

Study the Effect of the Folic Acid on Removal Some Metals Ions from Aqueous Solution and Blood Samples using Atomic Absorption Technique

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Summary-Seventy-five blood samples were collected from pregnant women's who used folic acid as a drug at Suez Hospital, Egypt. Iron, Copper, and Nickel metal ions were analyzed in blood sample using atomic absorption spectrometer after digestion using (1:3) concentrated perchloric acid and nitric acid. The ranges of metal ions in the blood samples analysis were as the following: Fe 0.10-0.380 mg/L, Cu of 0. 01-0.2 and Ni of 0.01-0.3 mg/L. The bonds and functional groups for folic acid that give rise to the absorption of ultraviolet radiation are appeared at regions from 200-400 nm .The essential infrared spectra data for folic acid indicated that folic acid exhibits a strong absorption band at 1569 cm^{-1} due to the stretching vibration of $\nu(\text{C}=\text{O})$ of free ketonic of the carboxylic group. This group is shifted or disappeared in the spectra of its complexes accompanied by the appearance of two bands in the $1613\text{--}1603\text{ cm}^{-1}$ range due to $\nu_{\text{as}}(\text{COO}^-)$ and one in the $1350\text{--}1413\text{ cm}^{-1}$ range assigned to $\nu_{\text{s}}(\text{COO}^-)$. Accordingly, the anti-symmetric and symmetric stretching vibration modes ($\nu_{\text{as}}(\text{COO}^-)$ and $\nu_{\text{s}}(\text{COO}^-)$) of the COO^- group should help in elucidating the structure of metals-folate complexes. The human body cannot break down heavy metals, which can build up to toxic levels in the body and interfere with normal functioning. Folic acid and other chelating drugs lower the blood levels of metals attaching to the heavy metal molecules, which helps the body to remove them through urination. The data obtained for statistical correlation coefficient between metal ions and folic acids in the blood samples indicating that inverse relationship between folic acid intake and metal nutrient.

Introduction

Vitamin B9 (Folic Acid) is one of the most biologically important compounds, and information about their metal ions complexation properties are rare in the literature. However, the interaction between some transition metal ions and vitamin B9 resulted in the formation of a number of binary transition metals-FA complexes in which number of drugs can interfere with FA supplement. The present study was done to determine the global stoichiometric stability constants of calcium zinc iron metal ions FA, and Gly binary and mixed ligand complexes using potentiometric technique, and by applying the row data on Hyper quad 2008 computer program. The elucidation of the plausible molecular structures of the vitamin B9-Gly complexes in solutions and the possible binding sites of FA to bind calcium, zinc, and iron metal ions, and the Gibbs free energy calculations of the complex species were done using Gaussian 09 molecular modeling soft-ware. In addition, calcium, zinc, and iron metal speciation studies were performed using hyper quad

Simulation and Speciation (HySS 2008) program, in order to evaluate the suitability of supplementation of vitamin B9 in pregnant women by studying the interaction of FA with iron, zinc, and calcium metal ions in the presence of Gly⁽¹⁾. Folic acid tends to chelate with metal ions. The work deals with measurement of pH and the equilibrium constants for the reaction between the folic acid and the transition metal ions manganese(II), nickel(II), cobalt(II), iron(II), copper(II), zinc(II) and the divalent ions cadmium(II) and magnesium(II). Later studies involving manganese, zinc, iron and ions with carcinogenic activity with folic acid have been also reported. It is well established that folic acid and related substances exhibit numerous dissociation constants in the 1-9 pH range. Folic acid behaves as an acid owing to the presence of one phenolic and two carboxylic groups. Absorbance measurements in the u.v.-vis region, coupled with ¹H and ¹³C-NMR studies (4,s), have been developed to measure pK values for folic acid. The literature values exhibit scattering [e.g. pK values of 8.38, 8.26 and 8.15 have been ascribed to the dissociation of the phenolic OH in the pteridyl moiety⁽²⁾. Recent observations suggest an inverse relationship between folic acid intake and zinc nutrient indicate an interaction between folic acid and zinc at the intestinal level. To define that interaction, we designed in vivo and in vitro transport studies in which folic acid transport in the presence of zinc, as well as zinc transport in the presence of folic acid was examined. These studies show that zinc transport is significantly decreased when folate is present in the intestinal lumen. Similarly folic acid transport is significantly decreased with the presence of zinc. To determine whether this intestinal inhibition is secondary to zinc and folate-forming complexes, charcoal-binding studies were performed. These studies indicate that zinc and folate from complexes at pH 2.0, but that at pH 6.0, these complexes dissolve. Therefore, our studies suggest that under normal physiological conditions a mutual inhibition between folate and zinc exists at the site of intestinal transport⁽³⁾. The folate dependency of many tumors has been therapeutically and diagnostically exploited by administration of anti-FR α antibodies, high-affinity antifolate^(4,5). Folate-based imaging agents and folate-conjugated drugs and toxins⁽⁶⁻⁸⁾. To understand how folate binds its receptors, we determined the crystal structure of human FR α in complex with folic acid at 2.8 Å resolutions. FR α has a globular structure stabilized by eight disulphide bonds and contains a deep open folate-binding pocket comprised of residues that are conserved in all receptor subtypes. The folate pteroyl moiety is buried inside the receptor, whereas its glutamate moiety is solvent-exposed and sticks out of the pocket entrance, allowing it to be conjugated to drugs without adversely affecting FR α binding. The extensive interactions between the receptor and ligand readily explain the high folate-binding affinity of folate receptors and provide a template for designing more specific drugs targeting the folate receptor system. Heavy metals are naturally occurring elements that have a high atomic weight and a density at least 5 times greater than that of water. Their multiple sources are industrial, domestic, agricultural, medical and technological applications have led to their wide distribution in the environment; raising concerns over their potential effects on human health and the environment. Their toxicity depends on several factors including the dose, route of exposure, and chemical species, as well as the age, gender, genetics, and nutritional status of exposed individuals. The aim of this paper is to study the complex formation between folic acid and a typical some metals ions

