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High-yield expression in *Escherichia coli* of soluble human MT2A with native functions

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Abstract

Metallothioneins (MTs) are a family of low molecular weight, cysteine rich heavy metal binding proteins with multifunction, such as metal detoxification and antioxidation, and are involved in a number of cellular processes including gene expression, apoptosis, proliferation and differentiation. However, high yield expression of human MT in *Escherichia coli* has not been established effectively. To produce large amounts of human MT protein at low cost, recombinant human metallothionein 2A (MT2A) protein with an N-terminal GST tag was successfully expressed at high levels in soluble form in *E. coli* and high purification of it was established by affinity chromatography under native conditions. The final yield was about 5 mg of the recombinant MT2A per liter of bacterial culture with the purity of 97.9%. Chemical and functional characteristics analysis of the recombinant human MT2A exhibited intact metal binding ability, hydroxyl radical scavenging ability and significant protective role against DNA damage caused by UVC radiation. Establishment of highly purified recombinant human MT2A protein with native characteristics at low cost would improve its function study and wide applications in protecting against oxidative damage and UV radiation.

Keywords: Human metallothionein 2A; Recombinant protein; Affinity chromatography; Hydroxyl radical scavenging; UVC radiation protection

Metallothioneins are ubiquitously expressed proteins containing 61–68 amino acid residues, and are characterized by high cysteine content, paucity of aromatic acid and selective binding of heavy metals such as zinc, copper and cadmium. All vertebrates examined contain two or more distinct MT[1] isoforms designated MT-1 through MT-4 [1]. In humans, at least 10 of 17 MT genes are functional, which encode multiple isoforms of hMT1, one isoform of hMT2A, hMT3 and hMT4 [2,3]. Between them, the hMT2A gene is highly expressed with a proportion of about 50% of total MT proteins [4].

The primary role of MT remains enigmatic as further functions were continually discovered since it was discovered about half a century ago. The metal-binding and redox properties of MT endow it wide-ranging functional capabilities in biosystems [5]. For example, constitutive MT plays a background role in metal metabolism and certain homeostatic mechanisms, whereas highly induced MT involves in adaptation to various environmental stresses [6]. The role for MT in heavy metal detoxification has been well demonstrated, however, it is not thought to be the sole or predominant function of the protein [7]. The biological role of MT in response to oxidative stress may be a cellular defense system, and studies have identified MT as an efficient free radical scavenger, suggesting a role in modulating reactive oxygen species [8]. Moreover, MT could act in a number of biochemical processes including gene expression, apoptosis, proliferation and differentiation [5,9,10].

The unique features of MTs have attracted the attention of many biologists and chemists to investigate its multiple

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[1] *Abbreviations used:* MTs, Metallothioneins; GST, glutathione *S*-transferase.

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function in biological process and its potential in application, such as detoxification, antioxidation, antiradiation and etc. However, to investigate its potential in application requires sufficient amounts of pure, active material. The supply of MT for research has relied on its purification from mammalian tissue, in particular because rather large quantities can be prepared from livers of animals in which MT has been induced by heavy metals [11,12]. Although direct purification from tissues is a preferred way to produce native proteins, it is limited by low recovery and this approach can't be applied to produce human MT. Large scale production of highly purified human MT proteins with intact functions in *Escherichia coli* has not been established though attempt in expressing the recombinant MT in bacteria and fungus has already been reported [13,14].

In this study, human MT2A was expressed as a GST fusion protein. Highly purified protein was produced by affinity chromatography after cleaved by a site-specific protease. The characteristics of metal binding, hydroxyl radical scavenging, and radiation protection are demonstrated to be indistinguishable from native protein. It indicates that rapid and efficient purification of human MT with native functions can be established with the high binding affinity approach and facilitated by expressing fusion protein as soluble form.

Materials and methods

Plasmid and reagents

Chemicals and reagents for bacterial growth were supplied from Sigma–Aldrich (St. Louis, MO, USA). Reagents for RNA isolation, reverse transcription, PCR, restriction enzymes digestion, and ligation were supplied by Promega Corporation (Madison, Wisconsin, USA). Primers for amplification and sequencing were synthesized by BioAsia Biotechnology Co., Ltd (Shanghai, China). Expression vector pGEX-6P-1, *E. coli* strain BL21, Glutathione Sepharose 4B matrix and PreScission Protease was purchased from Amersham Biosciences (Stockholm, Sweden). The Bradford protein assay kit was purchased from Bio-Rad (California, USA). PVDF blotting membranes and ECL were purchased from Amersham Biosciences (Stockholm, Sweden). The kit for measuring reactive oxygen species was purchased from Nanjing Jiancheng Bioengineer Institute (Nanjing, Jiangsu, China). The human liver tissue was obtained following partial hepatectomy from the General Surgery Department of Xiangya hospital following informed consent. The cell line of immortalized human lymphocyte transformed by EBV was kept by our laboratory.

Construction of the expression vector for hMT2A

The isolation of human liver RNA and reverse transcription were performed under the protocol provided by manufactures. Since the existence of MT isoforms, to obtain the cDNA sequence of MT2A isoform (GenBank Accession No. BC007034), we first designed primers according to the 5' and 3' untranslated regions, which are specific for each MT isoform gene [15]. Using this amplified cDNA fragment as a template, the full-length of human MT2A (hMT2A, 183 bp, without ATG start code, amino acids Asp-2 to Ala-61, followed by stop code) was amplified by primers designed just according to encoding region. To insert the fragment into pGEX-6p-1 plasmid, we designed primers with *Sal*I site according to its multicloning site. The amplified hMT2A fragment and pGEX-6p-1 vector were digested by *Sal*I. The PCR fragment was fused downstream to GST encoding sequence. After transforming into *E. coli* JM109 competent cells, colonies were screened by restriction map and the orientation was checked by sequencing (BioAsia Biotechnology Co., Shanghai, China). The produced plasmid was named pGEX-hMT2A.

Expression of the recombinant hMT2A fusion proteins

The pGEX-hMT2A plasmid was extracted, purified and transformed into *E. coli* strain BL21. To screen the more efficient clones, a small-scale expression was carried out and the fusion protein expression was checked out on 12% SDS–PAGE. Briefly, single transformed colony was grown into 3 ml of 2× YTA medium (16 g/l tryptone, 10 g/l yeast extract, 5 g/l NaCl, containing 100 μg/ml ampicillin) for overnight. Ten milliliter of 2× YTA medium was inoculated with 500 μl of the overnight cultures and grown at 37 °C with vigorous shaking until the OD_{600} was upon 0.5–0.7. Then expression was induced with 0.1 mM IPTG. We also inoculated one extra culture to serve as a non-induced control. After the cultures were grown for an additional 4–5 h, cells were harvested by centrifugation for 10 min at 4000*g*. About 40 mg of wet weight cells were harvested and then resuspended in 500 μl of ice cold PBS, and sonicated until the cloudy cell suspension becomes translucent. The lysate was centrifuged at 10,000*g* at 4 °C for 30 min. The supernatant was the soluble protein and was saved on ice. The pellet was resuspended in PBS and this was a suspension of the insoluble protein. Proteins were separated on 12% SDS–PAGE gel and visualized with Coomassie blue staining.

Orthogonal design method in screening for optimum expressing conditions

Orthogonal design method was employed to optimize the condition for soluble fusion protein expression. According to the variable factors that influence the yield of recombinant protein, cell density before inducing, IPTG concentration, inducing time and growth temperature were selected as independent factors. The four factors and four levels for each factor were arranged in an orthogonal experimental table L16 (4^5) [16,17]. Basing on the orthogonal design table, a total of 16 experiments