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Comparison of chelating ability of dipeptide (histidine-β-alanine) and (tetrakis(4-sulfonatophenyl)porphyrin) (TPPS4) for in vitro removal of toxic metals

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Abstract

Peptides are one of the best candidates for drug development due to their high specificity and low toxicity and porphyrins are significant macromolecules in biological systems with important roles. In this work, synthesis of dipeptide (histidine-*β*-alanine) was done by solid-phase peptide synthesis method (SPPS) and tetrakis(4-sulfonatophenyl)porphyrin($TPPS_4$) was synthesized by Adler method. The molecular structure of the dipeptide and porphyrin was defined using different methods such as UV-Vis, FT-IR, ^{1}H NMR and LC-Mass spectrometry for dipeptide. Kinetics study and comparison of the chelating ability of dipeptide (histidine-*β*-alanine) and $TPPS_4$ were investigated for removing of metal ions Al^{3+}, Cu^{2+}, Hg^{2+} and Pb^{2+} in vitro.

Keywords: Dipeptide; solid-phase peptide synthesis; tetrakis(4-sulfonatophenyl)porphyrin; chelating ability.

Introduction

Natural and synthetic peptides have shown promise as pharmaceutics with the potential to treat a wide variety of diseases. Peptides are usually selective and efficacious, therefore need only be present in low concentrations to act on their targets. The metabolism of peptides is superior to other small molecules due to the limited possibility for accumulation and result in relatively non-toxic amino acid, peptide metabolites. These properties contribute towards the overall low toxicity of peptides, with a limited risk of adverse interactions [1]. A solid-phase approach is the most popular way to synthesize peptides on small and large scales for research purposes [2]. Porphyrins are significant macromolecules applied in different fields such as photoconverter, photocatalysts, photosensitizes in diagnosis and photodynamic therapy and also, with different application as a drug [3-6]. In this paper, we report the comparison between dipeptide (his-*β*-alanine) and tetrakis (4-sulfonatophenyl) porphyrin ($TPPS_4$) as a chelating agents. The ability of dipeptides and porphyrin to chelate various metal ions can be investigated [7-9]. The reason for choosing these

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two compounds and comparing them is that both are naturally present in biological systems with important roles [10-13]. In this paper, molecular structure and comparison of the chelating property of synthesized dipeptide and porphyin were investigated.

Experimental

Chemicals

All chemicals were purchased from various companies as follows: Wang resin, *N*-Fmoc-*N*-trityl-L-histidine was purchased from the Bachem chemical company. Boc-*β*-alanine-OH was obtained from Aldrich company, hydroxybenzotriazole (HOBt) was purchased from Fluka company. Scavengers (anisole andphenol), trifluoroacetic acid (TFA), piperazine, *N*,*N*′-diisopropylcarbodiimide(DIC), diethyl ether, dichloromethane, *N*,*N*-dimethyl formamide, methanol, acetic anhydride and pyridine, propionicacid, benzaldehyde, pyrrole, sulfuric acid, acetone, sodium carbonate and metal salts ($AlCl_3$, $CuCl_2$, $HgCl_2$ and $Pb(NO_3)_2$) were obtained from Merck chemical company.

Sample characterization

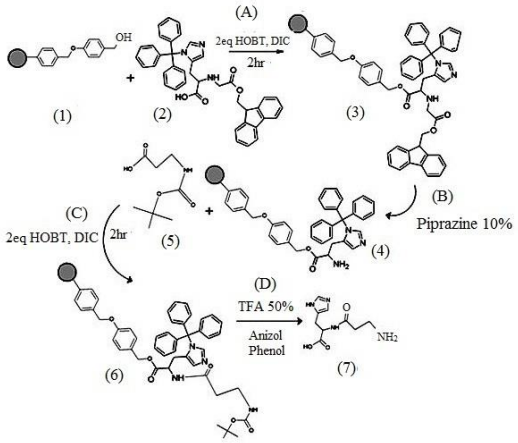
PG Instruments T80 Double Beam UV-Vis spectrophotometer was used for UV-Vis measurements. The infrared spectra were recorded on a Shimadzu FT-IR-8400S spectrophotometer in the range of 400–4000 cm^{-1}. Proton NMR spectra were recorded on a Bruker DRX250 (300 MHz) spectrometer in water. LC-MS analyses were performed on Agilent 6410 Triple Quadruple LC-MS (USA).

