Analytical Biochemistry 538 (2017) 42–52

Contents lists available at ScienceDirect

Analytical Biochemistry

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

Menadione-mediated WST1 reduction assay for the determination of metabolic activity of cultured neural cells

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ARTICLE INFO

Article history:
Received 9 May 2017
Received in revised form
15 September 2017
Accepted 18 September 2017
Available online 20 September 2017

Keywords:
Brain
Cell viability
Electron cycler
Menadione
Metabolic activity
WST1

ABSTRACT

Cellular reduction of tetrazolium salts to their respective formazans is frequently used to determine the metabolic activity of cultured cells as an indicator of cell viability. For membrane-impermeable tetrazolium salts such as WST1 the application of a membrane-permeable electron cycler is usually required to mediate the transfer of intracellular electrons for extracellular WST1 reduction. Here we demonstrate that in addition to the commonly used electron cycler M-PMS, menadione can also serve as an efficient electron cycler for extracellular WST1 reduction in cultured neural cells. The increase in formazan absorbance in glial cell cultures for the WST1 reduction by menadione involves enzymatic menadione reduction and was twice that recorded for the cytosolic enzyme-independent WST1 reduction in the presence of M-PMS. The optimized WST1 reduction assay allowed within 30 min of incubation a highly reliable detection of compromised cell metabolism caused by 3-bromopyruvate and impaired membrane integrity caused by Triton X-100, with a sensitivity as good as that of spectrophotometric assays which determine cellular MTT reduction or lactate dehydrogenase release. The short incubation period of 30 min and the observed good sensitivity make this optimized menadione-mediated WST1 reduction assay a quick and reliable alternative to other viability and toxicity assays.

Introduction

2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium sodium salt (WST1) is a charged and water-soluble derivative of 3-(4,5-dimethylthiozal-2-yl)-2,5-diphenyltetrazolium bromide (MTT). WST1 is less toxic than MTT and results in the formation of extracellular WST1 formazan without the need of cell solubilization with organic solvents [1,2], which makes it a time-saving and non-toxic alternative to the widely used MTT reduction assay [1]. However, as the water-soluble WST1 is not taken up by cells, intracellular reduction of WST1 is prevented [3] and a membrane-permeable electron cycler is usually required to allow extracellular WST1 reduction (Fig. 1). Such electron cyclers can either be linked to plasma membrane electron transport (PMET) or permeate the cell membrane in their oxidized form [3]. In cells, oxidized electron cyclers are reduced by cellular electron sources, permeate the cell membrane in the reduced form, reduce the WST1 outside of the cells and subsequently enter the cells again to start a new cycle of electron transfer from the cells to the extracellular WST1 [4]. For the initially described WST1 assay, 1-methoxy-5-methylphenazinium methyl sulfate (M-PMS) was used as an electron cycler [1] and is still a frequently used electron cycler in commercially available WST1 reduction assay kits. Nevertheless, other electron cyclers

Abbreviations used: 3-BP, 3-bromopyruvate; ANOVA, analysis of variance; DMEM, Dulbecco's modified Eagle's medium; GFAP, glial fibrillary acidic protein; FCS, fetal calf serum; LDH, lactate dehydrogenase; M-PMS, 1-methoxy-5-methylphenazinium methyl sulfate; MTT, 3-(4,5-dimethylthiozal-2-yl)-2,5-diphenyltetrazolium bromide; PMET, plasma membrane electron transport; PMS, phenazine methosulfate; RT, room temperature; SD, standard deviation; SN, supernatant; WST1, 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium sodium salt; WST8, 5-(2,4-disulfophenyl)-3-(2-methoxy-4-nitrophenyl)-2-(4-nitrophenyl)-2H-tetrazolium sodium salt; XTT, 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino)carbonyl]-2H-tetrazolium sodium salt.

