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Tannic acid (TA): A molecular tool for chelating and imaging labile iron

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ABSTRACT

This report presents the potential utilization of tannic acid (TA) as a natural iron chelator. TA is capable of binding with small ferric complexes without competitive binding with endogenous iron-containing molecules such as ferritin and transferrin. It was observed that the extracellular iron binding of TA resulted in the formation of self-assembled Fe^{3+}-TA complexes, which were then taken up by HepG2 cells via phagocytosis pathway with autophagy-inducing properties. Obviously, TA was found to inhibit iron-induced HepG2 cell growth. However, cellular interactions and biological responses to the treatment were found to depend on availability of iron. Based on the results of the iron efflux experiment, it can be stated that TA has the capability to mobilize iron from cells in the form of assembled Fe^{3+}-TA complexes. Interestingly, TA-mediated cellular iron influx and efflux were successfully monitored via MRI. The results of this study suggest that TA can be used as a molecular tool for chelating and imaging labile iron. This might be a promising approach for prevention and treatment of iron-associated cancer or other iron overload disorders.

1. Introduction

There is no doubt that iron is an essential element for the human body. It plays crucial roles in various cellular functions including cellular metabolism, cellular replication and growth, etc. (National Institutes of Health, 2016; Chellan and Sadler, 2015). Utilization of iron in the human body is tightly regulated by iron homeostasis (Pantopoulos et al., 2012). There is increasing evidence that a range of diseases including age-related, hematological, and non-hematological disorders is associated with deregulated iron homeostasis. Therefore, targeting iron homeostasis is promising in the treatment of various diseases. Normally, free iron or small iron complexes are not allowed to be present in high concentrations in healthy condition and molecular chaperone has to get involved in every step of iron homeostasis (Brissot et al., 2012; Gammella et al., 2017). Such small iron molecules (the so-called non-transferrin-bound iron, NTBI), which are able to easily cycle iron redox states, are capable of alternating cellular redox status and can participate in the generation of reactive oxygen species, causing cellular and tissue damage (Dixon and Stockwell, 2014). In addition, they play an important role in disease progression via promotion of cellular growth and proliferation (Torti and Torti, 2013). Recently, treatment of diseases associated with NTBI (both hematological and non-hematological disorders) can be carried out via the iron-chelating approach by using iron chelators. Generally, there are three major classes of chelators developed for medicinal usage, ranging from siderophores, synthetic chelators, and plant-derived chelators (natural iron chelators) (Hatcher et al., 2009). Among them, plant-derived chelators, including plant polyphenols and flavonoid compounds, are of interest because of their high affinity and selectivity for binding ferric iron as well as their therapeutic potential. Tannic acid (TA) is a large polyphenolic molecule consisting of a mixture of large gallotannins, trigallic acid, m-digallic acid, and gallic acid. Unlike small phenolic compounds, interaction between ferric ions and TA can undergo a process of self-assembly to form large self-assembled Fe^{3+}-TA complexes. The large assembled Fe^{3+}-TA structure may be considered beneficial for enhancing the MRI signal because it has a paramagnetic center with capability of enhancing the rate of water-proton exchange by slowing down rotational diffusion (Guo et al., 2014; Merbach et al., 2013). According to the study of its absorption, metabolism and toxicology, moreover, TA was found to be rapidly absorbed in the gastrointestinal tract into the bloodstream and rapidly excreted, with low acute, subchronic and chronic oral toxicity (Nakamura et al., 2003; Wagner, 2006). Therefore, it would be of great interest to study the feasibility of TA as a natural iron chelator for treating and imaging NTBI-related diseases.

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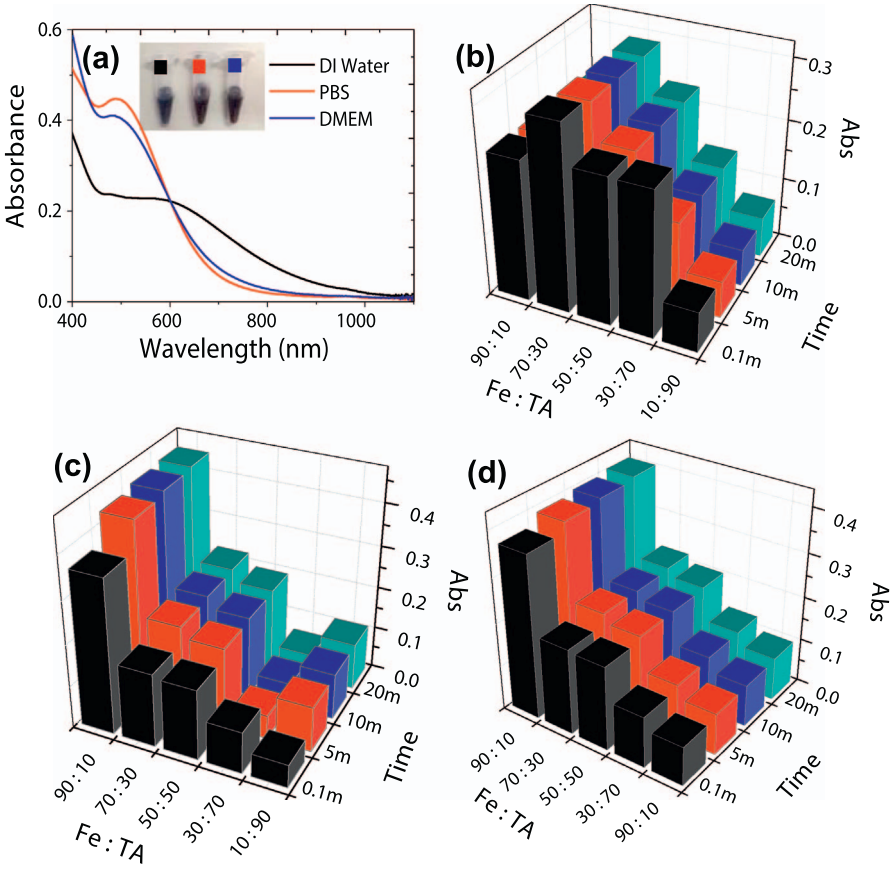