Methods

Dipeptide synthesis

Dipeptide (histidine-*β*-alanine**)** was manually synthesized on solid phase using standard Fmoc and Boc strategy [14,15]. Briefly, the peptide sequence his-*β*-alanine was assembled on Wang resin **(1)**.*N*-Fmoc-*N*-trityl-L-histidine (2.47 g, 2 equivalents)(2), and two equivalents of HOBt (0.26 g) and DIC(1mL), as a coupling reagent, were added to the reaction vessel. The mixture was shaken for 2 h. Then, first amino acid is loaded to the resin, the unreacted sites must be end-capped with 2 equivalents acetic anhydride and 2 equivalents pyridine to ensure that future reactions do not react at those unloaded sites. Removal of the Fmoc group **(3**) was obtained by the addition of 10% piperazine, shacked for 30 min under nitrogen atmosphere. The monitoring of the completion of the Fmoc cleavage was performed with color detector (acetaldehyde/chloranil) for detection of free terminal amino groups **(4)**[16]. Second amino acid Boc-*β*-alanine-OH(0.189 g, 2 equivalents)**(5)** was treated with coupling reagents HOBt and DIC added to the resin and shaken for 1.5 h at room temperature. In the last step of the synthesis, peptide was cleaved from the resin with a mixture of trifluoroacetic acid TFA (1 mL), anizol (0.3 mL), phenol(0.3 g) and was shaken mechanically at room temperature for 2 h. One important consideration when selecting the cleavage cocktail are scavengers, such as anizol, phenol, triisopropylsilane (TIPS), 1, 2-Ethanedithiol (EDT), thioanisole or water. Scavengers are often added because during the course of cleavage, highly reactive cationic species can be generated that can cause damage to the structure. The purpose of a scavenger is to extinguish any reactive species that may be generated during revelation from TFA cleavage[17]. At the end of 2

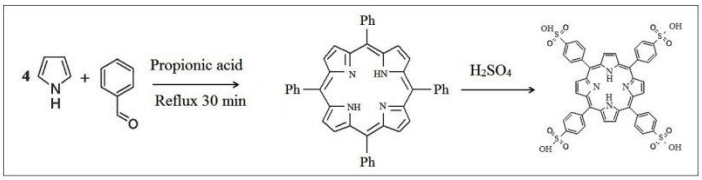
h, the resin was filtered and washed with DMF (5 mL × 3*) and DCM (5 mL × 3). The filtrate was evaporated under reduced pressure and the resulting mixture precipitated by adding diethylether. The presence of the peptide was confirmed by LC–MSanalysis [18,19].



Scheme 1. Steps of dipeptide(his-*β*-alanine) synthesis

Synthesis of (tetrakis(4-sulfonatophenyl) porphyrin) (TPPS$_4$)

Pyrrole 1.9 mL and benzaldehyde 2.7 mL were reacted for 30 minutes in 100 mL of refluxing propionic acid. After cooling down, the product, meso-tetraphenyl porphyrin (TPP), was precipitated in saturated sodium acetate solution, and was washed with methanol-water solution and dried. Thep-sulfonation on phenyl rings was achieved by reacting 0.1 g tetraphenyl porphyrin with 4 mL sulfuric acid and it was kept in Bain Marie for 8 h. The product was neutralized with Na_2CO_3 solution and extracted using methanol as solvent. Solid TPPS$_4$ was obtained after methanol evaporation [20,21] (Scheme 2).



Scheme 2. Steps oftetrakis(4-sulfonatophenyl)porphyrinsynthesis (TPPS$_4$)

Results and discussion

Characterization of synthesized dipeptide (histidine-β-alanine)

Dipeptide was successfully synthesized *via* the standard BOC method [14,15]. The synthesis of dipeptide (histidine-*β*-alanine) was structurally confirmed by UV-Visible, FT-IR, ^{1}H NMR and LC-Mass techniques. The UV-Visible absorbance spectra of histidine-*β*-alanine was obtained in water at 25 °C, the UV absorptions appeared at 214 and 268 nm which can be related to electronic transitions of π→π* and n→π*respectively. The following spectral data for dipeptide was obtained from the FT- IR spectra (KBr, cm^{-1}) with ν_{max}: 3238 (NH_2), 2613-3300(OH), 1643 (N-C=O), 1564 (-C=N), and from ^{1}H NMR spectra (300 MHz, D_2O, δ)*:* 7.48 (imidazole ring); 4.69(2H, CH_2N), 4.24 (d, CO_2H). The LC–MS analysis

revealed a single mass peak in $[M+H]^+$ and $[M]^-$ which corresponds to the calculated molecular weight of the dipeptide, $C_9H_{14}N_4O_3$, calculated: 226.23, found: *m*/*z* $[M+H]^+$: 227.000 and *m*/*z*$[M]^-$: 224.800. For all above data, please refer to the supplementary information.