by using FTIR and UV spectroscopies and atomic absorption spectrometer, then biological application of folic acid in the blood samples will be discussed in this work.

Materials and methods

Materials

Standard solutions of the heavy metals

The stock solutions of heavy metals (Fe, Cu and Ni) used to make the standards curve were prepared by in the range of 0.01-1mg/L. The stock solutions were further diluted with double distilled water to obtain working solutions at several concentration levels. The stock solution can be prepared by dilution. All stock solutions, working solutions, standards were immediately stored at 4°C. The following metals standard solutions were prepared with concentrations of 0.01, 0.001mg/L for Fe⁺³, Ni⁺² and Cu⁺² (Aldrich), while folic acid tablet was used in this work of 0.001mg/L folic acid.

Instruments

1. The pH of mixture was measured using pH-meter
2. Spectrophotometric Measurements (FTIR and UV)

Absorption spectra were taken with the UV-Vis spectrophotometer (1650 PC, Shimadzu, Japan). The absorbance of folic acid and metal complexes were measured in a wave length (200-400 nm). FTIR spectroscopy⁽⁹⁾: Microscopic FTIR-spectra of the complex formed between folic acid and metals ions as well as the spectra of folate metals complexes were recorded at FTIR spectrophotometer (IR Prestige – 21. Shimadzu, Japan) after packed with potassium bromide at room temperature, while the samples of complex were prepared by mixing the appropriate quantity of folic acid with metals ions.

3. Atomic absorption spectrometer (Model A6600 AVANTAPM-GBC).

Procedures

Each blood sample (3mL) was transferred into 100mL conical flasks. perchloric acid and nitric acid were added in ratio 1:3 as follows: 2mL perchloric acid (70% v/v) and 6mL of nitric acid (72% v/v). The conical flask was covered with dish and the mixture digested at low temperature on hot plate till clear solution was obtained. The digest was made up to 25mL with deionized water in measuring flask. The heavy metals analyzed in blood samples using atomic absorption spectrometer (Model A6600 AVANTAPM-GBC).

Recovery of metal ions Experiment

Reaction in patch experiment folate metal complex were prepared by mixing (0.001mg/L) folic acid and metals ions solutions in a range of (1×10^{-2} to 1×10^{-3} mg /L) then, was mixed using magnetic stirrer. Three 10 mL double distilled water were used in this work. Each sample wastaken in two portions of 2 mL into two conical flasks. One milliliter of mixed standard solution containing 20 mg/L nickel, 20 mg/LIron and 20 mg/Lcopper was added to spike one set of portions (i.e., three conical flasks) of the samples which containing 2mL folic acid. The other set was left unspiked. Themixture solutions were stirringon magnetic stirrer. At the end of the mixing, they were all made up to 25 mL using conical flask and the concentrations of heavy metals determined using atomic absorption spectrometer.

$$\% \text{ Recovery} = \frac{C_0 - C_x}{C_0} \times 100 \quad (1)$$

where

C_0 = Initial Concentration (mg/L) of heavy metal determined in liquid samples

C_x = The final concentration (mg/L) of heavy metal determined after addition offolic acid

Precision Analysis One milliliter of the mixed metals standard solution was pipetted into a 25mL measuring flask and made up to mark, to give 0.5 mg/L of Iron, Copper and nickel. This was analyzed 5 times for the heavy metals. The results obtained were subjected to statistical analysis using the mean value and standard deviation value (S it refers tstandard deviation, M= mean value, n=number of variables).

