Free Radical Research

Taylor & Francis
healthsciences

Derivatives of Xanthic Acid are Novel Antioxidants: Application to Synaptosomes

CHRISTOPHER M. LAUDERBACK [a], JENNIFER DRAKE[a], DAOHONG ZHOU [b,c], JANNA M. HACKETT [a], ALESSANDRA CASTEGNA [a], JAROSLAW KANSKI [a], MARIA TSORAS[a], SRIDHAR VARADARAJAN [a] and D. ALLAN BUTTERFIELD[a,d,*]

[a]Department of Chemistry and Center of Membrane Sciences, University of Kentucky, Lexington, KY 40506, USA;

xanthic acid, has been reported to have anti-apoptotic and anti-inßammatory properties that are attributed to speciÞc inhibition of phosphatidyl choline phospholipase C (PC-PLC). However, because oxidative stress is involved in both of these cellular responses, the possibility that xanthates may act as antioxidants was investigated in the current study. Finding that xanthates efÞciently scavenge hydroxyl radicals, the mechanism by which D609 and other xanthate derivatives may protect against oxidative damage was further examined. The xanthates studied, especially D609, mimic glutathione (GSH). Xanthates scavenge hydroxyl radicals and hydrogen peroxide, form disulÞde bonds (dixanthogens), and react with electrophilic products of lipid oxidation (acrolein) in a manner similar to GSH. Further, upon disulÞde formation, dixanthogens are reduced by glutathione reductase to a redox active xanthate. Supporting its role as an antioxidant, D609 signiÞcantly p 0 01 reduces free radical-induced changes in synaptosomal lipid peroxidation (TBARs), protein oxidation (protein carbonyls), and protein conformation. Thus, in addition to inhibitory effects on PC-PLC, D609 may prevent cellular apoptotic and inßammatory cascades by acting as antioxidants and novel GSH mimics. These results are discussed with reference to potential therapeutic application of D609 in oxidative stress conditions.

Keywords D609; Xanthates; Glutathione; Antioxidant; Oxidative stress; Synaptosomes

Abbreviations GSH, reduced glutathione; GSSG, oxidized glutathione; ROS, reactive oxygen species; NAC, N-acetyl cysteine; D609, tricyclodecan-9-yl-xanthogenate; PC-PLC, phosphatidyl choline phospholipase C; TA, terephthalic acid; DTNB, 5,5-dithiobis(2-nitrobenzoic acid); PBS, phosphate buffered saline; RT, room temperature; TBARs, thiobarbituric acid reactive substances; EPR, electron paramagnetic resonance; Mal-6, 2,2,6,6-tetramethyl-4-maleimidopiperidin-1-oxyl; DNPH, 2,4-dinitrophenyl hydrazine; DNP, 2,4-dinitrophenyl hydrazone

INTRODUCTION

Glutathione (GSH) is a ubiquitously expressed tripeptide (L-g-glutamyl- L-cysteinylglycine) and, at concentrations of 0.5Ð10 mM, is one of the most abundant intracellular thiols. [1] Among its many functions,[2] GSH scavenges reactive oxygen species (ROS) and maintains the intracellular redox state. Depletion of GSH is known to be damaging to cells and eventually leads to cell death.[3Ð7] Because GSH is a major defense against cellular oxidative injury, loss of GSH shifts the oxidantÐantioxidant balance in favor of the oxidant, a condition known as oxidative stress![8,9] Increased ROS generation induces oxidative modiÞcation to biomolecules such as proteins, lipids and DNA and results in cellular dysfunction.

*Corresponding author. Address: Department of Chemistry and Center of Membrane Sciences, University of Kentucky, Lexington, KY 40506, USA. Tel.: 1-859-257-3184. Fax: 1-859-257-5876. E-mail: dabcns@uky.edu

ISSN 1071-5762 print/ISSN 1029-2470 online 2003 Taylor & Francis Ltd
DOI: 10.1080/1071576021000040664

Therefore, maintenance of high intracellular GSH levels is of critical importance to cellular integrity.

Oxidative stress is implicated in a number of human diseases including many neurodegenerative disorders.[10,11]

3 to 4 month old gerbils and homogenized by 12 passes with a motorized teßon pestle in isolation buffer (0.32 M sucrose, 4mg/ml leupeptin, 4 mg/ml pepstatin, 5 mg/ml aprotinin, 20 mg/ml trypsin

Statistical Analysis

ANOVA was used for the statistical evaluation of data and, except where indicated, signiÞcance is equivalent to p 0 01

RESULTS

D609 Interacts with Hydrogen Peroxide

D609 has been reported to be protective in models of apoptosis and inßammation,[4,23Ð25] two cellular responses that involve ROS generation. Consequently, studies were conducted to determine the ROS scavenging capabilities of D609 and several xanthate analogs (Fig. 1). Previous data[31] has shown that derivatives of xanthic acid scavenge hydroxyl radicals. D609 also interacts directly with hydrogen peroxide. When D609 was incubated with 10 mM H_2O_2, ßuoresence of resoruÞn was decreased with increasing concentrations of D609 (Fig. 2). This suggests that D609 interacted with H_2O_2, preventing H_2O_2 from acting as a substrate for horseradish peroxidase. At 100 mM D609, H_2O_2 was virtually undetectable (Fig. 2), suggesting that D609 had scavenged H_2O_2.

