Free Radical Biology and Medicine 67 (2014) 437–450

Contents lists available at ScienceDirect

Free Radical Biology and Medicine

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

Original Contribution

A mitochondria-targeted mass spectrometry probe to detect glyoxals: implications for diabetes ☆

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ARTICLE INFO

Article history:
Received 3 August 2013
Received in revised form
22 November 2013
Accepted 25 November 2013
Available online 4 December 2013

Keywords:
Mitochondria
Exomarker
Methylglyoxal
Glyoxal
Hyperglycemia
MitoG
Free radicals

ABSTRACT

The glycation of protein and nucleic acids that occurs as a consequence of hyperglycemia disrupts cell function and contributes to many pathologies, including those associated with diabetes and aging. Intracellular glycation occurs after the generation of the reactive 1,2-dicarbonyls methylglyoxal and glyoxal, and disruption of mitochondrial function is associated with hyperglycemia. However, the contribution of these reactive dicarbonyls to mitochondrial damage in pathology is unclear owing to uncertainties about their levels within mitochondria in cells and in vivo. To address this we have developed a mitochondria-targeted reagent (MitoG) designed to assess the levels of mitochondrial dicarbonyls within cells. MitoG comprises a lipophilic triphenylphosphonium cationic function, which directs the molecules to mitochondria within cells, and an *o*-phenylenediamine moiety that reacts with dicarbonyls to give distinctive and stable products. The extent of accumulation of these diagnostic heterocyclic products can be readily and sensitively quantified by liquid chromatography–tandem mass spectrometry, enabling changes to be determined. Using the MitoG-based analysis we assessed the formation of methylglyoxal and glyoxal in response to hyperglycemia in cells in culture and in the Akita mouse model of diabetes in vivo. These findings indicated that the levels of methylglyoxal and glyoxal within mitochondria increase during hyperglycemia both in cells and in vivo, suggesting that they can contribute to the pathological mitochondrial dysfunction that occurs in diabetes and aging.

Glycation, the nonenzymatic formation of sugar–protein and sugar–nucleotide adducts, plays a major role in disrupting cell function and causing tissue damage in a range of pathologies such as diabetes, aging, and neurodegeneration [1–3]. Glycation increases in response to the elevation in glucose that occurs in unregulated diabetes and is a major cause of diabetic complications [4,5]. Within the cell excessive glucose can lead to molecular damage through the formation of 1,2-dicarbonyl compounds such as methylglyoxal from the triose phosphate intermediates of glycolysis [1,6] or from the metabolism of acetone generated during ketosis [7]. These reactive 1,2-dicarbonyls often exist in modified chemical forms in situ including reversible hemiacetals, hemithioacetals, and hemiaminals with small biomolecules and with reactive moieties on proteins and nucleic acids [8,9]. In addition they can react directly with free amine functions on proteins and nucleic acids, thereby generating substantial permanent modifications such as arginine-derived hydroimidazolones and lysine cross-links on proteins [10] and guanine-derived imidazopurinones on DNA [11]. Such modifications are thought to result in biochemical dysfunction by altering protein structure and activity and by inducing genomic mutations [2]. These markers of glycation damage are elevated in many clinical samples from diabetic patients and also in animal models of diabetes and aging [2,4,9,12,13], consistent with a contribution from these reactions to cell damage and pathology.

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http://dx.doi.org/10.1016/j.freeradbiomed.2013.11.025

An important role for methylglyoxal and glyoxal in pathology is further supported by the existence of the glyoxalase enzyme system, which specifically degrades these two dicarbonyls [14]. Loss of the glyoxalase degradation pathway renders organisms more susceptible to glycation and subsequent damage, whereas its overexpression increases life span in *Caenorhabditis elegans* [15]. Thus dicarbonyl-associated glycation of proteins and nucleic acids is a significant contributing factor in a range of pathologies, particularly those associated with diabetes or aging.

In hyperglycemia, there is considerable evidence for mitochondrial damage and elevated oxidative stress that contribute to pathology, and this has been in part ascribed to mitochondrial glycation due to methylglyoxal and glyoxal [16–21]. Furthermore these reactive dicarbonyls disrupt mitochondrial function in vitro [22–24]. Therefore, understanding the contribution from glycation damage by reactive dicarbonyls to mitochondrial dysfunction is important for analyzing and understanding the pathology associated with hyperglycemia. However, the mechanistic details are uncertain, and it has proven difficult to specifically evaluate the importance of these processes. This is in part due to the uncertainties related to the distribution of methylglyoxal and glyoxal between the cytosol and the mitochondria. To assess the importance of mitochondrial damage caused by methylglyoxal and glyoxal we have developed a mitochondria-selective molecule, MitoG, to assess relative changes in the levels of these damaging species within mitochondria in cells and in vivo.

