Significant effects of EDTA and bathocuproine disulfonate were observed

GRAPHICAL ABSTRACT



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ABSTRACT

Kinetics of reduction of the stable radical cation derived from 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) in reaction with the anti-rheumatic drug bucillamine (BUC) and two reference thiols – cysteine (Cys) and glutathione (GSH) was followed spectrophotometrically in acidic medium with 10-fold molar excess of the reductant. Decay of the radical is governed by pseudo-first order kinetics with small deviation in the case of GSH. H^+ ions displayed second order inhibition of the reaction with all the studied compounds. The reaction of BUC exhibits zero order kinetics to the radical at lower acidities with a moderate acceleration of the reaction rate by H^+ ions. A significant catalytic effect of Cu^{2+} ions on the reactions with all the reductants was observed. The most sensitive to Cu^{2+}-catalysis was the reaction of BUC with the radical cation, while Cu^{2+} ions showed much lower effect on the reaction with GSH. The presence of EDTA strongly inhibited the reactions and equalized the reaction rates for all the reductants. A Cu(I) selective chelator bathocuproine disulfonate reduced the reaction rate with Cys, but accelerated the reaction with BUC at the lower acidities. The experimental results were rationalized in the framework of the mechanism of reductive chelation. The conclusions may have important consequences for interpretation of antioxidant capacity assays, such as TEAC, utilizing the ABTS derived radical cation.

1. Introduction

Thiol-type antioxidants, constituting a class of organic sulfur derivatives having sulfhydryl functional groups, play a crucial role in protecting cells from oxidative damage by interacting with the

* Corresponding author.
E-mail address: valent@fns.uniba.sk (I. Valent).

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electrophilic groups of reactive oxygen species (ROS) as a first and major member of physiological antioxidative defense system [1]. Decreased levels of thiol compounds in the organism have been shown to cause various disorders. Biologically derived thiols such as glutathione (GSH), cysteine (Cys), and homocysteine are often called biothiols. The side chain functional group —CH_2—SH of cysteinyl residues serves as an active site for most biologically important thiols. The integrity of the —SH groups of intracellular and plasma membrane proteins and soluble thiols are essential to a large number of biological functions. Low molecular weight nonprotein thiols have been shown to have a protective effect against free radical-, radiation- and chemically reactive metabolite-induced toxicity [2].

Bucillamine, *N*-(2-mercapto-2-methylpropionyl)-L-cysteine (Fig. 1a), is a cysteine derivative with two thiol (sulfhydryl) groups per molecule and is known as an efficient anti-rheumatic drug [3] with immunological effects [4]. The exact mechanism of bucillamine action is unknown, but many studies to date suggest that it is an immunomodulator [2,5]. Bucillamine (BUC), also referred to as SA96, is rapidly metabolized to SA981 which is a disulfide compound formed by intramolecular binding of two sulfhydryl groups (Fig. 1b). The disulfide structure of bucillamine metabolites plays a critical role in the pharmacological action of the drug [5]. Most likely, the two sulfhydryl groups which are predisposed to close a stable ring underlie to a different pharmacological action of bucillamine in comparison with D-penicillamine, another substituted cysteinyl anti-rheumatic drug [5].

Bucillamine is capable of replenishing the thiol group in glutathione, thereby assisting defense against oxidant injury [6]. BUC as the effective thiol donor has potential to attenuate or prevent damage during myocardial infarction, cardiac surgery and organ transplantation [6,7]. On the other hand, the drug has been reported to act as an inducer of apoptosis via generation of ROS in the presence of copper ions [8]. Kładna et al. [9] showed that BUC can directly scavenge ROS or inhibit reactions generating them. However, the drug may have pro-oxidant activity under some conditions [9].

In studies of antioxidant properties of various compounds with respect to their radical scavenging capacity, a radical cation derived from 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid), ABTS (Fig. 2), is frequently used [10]. $ABTS^{\bullet+}$ has been suggested [11] as a useful reference free radical for studies of reactions of organic radicals with sulfhydryl compounds. The aim of this contribution was to examine the reaction of BUC and two reference sulfhydryl compounds (Cys and GSH) with $ABTS^{\bullet+}$ as potential models for more precise kinetic and mechanistic studies of reactions of cysteine-derived drugs with free radicals. Particular attention was given to effect of cupric ions as the catalytic action of copper on oxidation reactions of Cys [12] and its derivatives including BUC [8] is well-known. Despite the amount of experimental data that have been collected so far, detailed mechanisms of these reactions are not fully understood.