Xanthates Mimic Glutathione

Glutathione is well-known for its protection against cellular oxidative damage.[1] A cysteine-containing tri-peptide, GSH utilizes the sulfur atom of its cysteine residue for its molecular mechanism of protection, i.e. radical scavenging and nucleophilic attack of reactive products of lipid peroxidation. Because xanthates also contain a free sulfur atom, studies were designed to investigate GSH mimicry by the xanthates.

DTNB is a reagent widely used for the speciÞc detection of free thiol groups. GSH reacts readily with DTNB to produce a strong absorbance at *l* 412 nm (Fig. 3A). However, oxidized GSH (GSSG) is unreactive toward DTNB. Derivatives of xanthic acid exhibit a similar pattern of DTNB reactivity. The xanthates used in this study also react with DTNB and show decreased B

study both scavenge radicals and form disulÞde bonds in a similar manner to GSH, the reactions of D609 and GSH with acrolein, a toxic product of lipid peroxidation,[33] were monitored. If nucleophilic addition to the aldehyde by the free thiol of GSH or D609 occurred, a decrease in DTNB reactivity would result. Incubation of D609 with an equimolar concentration of acrolein for 15 min at RT resulted in a signiÞcant decrease ($p < 0.01$; 48%) in DTNB reactivity (Fig. 5) while the DTNB reactivity of GSH was completely eliminated (data not shown). This suggests that D609 is capable of detoxifying aldehydic products of lipid peroxidation by a mechanism similar to GSH, however, not with the same efÞcacy as the latter. D609 (100 mM) was also capable of signiÞcantly decreasing protein carbonyl formation ($p < 0.01$) caused by a physiologically relevant acrolein concentration (50 nM)[34] in synaptosomal membranes (data not shown).

Xanthates Prevent TBRAs Formation in a Structure-dependent Manner

Mal-6 bound to W sites is weakly restricted, and is manifested as narrow lines in the EPR spectrum. Alternatively, Mal-6 bound to S sites have strongly hindered motion, which results in broadened lines in the EPR spectrum. The resulting intensities of the respective W and S peaks of the $M_I = 1$ low Þeld resonance lines yield the W/S ratio, a parameter that is highly sensitive to protein conformational changes.[29] Decreased values of the W/S ratio, which reßect an overall decreased motion of spin-labeled sites on proteins, arise from increased inter- and intra-molecular protein interactions, decreased segmental motion of spin labeled proteins, protein–protein crosslinking, or changes in the structure of the lipid bilayer. [29,37]

chain (R-group). Consistent with this notion the methylated-D609 failed to scavenge radicals.

the differences in reactivity toward the electrophilic lipid peroxidation product acrolein (Fig. 5).

[28] Lauderback, C.M., Breier, A.M., Hackett, J., Varadarajan, S., Goodlett-Mercer, J. and ButterÞeld, D.A. (2000) ÒThe pyrrolopyrimidine U101033E is a potent free radical scavenger and prevents Fe(II)-induced lipid peroxidation in synaptosomal membranesÒ, Biochim. Biophys. Acta1501, 149Ð161.
[29] ButterÞeld, D.A. (1982) ÒSpin labeling in diseaseÓ,Biol. Magnetic Reson4, 1Ð78.
[30] ButterÞeld, D.A. and Stadtman, E.R. (1997) ÒProtein oxidation processes in the aging brainÓ,Adv. Cell Aging Gerontol.2, 161Ð191.
[31] Zhou, D., Lauderback, C.M., Yu, T., Brown, S.A., ButterÞeld, D.A. and Thompson, J.S. (2001) ÒD609 inhibits ionizing radiation-induced oxidative damage by acting as a potent antioxidantÓ, J. Pharmacol. Exp. Ther.298, 103Ð109.
[32] ButterÞeld, D.A., Castegna, A., Lauderback, C.M. and Drake, J. (2002) ÒReview: evidence that amyloid beta-peptide-induced lipid peroxidation and its sequelae in AlzheimerÕs disease brain contributes to neuronal deathÓ,Neurobiol. Aging 23, 655Ð664.
[33] Uchida, K., Kanematsu, M., Morimitsu, Y., Osawa, T., Noguchi, N. and Niki, E. (1998) ÒAcrolein is a product of lipid peroxidation reaction. Formation of free acrolein and its conjugate with lysine residues in oxidized low-density lipoproteinsÓ, J. Biol. Chem273, 16058Ð16066.
[34] Lovell, M.A., Xie, C. and Markesbery, W.R. (2001) ÒAcrolein is increased in AlzheimerÕs disease brain and is toxic to primary hippocampal culturesÓ, Neurobiol. Aging22, 187Ð194.