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https://doi.org/10.1016/j.ab.2017.09.011
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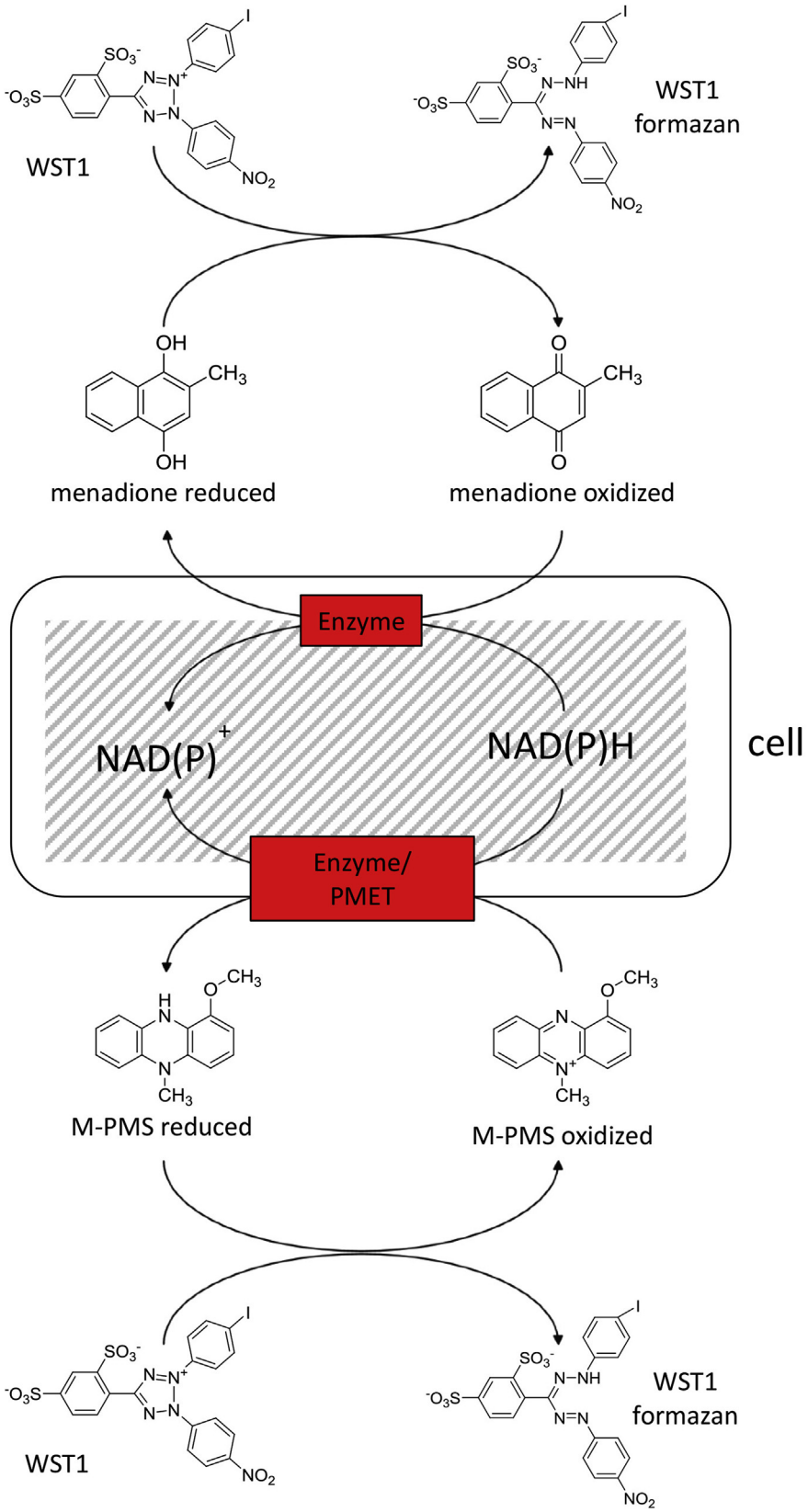

Fig. 1. WST1 reduction to WST1 formazan in the presence of the electron cyclers menadione or methoxy-5-methylphenazinium methyl sulfate (M-PMS). As the negatively-charged WST1 cannot cross the cell membrane, the presence of a membrane-permeable electron cycler, such as menadione or M-PMS, is required to facilitate extracellular WST1 reduction. Such electron cyclers can either link with plasma membrane electron transport (PMET) or permeate the cell membrane in their oxidized form into the cell where they are reduced by intracellular electron sources such as NADH or NADPH. A cytosolic enzyme is involved in the intracellular menadione reduction, which is required for the menadione-mediated extracellular WST1 reduction.