Characterization of the synthesized (tetrakis(4-sulfonatophenyl)porphyrin)($TPPS_4$)

The synthesis of $TPPS_4$ approved using UV-Visible spectra, λ_{max}= 412 nm (Soret band), 512, 550, 578, and 632 nm (Q bands) shown in Figure 1. FT-IR spectra (KBr, cm^{-1}),ν_{max}: 1000-1380 (=C-N, S=O), 1336 (C=C exocyclic pyrrol), 1382 (C=C endocyclicpyrrol), 1560 (-C=N), 2800-3010 (C-H aliphatic & aromatic), 3000-3600 (N-H pyrrol,O-H). ^{1}H-NMR spectra (300 MHz, D_2O) δ=8.12 (d, d, 16H, C_6H_4), δ=7.63 (S, 8H, *β*- Pyrrole). For all above data, please refer to the supplementary information.

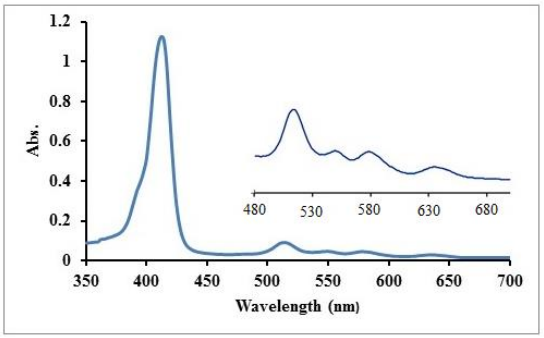


Figure 1. UV-Vis absorption spectrum of $TPPS_4$

Optimization of concentration of dipeptide and porphyrin

Different concentrations of metal salts: $AlCl_3$, $CuCl_2$, $HgCl_2$ and $Pb(NO_3)_2$, (10^{-1}, 10^2, 10^{-3}, 10^{-4} and 10^{-5} M) were prepared to study the kinetics with chelators [dipeptide (histidine-*β*-alanine) and $TPPS_4$]. The changes of absorption in the wavelength of 214 nm (maximum wavelength of dipeptide and 412nm (maximum wavelength of $TPPS_4$) were investigated by UV-Visible spectrophotometer while theconcentration of chelators were kept constant at ~10^{-5} M vs. metal ions. The kinetic study was done using a kinitic mode of spectrophotometer with amaximum wavelength of chelators.

Study of in vitro chelating property of dipeptide (histidine-*β*-alanine) and Tetrakis(4-sulfonatophenyl)porphyrin($TPPS_4$)

The UV-Visible absorbance spectrum was investigated to study kinetics of [histidine-*β*-alanine (~10^{-5}M) and $TPPS_4$ (~10^{-5}M)] with different concentrations (~10^{-1} to ~10^{-5}M) of metal ions (Al^{3+}, Cu^{2+}, Hg^{2+} and Pb^{2+}). The interactions of the dipeptide and $TPPS_4$ with various concentrations of metal ions show that, the maximum absorption band of dipeptide (214 nm) and $TPPS_4$ (412 nm) reduced and shifted to shorter and longer wavelengths. In respect to the size of metal ions, we observed significant changes on the four Q bands in the visible region in such that they reduced to two bands due to the structural symmetry increase from C_{2v} to D_{4h} point groups [9]. The results show that, at initial times (about 5-10 minutes), chelating with metals, which have the same concentration with chelators (10^{-}

^{5}M) were faster, but these reactions of metals with concentrations higher than chelators were slower. To compare the chelating ability of dipeptide and $TPPS_4$ (Figures 2-4), the optimal concentration of metals was found to be ~10^{-3} M for chelators. In terms of rate of reaction, the experimental process of chelating shows that the reactions are completed in the first ten minutes. In this regard, based on the rate law, the reactions were found to be first order. Using the integrated first order rate law of $Ln[A]= kt+Ln[A_0]$, the rate constant (k) was determined from the plot of $-Ln[A/A_0]$ vs. time which gives a straight line with a slope of k.

In Figure 2, it is shown that the order of rate constant increase for (Al^{3+}) is such $k_{dipeptide}>k_{TPPS4}$, which indicates that the reaction of dipeptide with Al^{3+} is faster than $TPPS_4$.

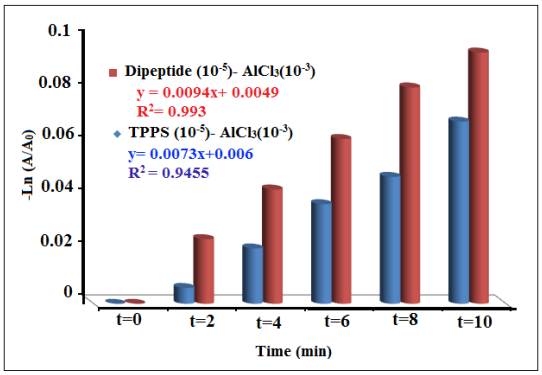


Figure 2. Kinetics study of dipeptide (histidine-β-alanine) (10^{-5} mol L^{-1}) and $TPPS_4$ (10^{-3} mol L^{-1}) with $AlCl_3$

Study of chelation ability of the chelators with Cu^{2+} was shown in Figure 3 $k_{TPPS4}>k_{dipeptide}$, which represents that the reaction of $TPPS_4$ with Cu^{2+} is faster than dipeptide.