2. Materials and methods

Bucillamine was provided by Santen Pharmaceutical Co., Osaka, Japan and was used without purification as well as L-cysteine (97%, Aldrich), glutathione (puriss, Fluka), ABTS diammonium salt (purum, >99%, Fluka), $K_2S_2O_8$ (p.a., 99%, Merck), perchloric acid (p.a., 70–72%, Merck), disodium ethylenediaminetetraacetate dihydrate (complexon III, p.a., Lachema), bathocuproine disulfonate ($Na_2BCS \cdot xH_2O$, Aldrich), and $CuSO_4 \cdot 5H_2O$ (p.a., Lachema).

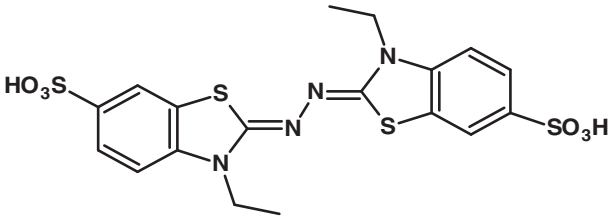

Fig. 2. Structure of 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid), ABTS.

$ABTS^{\bullet+}$ was pre-formed by addition of 18.3 mg of $ABTS(NH_4)_2$ into an aqueous solution of $K_2S_2O_8$ (3.3 mg in 5 ml H_2O). The resulting mixture was stored overnight in the dark below 0 °C. This way a primary stock solution of the radical (approximately of 4.7 mmol dm^{-3}) was prepared which was defrosted before an experiment and used to prepare a working stock solution of the required concentration. Remaining primary stock solution may be frozen back and stored for a repeat use. The primary stock solution of $ABTS^{\bullet+}$ prepared by this procedure contains about 25 molar% of unreacted ABTS which supports stability of the radical [13].

UV–vis spectra were recorded on a Lambda 25 Perkin-Elmer UV/VIS spectrophotometer and an Agilent 8453 diode-array spectrophotometer. Both spectrophotometers were used also for kinetic measurements performed at the temperature of 20 °C. The temperature was regulated by water circulation using a Julabo F12 thermostat when the Lambda 25 spectrophotometer was used, while the diode-array spectrophotometer was equipped with an Agilent 89090A temperature controller based on the Peltier effect. Measurements were carried out in a 10 mm QS stoppered cuvette. Kinetic runs were recorded on the Lambda 25 spectrophotometer immediately after rapid mixing of the reactants in the cuvette by following the absorbance at 734 nm without a stirring of the reaction solution during the measurement. Alternatively, or in parallel, kinetics were followed using the diode-array spectrophotometer and recording full spectra 190–1100 nm, usually with 0.5–2 s cycle time. In this case, the reaction solution was continuously stirred at 600 rpm by 7 mm teflon-coated stirring bar. The time dependences of absorbance at 734 and 340 nm were selected from the acquired data for kinetic analysis. These wavelengths correspond to the absorption maxima of $ABTS^{\bullet+}$ and ABTS, respectively. Although, the reported values for the absorption maximum of the radical cation in this region slightly vary from 725 nm [14] to 734 nm [10], the plateau is relatively wide and a value for the molar absorptivity of the radical $\varepsilon_{728} = 1.50 \times 10^4$ mol^{-1} dm^3 cm^{-1} [15] can be accurately used for estimation of concentration of $ABTS^{\bullet+}$ in aqueous solutions.

Stock solutions of BUC, Cys, and GSH were prepared by weight. A stock solution of perchloric acid was standardized by titration with a solution of sodium hydroxide (p.a., Centralchem, Slovakia) using a Titrino automatic titrator (type 785 DMP, Metrohm). The volumetric NaOH solution was standardized by titration of a weighted amount of potassium hydrogen phthalate (p.a., Merck).

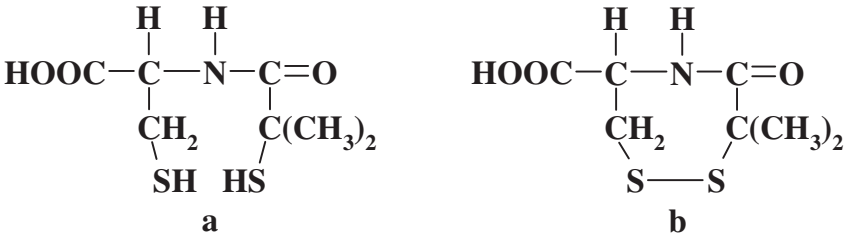

Fig. 1. Structure of bucillamine (a) and SA981 (b).