Results and discussion

The essential infrared data are summarized in Table 1. Folic acid exhibits a very strong absorption band at 1569 cm^{-1} due to the stretching vibration of $\nu(\text{C}=\text{O})$ of free ketone of the carboxylic group ⁽⁹⁾. This group is shifted or disappeared in the spectra of its complexes accompanied by the appearance of two bands in the $1613\text{--}1603 \text{ cm}^{-1}$ range due to $\nu_{\text{as}}(\text{COO}^-)$ and one in the $1350\text{--}1413 \text{ cm}^{-1}$ range assigned to $\nu_{\text{s}}(\text{COO}^-)$. Accordingly, the antisymmetric and symmetric stretching vibration modes ($\nu_{\text{as}}(\text{COO}^-)$ and $\nu_{\text{s}}(\text{COO}^-)$) of the COO^- group should help in elucidating the structure of complexes ⁽¹⁰⁾.

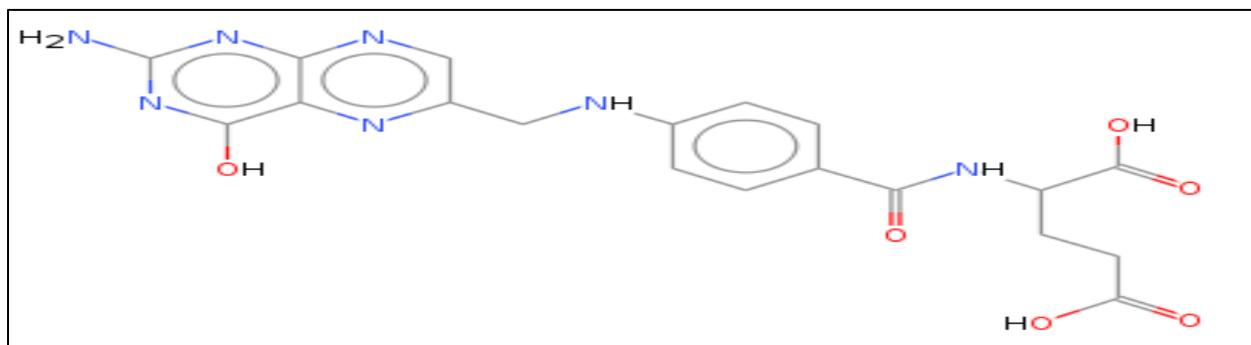


Fig 1: The chemical structure of folic acid (2-[[4-[(2-amino-4-oxo-1H-pteridin-6-yl)methylamino]benzoyl]amino]pentanedioic acid)

Table1: IR frequencies (Cm^{-1}) of folic acid (FO) and its metal complexes

| Frequency cm^{-1} | Folic acid | Fe folate complex | Cu folate complex | Ni folate complex |
|----------------------------|------------|-------------------|-------------------|-------------------|
| $\nu(\text{OH})$ | 3415 | 3381 | 3348 | 3677 |
| $\nu(\text{NH})$ amide | 3230 | 3175 | 3220 | 3433 |
| $\nu(\text{C-H})$ | 3106 | 3090 | 3030 | 2920 |
| $\nu(\text{C=O})$ amide | 1662 | 1658 | 1655 | 1568 |
| $\nu(\text{COO}^-)$ | 1569 | 1613 | 1603 | 1455 |

The direction of the frequency shift of the $\nu_{\text{as}}(\text{COO}^-)$ and the $\nu_{\text{s}}(\text{COO}^-)$ bands with respect to those of the free ion depends on the coordination mode of the COO^- . The direction of the frequency shift of the $\nu_{\text{as}}(\text{COO}^-)$ and the $\nu_{\text{s}}(\text{COO}^-)$ bands with respect to those of the free ion depends on the coordination mode of the COO^- group with the metal ion. Nakamoto and McCarthy⁽¹¹⁾ claimed that if the coordination is mono dentate the $\nu_{\text{as}}(\text{COO}^-)$ and $\nu_{\text{s}}(\text{COO}^-)$ will be shifted or disappear in the spectra of its complexes accompanied by the appearance of two bands in the 1569–1631 cm^{-1} range due to $\nu_{\text{as}}(\text{COO}^-)$ and one in the 1350–1413 cm^{-1} range assigned to $\nu_{\text{s}}(\text{COO}^-)$. Accordingly, the antisymmetric and symmetric stretching vibration modes ($\nu_{\text{as}}(\text{COO}^-)$ and $\nu_{\text{s}}(\text{COO}^-)$) of the COO^- group should help in elucidating the structure of complexes⁽¹⁰⁾.

