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Interaction of heme and heme–hemopexin with an extracellular oxidant system used to measure cell growth-associated plasma membrane electron transport

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Abstract

Since redox active metals are often transported across membranes into cells in the reduced state, we have investigated whether exogenous ferriheme or heme bound to hemopexin (HPX), which delivers heme to cells via receptor-mediated endocytosis, interact with a cell growth-associated plasma membrane electron transport (PMET) pathway. PMET reduces the cell-impermeable tetrazolium salt, WST-1, in the presence of the mandatory low potential intermediate electron acceptor, *m*PMS. In human promyelocytic (HL60) cells, protoheme (iron protoporphyrin IX; 2,4-vinyl), mesoheme (2,4-ethyl) and deuteroheme (2,4-H) inhibited reduction of WST-1/*m*PMS in a saturable manner supporting interaction with a finite number of high affinity acceptor sites (Kd 221 nM for naturally occurring protoheme). A requirement for the redox-active iron was shown using gallium-protoporphyrin IX (PPIX) and tin-PPIX. Heme–hemopexin, but not apo-hemopexin, also inhibited WST-1 reduction, and copper was required. Importantly, since neither heme nor heme–hemopexin replace *m*PMS as an intermediate electron acceptor and since inhibition of WST-1/mPMS reduction requires living cells, the experimental evidence supports the view that heme and heme–hemopexin interact with electrons from PMET. We therefore propose that heme and heme–hemopexin are natural substrates for this growth-associated electron transfer across the plasma membrane.

Keywords: PMET; Heme; Hemopexin; Plasma membrane electron transport; Oxidoreductase

1. Introduction

Proliferating mammalian cells transfer electrons across the plasma membrane from intracellular reductants like NADH to extracellular electron acceptors such as oxygen [1–3]. These plasma membrane electron transport (PMET) systems facilitate cellular responses to physiological stimuli and have been implicated in the activation of signaling cascades and in the control of cell growth [4,5]. At least two PMET pathways have been shown to be associated with cell growth. One reduces ferricyanide [6] and has been shown to use the voltage-dependent anion channel, VDAC1 [1]. The other is comprised of at least three components, involves plasma membrane ubiquinone [7,8], and oxygen can be a terminal electron acceptor [9]. This PMET pathway has been shown to be quite distinct from mitochondrial electron transport [10] and recycles cytosolic NADH to maintain glycolysis and cell growth, particularly in glycolytic cells [9]. It is measured by reduction of the water soluble tetrazolium salt, WST-1 [2,(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium), in the presence of an intermediate electron acceptor [IEA; *m*PMS (1-methoxy-5-methylphenazinium methylsulfate)]. The standard 1 electron reduction potential of *m*PMS is +70 mV and of WST-1 is −141 mV (obtained by adding +199 mV to −340 mV versus Ag/AgCl and saturated KCl, [11]). The IEA accepts electrons from a terminal electron donor enzyme in the pathway to reduce WST-1 directly (Figs. 1A and B; ref. [12]). WST-1 contains negatively charged sulfonate moieties, excluding it from the cell via the plasma membrane potential (50–70 mV, negative inside) but allowing it to accept electrons from *m*PMS [11,12]. Activated *m*PMS will pick up low potential electrons from plasma membrane electron donors to reduce WST-1 directly in two separate one electron transfer events involving both WST-1 and extracellular PMS radicals [M.V. Berridge, M. Davies, unpublished data]. A requirement for ubiquinone (standard one electron

