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Full Length Article

In vitro DNA binding, antioxidant, antimicrobial and anticancer assessment of amino acid functionalized magnetic nanoparticles

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ABSTRACT

Biofunctionalized magnetic nanoparticles (FMNPs) were synthesized through *in situ* coprecipitations of Fe (II) and Fe (III) salts under air followed by the addition of amino acids (AA): serine (Ser), alanine (Ala), cysteine (Cys), histidine (His), and methionine (Met) via bottom-up approach without following any sophisticated route. However, the effect of amino acid side chain on the formation of γ-Fe_2O_3 (maghemite) and Fe_3O_4 (magnetite) was attained through XRD, HR-TEM, SEM, VSM, FTIR, DLS and Mercury Porosimeter (MP). Surprisingly, our study depicts that except His; Ser, Ala, Cys and Met produced more than 50% of magnetite phase and inhibited the oxidation of Fe_3O_4 in the presence of air. Furthermore, AA@MNPs were used for *in vitro* biological assessment such as salmon testes DNA (ST-DNA) binding efficiency, DPPH· scavenging activity, antimicrobial and anticancer properties using UV–visible spectrophotometry and colorimeter respectively. The ST-DNA binding efficiency was observed at λ_{max} 259 nm and quantitative interaction was determined using 15–125 μM AA@MNPs and 500 μg/ml DNA at physiological temperature, shows stacking interaction. The Cys@MNPs have exhibited highest DPPH· scavenging activity with SC50 2.5 μg/mL at λ_{max} 525 nm. Antimicrobial property of AA@MNPs was studied on gram-positive (*B. subtilis*), gram-negative (*E. coli*) bacteria and fungi (*C. albicans*) using disc diffusion assay by measuring zone of inhibition (ZOI) in mm, mentioned as a diameter. Alongside, minimum inhibition concentration (MIC) was estimated by the dilution method for those strains sensitive against AA@MNPs. Although, AA@MNPs showed ~100% control growth of human lung cancer cell line (A549) with LC50, TGI and GI50 >80 μg/ml shown cells compatibility. These significant results revealed the impact of AA side chain with different surface activities of FMNPs.

1. Introduction

Currently, the MNPs have been extensively investigated owing to their unique physicochemical activities in the field of magnetic recording media and biomedical application [1,2]. To develop efficient biomedical applications, their stability is strongly required in colloidal phases, specific for cellular uptake and target specific. The enhancement of the colloidal stability depends on the dimension and surface chemistry of MNPs, which could achieve by avoiding aggregation due to gravitational forces [3].

However, the Surface functionalization using various targets specific analogs or biomolecules are reported as operative to facilitate the above-mentioned activity [4,5]. MNPs surface functionalization was carried out using polymers (polyethylene glycol, polyethyleneimine) [6,7], 1st tier dendrimers (TTDMM) [8,9], surfactant (CTAB) [10], organic acids (formic acid, oxalic acid, citric acid) [8] along with the biomolecules like dextran [11], albumin [12], chitosan [13], enzymes [14], antibodies [15], are being capped *in* or *ex situ* routes. Literature illustrates various synthetic methodologies and capping agents, which are being widely preferred for developing various applications [16,17]. Despite, many properties and processes reported elsewhere yet many advance understanding about the interaction mechanism through the capping and their profound applications are remaining to find. However, the systematic studies with a series of functionalizing agents could further enhance the understanding and role of various chemical entities on the properties and application of FMNPs.

In light of the above, previously we have reported the studies of bare MNPs with the series of aliphatic organic acids (formic, oxalic, citric acid) and Trimesoyl 1,3,5-tridi (methyl, ethyl, propyl, butyl and hexyl) malonate ester 1st tier dendrimers in aprotic polar

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medium at physiological temperature. The studies on choosing series have facilitated in finding out the degree of freedom at the working coordinates of the MNPs *vis-a-vis* members of the series along with the charge-charge interaction, especially London dispersive forces and electrostatic interaction formed on the dispersion in DMSO and could further develop various biomedical and biochemical applications [8,9]. Consequently, we have found exceptionally higher absorption of the 1st tier dendrimers and aliphatic organic acids through electrostatic interaction. The adsorption study of amino acids on the bare MNPs surface at pH 6 depicts the role of electrostatic interaction formed between the surfaces of positively charged MNPs and negatively charged amino acids are reported [18]. In earlier research article we have reported that the larger numbers of —COOH group enhances the MNPs capping through and stabilization while increasing numbers of alkyl chain length of 1st tier dendrimers destabilized the MNPs [19]. Thus, the study proved the active role of —COOH on MNPs stabilization and thus, in this research article we have studied the impact of L-amino acid series having —COOH, —NH_2 and various side chain groups on MNPs functionalization along with the potential towards biological applicability.