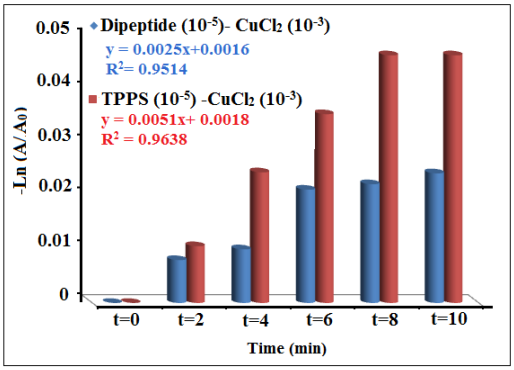


Figure 3. Kinetics study of dipeptide (histidine-β-alanine) (10^{-5} mol L^{-1}) and $TPPS_4$ (10^{-3}mol L^{-1}) with $CuCl_2$

Study of chelation ability of chelators with Pb^{2+} was shown in Figure 4 $k_{dipeptide}>k_{TPPS4}$, which represents that the reaction of dipeptide with Pb^{2+} is faster than $TPPS_4$.

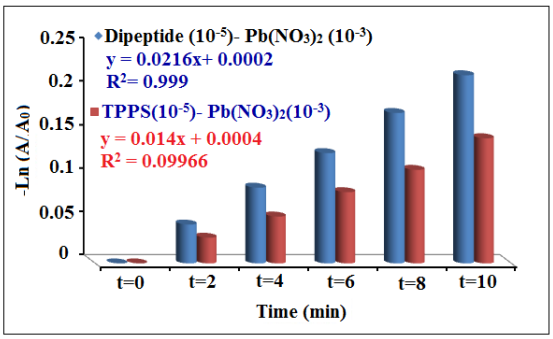


Figure 4. Kinetics study of dipeptide (histidine-*β*-alanine) (10^{-5} mol L^{-1}) and $TPPS_4$ (10^{-3}mol L^{-1}) with $Pb(NO_3)_2$

Study of chelation ability of chelators with Hg^{2+} was shown in Figure 5 $k_{TPPS4}>k_{dipeptide,}$ which represents that reaction of $TPPS_4$ with Hg^{2+} is faster than dipeptide.

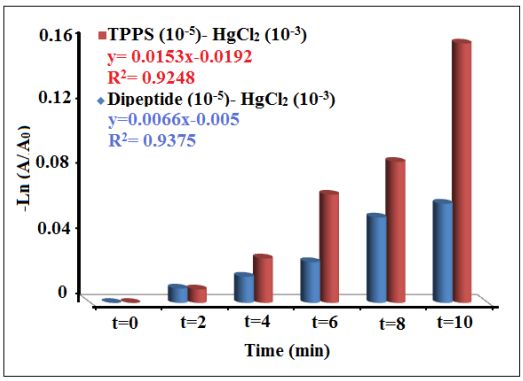


Figure 5. Kinetics study of dipeptide (histidine-*β*-alanine) (10^{-5} mol L^{-1}) and $TPPS_4$ (10^{-3} mol L^{-1}) with $HgCl_2$

Comparision of chelating ability of the chelators can depend on the size of the metallic cation, ligand-to-metal ratios, and the ionic strength of the supporting solution [22] and structure of the chelator. The results indicate that, for Al^{3+} and Pb^{2+}, the rate constants of dipeptide are relatively higher than $TPPS_4$ and for Cu^{2+} and Hg^{2+}, $TPPS_4$ show maxiumum chelating rate. The order of the observed strength chelating adsorption are: Al^{3+}(10^{-3}M) and Pb^{2+}(10^{-3} M): $k_{dipeptide}>k_{TPPS4}$; Cu^{2+}(10^{-3}M) andHg^{2+}(10^{-3} M): $k_{TPPS4}>k_{dipeptide}$.

Conclusion

In this work, dipeptide (histidine-*β*-alanine and $TPPS_4$ as chelating agents were synthesized. The chelating properties of dipeptide and $TPPS_4$ (in vitro) by UV-Vis absorbance spectra were investigated. The results revealed that chelating activity depends on the concentration of metals, molar ratio, kind of metals and structure of the chelator. It was found that both of the natural biologic chelators can be selected as chelating agentor removal of Al^{3+}, Cu^{2+}, Hg^{2+} and Pb^{2+} with different doses and different effective times.

Acknowledgements
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