Biological Applications

The blood samples were taken from pregnant women's (at Suez city-Egypt) who intake folic acid drug. Atomic absorption data are given in Table 2 proved that the values for the concentration of metal ion in blood sample in the presence of the folic acid are lower than those in absence of the folic acid. This means that folic acid withdraws the metal ion from the body forming the folate

complex in the blood⁽¹²⁻¹⁷⁾. It may be concluded that the folic acid in the complexed form absorbed more efficiency in body than the folic acid itself. Comparison between the normal, folic acid case and folic acid metal complex case percentage of Cu and Fe elements in the blood samples. Inspection of the results shown in Table 2 reveals that the copper and iron contents in blood decrease significantly with folic acid. In addition to, the absorption of folic acid complexes is much better than folic acid itself. This may be explained as being due to the high solubility differences between folic acid which is considered as an insoluble material, and its copper and iron complexes. As given in Table 3 it can be concluded that the maximum Recovery % of metal ions was 65.97% for Cu^{+2} and the minimum one was 50.8% for Ni^{+2} and Fe^{+3} respectively.

Table 2: The data of some heavy metals (mg/L) in blood samples before and after treated with folic acid collected from pregnant women at general Suez hospital.

| Blood sample | Fe (mg/L) before taking folic acid tablet | Fe(mg/L) after taking folic acid tablet | Cu (mg/L) before taking folic acid tablet | Cu (mg/L) after taking folic acid tablet | Ni (mg/L) before taking folic acid tablet | Ni (mg/L) taking folic acid tablet |
|-------------------|---|---|---|--|---|------------------------------------|
| Number of samples | 75 | 75 | 75 | 75 | 75 | 75 |
| Mean± SD | 0.374±0.326 | 0.190±0.165 | 0.093±0.081 | 0.060±0.052 | 0.288±0.251 | 0.19±0.165 |
| Range | 0.10-0.380 | 0.01-0.2 | 0.01-0.10 | 0.01-0.10 | 0.01-0.3 | 0.01-0.3 |

SD : Standard deviation

Table 3: The Recovery % of metal ions from aqueous solution using folic acid

| Blood sample | Fe^{+3} | Cu^{+2} | Ni^{+2} |
|-------------------|------------------|------------------|------------------|
| Number of samples | 75 | 75 | 75 |
| Recovery % | 50.8 | 64.5 | 65.97 |

Table 4: Pearson rank correlation coefficient matrix of folic acid, iron, copper, and nickel in blood samples for pregnant women at Suez-Egypt.

| Metal/folic | Fe | Cu | Ni | Folic acid |
|-------------|--------|--------|--------|------------|
| Fe | 1 | | | |
| Cu | 0.322 | 1 | | |
| Ni | 0.223 | 0.455 | 1 | |
| Folic acid | -0.523 | -0.321 | -0.221 | 1 |

Various degrees of correlations are found between the metal ions and folic acid (Table 4.). The correlation coefficient matrix between metal ions concentrations and the folic of the blood samples showed some different correlations coefficients values, both positive and negative values. There were positive relationships ($P < 0.05$) between the metal ions; Fe, Cu and Ni ($r = 0.322$ and $r = 0.223$ $P < 0.05$). On the other hand, strong negative correlation obtained between folic acid and Fe ($r = -0.523$, $P < 0.05$). Moderate negative correlation found between folic acid and both Cu and Ni metal ions ($r = -0.321$ and $r = -0.221$, $P < 0.05$) respectively as given in Table 4. From look at Table 4 it can be concluded that as the concentration of folic acid increases in the blood samples the level of metals ions decreases (negative correlations).

Conclusion

The complexation between metal ions (Fe(III) and Cu(II)) with folic acid resulted in the formation (1 : 2) molar ratio (metal : folic acid). Folic acid acts as a bidentate ligand via two monodentate carboxylate groups giving the general formula: $[M (FO)_2 (H_2O)_n] \cdot xH_2O$, where FO = folate anion; $n = 2$ and $x = 2$ or 3. The resulted folate compounds were assigned by infrared, ultraviolet techniques. The transition metals folic acid complexes are more preferred than folic acid itself as a drug in the human body because the absorption efficiencies of the transition metal complexes are higher than those the folic acid. The human body cannot break down heavy metals, which can build up to toxic levels in the body and interfere with normal functioning. Folic acid and other chelating drugs lower the blood levels of metals attaching to the heavy metal molecules, which helps the body remove them through urination. This finding was supported by strong negative correlation between folic acid and Iron ion in the studied blood samples.

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