Fig. 1. (a) The UV-vis absorption spectra of solutions obtained by mixing ferric chloride and TA in different media. (b–c) The absorbance at proper wavelength of mixed solutions containing different ferric chloride: TA ratios at different lengths of reaction time in DI water, PBS (pH 7.4), and DMEM (without phenol red), respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

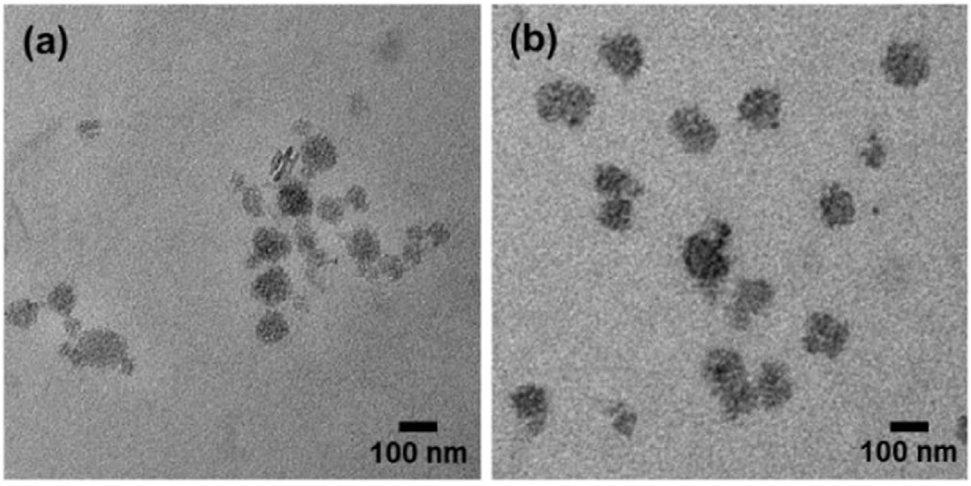

Fig. 2. TEM images of the self-assembled Fe-TA complexes obtained by mixing ferric chloride and TA in completed DMEM medium using different Fe:TA molar ratios (a) 10 μM ferric chloride and 10 μM TA (b) 100 μM ferric chloride and 10 μM TA.

2. Materials and Methods

2.1. Chemicals

Ferric chloride hexahydrate ($FeCl_3{\cdot}6H_2O$) and ferrous chloride tetrahydrate ($FeCl_2{\cdot}4H_2O$) were purchased from Fisher Chemicals; tannic acid ($C_{76}H_{52}O_{46}$) from LobaChemie; hydrochloric acid (HCl) and sodium hydroxide from RCI Labscan; ethanol from QRec; L-cysteine from Merck; ammonium persulfate (APS), acrylamide, *N*,*N*′-methylenebisacrylamide, 2′,7′-dichlorodihydrofluorescein diacetate (H_2DCFDA), ferritin, transferrin, monodansylcadaverine (MDC), and dimethyl sulfoxide (DMSO) from Sigma-Aldrich; Dulbecco's modified Eagle's medium (DMEM) from HyClone™ Thermo Scientific and Caisson Laboratories; fetal bovine serum (FBS), penicillin–streptomycin, and trypsin-EDTA from Caisson Laboratories; *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED) from Invitrogen; acridine orange from AMRESCO; ribonuclease A (Rnase A), collagen type I, Triton X, propidium iodide (PI), and annexin V-FITC apoptosis detection kit from US Biological; and sodium azide (NaN_3) from BDH Laboratory.

2.2. Interaction of TA With Different Iron Complexes in Different Media

Different mole ratios of ferric chloride and tannic acid were mixed together in different media including DI water, PBS (pH 7.4), and DMEM (without phenol red) at room temperature. At the desired time, the UV-vis absorption spectra were measured on a UV-vis