As amino acids (AA) are the basic building blocks of protein and peptides, thus seems essential for inducing biological activities, to manufacture pharma/agrochemical compounds and to develop biosensors [20]. Considering, wider applications of differently charged amino acids, we have chosen them for *in situ* MNPs functionalization through serine (Ser), alanine (Ala), cysteine (Cys), histidine (His), and methionine (Met). The purpose of choosing this series was to investigate the role of side chain along with functional groups of AAs on MNPs synthesis and their effect on biological activities. This study may lead us to understand the role of metal/metal oxide NPs in the origin of life by enhancing the knowledge about the complexation of basic biomolecules with metal/metal oxides. Lambert and Basiuk et al. have reported the polymerization of AA on the surface of minerals to know their role in the origin of the life [21,22].

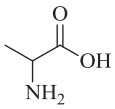
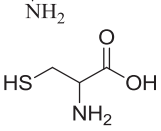
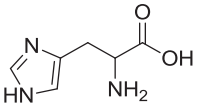
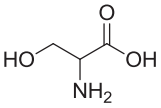
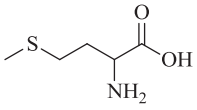
Many research groups have observed the role of AA side chain on binding affinity and functionalization towards the surface of metal oxides [23–25]. Reportedly, functionalization with polar AA is influenced by electrostatic effect, which depends on the pH variation and ionic strength, while nonpolar AA demonstrates their affinity to the hydrophobic surface due to mild electrostatic interactions [26,27]. Positively charged AA for an instance arginine and lysine are supposed to bind through the —COOH to develop electrostatic interactions. However, it was noticed that negatively charged amino acids like glycine and glutamic acid have formed stronger electrostatic interaction than of the arginine and lysine [21]. Basiuk et al. studied the formation of hydrogen and covalent bonding between amino acids and the surface metal oxides and Viota et al. found that the presence of AA influences the particle size distribution and colloidal stability [22,28]. However, for the cysteine there are two different mechanisms for MNPs interaction, such as the formation of sulfide bond through thiol group (—SH) and binding through —COOH on the surface of MNPs [29]. In another case a negatively charged formic acid showed dissociative adsorption on the surface of MNPs instead of electrostatic interaction [30]. Moreover, it was also reported that the complexation of amino acids with metal oxide may increase the thermal stability [31].

In our study, the functionalization of MNPs with AA was determined by observing the impact of AA side chain (Table 1) on the formation of magnetite and maghemite phase under atmospheric condition. In addition to above, for the first-time surface area of each AA@MNPs were calculated using mercury porosimeter (MP) which matches with theoretical surface area calculated from XRD. The HR-TEM data prove the effect of AA side chain on the size and shape of the MNPs as in our study it has been used as a capping agent this data was supported by DLS and XRD. Moreover, FTIR study shows that the all AA@MNPs except Cys@MNPs had a bidentate bridge formation through electrostatic interaction and hydrogen bonding. Additionally, it was also noticed that the biological activity of AA@MNPs depicts the role of surface functional group forming strong static interactions with ST-DNA, notable antioxidant property, and the antimicrobial property. This study may open the use of smart biocompatible AA@MNPs for various biomedical and environmental applications.

2. Materials and methods

Ferrous sulfate ($FeSO_4{\cdot}7H_2O$), anhydrous Ferric chloride ($FeCl_3$), Sodium hydroxide (NaOH), L-histidine, L-cysteine, L-alanine, L-methionine, and L-serine were procured from Sigma-Aldrich with 99.99% purity; 75% (v/v) aq. ethanol was procured from

Table 1
Different amino acids and its side chain properties.

Name of AA	Structure	Side chain	Functional group	Properties
Alanine		—CH_3	Methyl	Neutral Nonpolar
Cysteine		—CH_2SH	Sulfhydryl	Neutral Slightly polar
Histidine		—$C_4H_5N_2$	Imidazole	Basic polar
Serine		—CH_2OH	Hydroxyl	Polar uncharged
Methionine		—CH_2SCH_2	Thioether	Neutral nonpolar