To target mitochondria we used the lipophilic triphenylphosphonium (TPP) cation functionality, which has been shown to direct a wide variety of antioxidants, probes, and bioactive molecules to mitochondria in cells, animal models, and patients after intravenous, oral, or intraperitoneal administration [25–27]. Uptake occurs directly through the phospholipid bilayer and does not require a protein carrier. The extent of accumulation in mitochondria is determined by the membrane potential and can be adequately described by the Nernst equation, which indicates an ~10-fold increase in accumulation per 60 mV membrane potential under

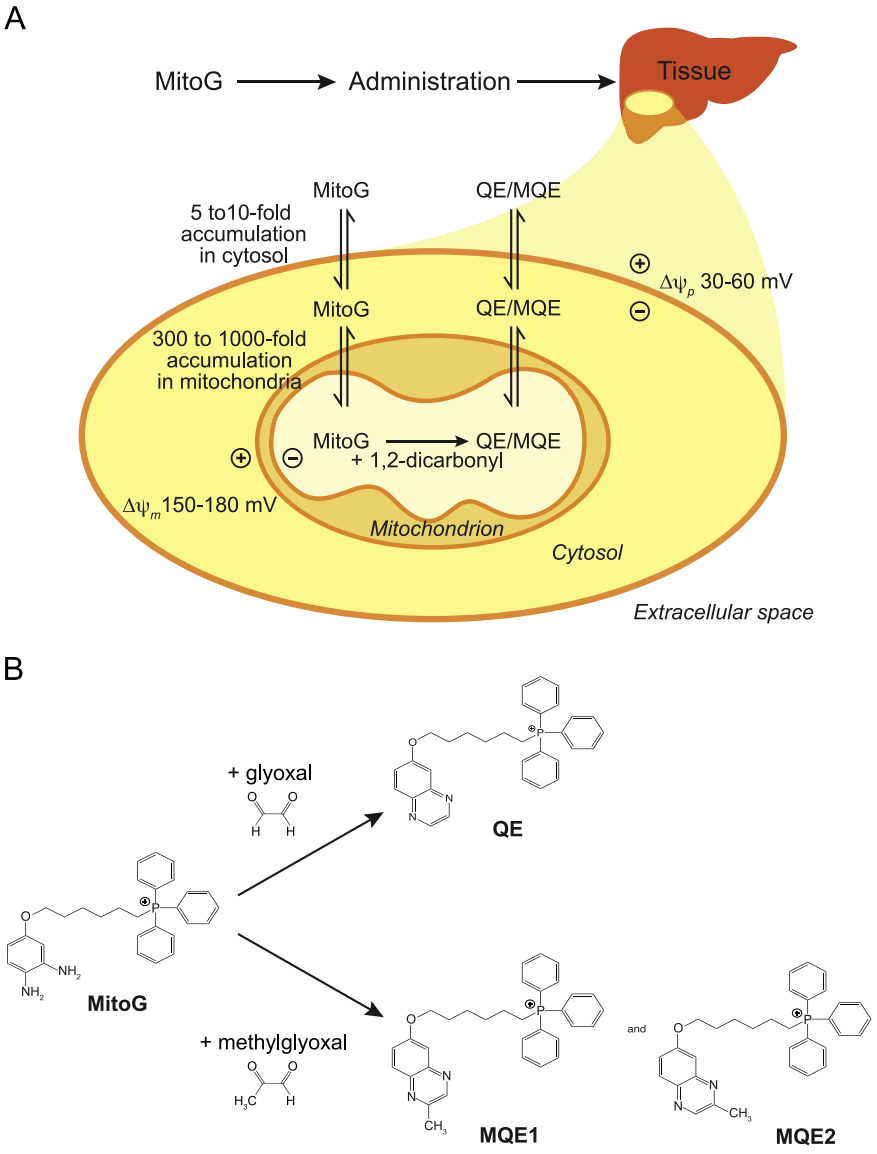

Fig. 1. Rationale and mechanism for the detection of intramitochondrial dicarbonyls. (A) MitoG, a mitochondria-targeted glyoxal and methylglyoxal trap, consists of a mitochondria-targeting TPP moiety and a phenylenediamine group that reacts with 1,2-dicarbonyls. The TPP moiety of MitoG leads to its uptake into tissues where it accumulates within mitochondria, driven by both the plasma and the mitochondrial membrane potentials. (B) Within mitochondria MitoG can then react with glyoxal or methylglyoxal to form the quinoxaline products, QE and MQE (present as two isomers, MQE1 and MQE2). These products can then be quantified by LC–MS/MS relative to deuterated internal standards to provide a measure of the amount of free glyoxal and methylglyoxal present within mitochondria in cells and in vivo.