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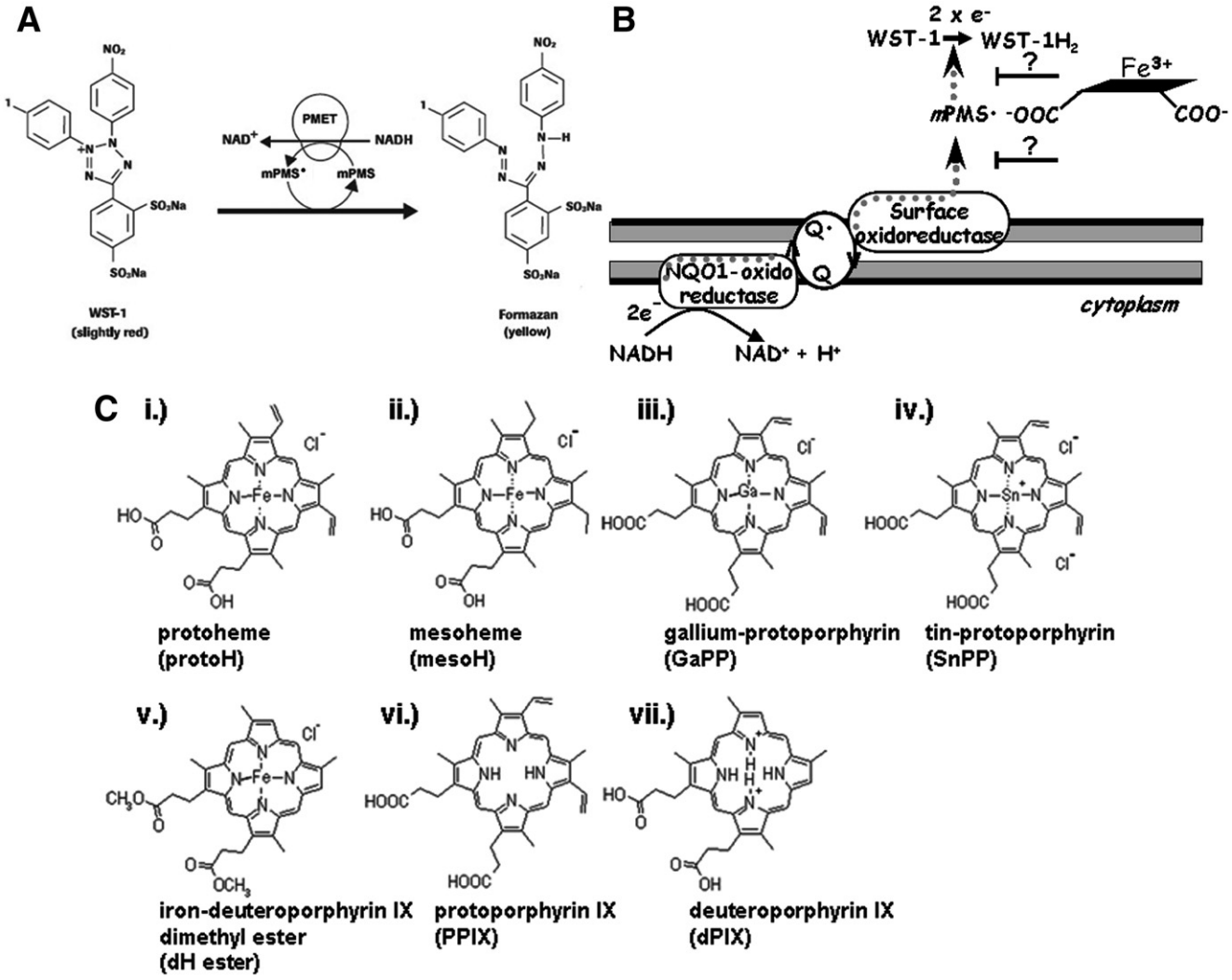

Fig. 1. Reduction of cell-impermeable WST-1 by plasma membrane electron transport. Panel A: diagrammatic representation of WST-1 reduction involving the intermediate electron acceptor, *m*PMS, (modified from information provided by Roche Diagnostics GmbH). Panel B: current model of PMET from intracellular NADH to extracellular WST-1: model of sites of interaction of heme with PMET. Diagram of ubiquinone-dependent electron transfer across the plasma membrane based on experimental evidence (for recent review see ref. [8]). Electrons are transferred (dotted line) from cytosolic NADH via ubiquinone (Q), shown as the radical semiubiquinone, via membrane protein components of PMET to the intermediate electron acceptor *m*PMS generating the *m*PMS radical, which then reduces WST-1. Two electrons and a proton are required to reduce WST-1. Two possible sites of interaction of either heme or heme–hemopexin with the electron path for WST-1 reduction are indicated by the schematic heme molecule shown. Panel C: Structures of porphyrin and heme analogs used. (i) Protoheme {2,4-vinyl, protoH; iron protoporphyrin IX chloride}; (ii) mesoheme {2,4,-ethyl, mesoH; iron mesoporphyrin IX chloride; 8,13-Bis(ethyl)-3,7,12,17-tetramethyl-21H,23H-porphine-2,18-dipropionic acid iron (III) chloride}; (iii) gallium-protoporphyrin (GaPP); (iv) tin-protoporphyrin (SnPP); (v) iron-deuteroporphyrin IX dimethyl ester (2,4,-H, dH-ester;); (vi) protoporphyrin IX {PPIX; 8,13-Bis(vinyl)-3,7,12,17-tetramethyl-21H,23H-porphine-2,18-dipropionic acid}; and (vii) deuteroporphyrin IX {2,4,-H, dPIX; 3,7,12,17-Tetramethyl-21H,23H-porphine-2,18-dipropionic acid}. Not shown is deuteroheme (2,4,-H, deuteroH). Some tetrapyrroles are supplied from Frontier Scientific as the mono or dichloride as shown.

reduction potential +36 mV) in PMET, first postulated in 1992 [13,14], has been confirmed genetically in yeast [15]. Furthermore, a complex of proteins in the plasma membrane that includes a constitutively expressed NADH-dependent oxidase (CNOX)-like member of the ECTO-NOX family of cell surface oxidases has been shown to be involved in the NADH-dependent reduction of WST-1 [16]. Although oxygen can be reduced by these NOX-like surface oxidoreductases [9], the efficiency is poor and other natural substrates remain to be identified.

Redox-active metals like iron and copper are often transported in the reduced state, [17,18] with the transporters being associated with metal reductases. In duodenal enterocytes, cytochrome b (Dcytb) has been proposed to reduce dietary ferric iron for iron absorption via transport across the basolateral membrane by the divalent metal transporter, DMT1 [19]. In erythroid cells, another ferri-reductase, Steap3, related to the F(420)H(2): $NADP^+$ oxidoreductases and yeast FRE metallo-reductases, acts in concert with diferric-transferrin generating Fe(II) as the exported species from endosomes [20]. In several cell types including macrophages and enterocytes, iron export from cells utilizes the multi-copper oxidase, hephaestin, together with the Fe(II) exporter, ferroportin, for iron loading of transferrin (for recent reviews see ref. [21,22]). Transporters for