ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Ionic Strength Dependence of Formation Constants, Protonation and Complexation of Leucine with Iron.

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ABSTRACT

The stability constants of the complexes between iron ion and leucine was determined potentiometrically at various ionic strengths of I=0.1, 0.3, 0.5, 0.7 and 0.9 mol.dm^{-3} and 20°C. The sodium perchlorate solution was used to maintain the ionic strength. The parameters based on these formation constants were calculated and the dependence of protonation and the stability constants on ionic strength are described by a Debye-Huckel type equation.

Keywords: Ionic, Strength, Protonation, Complexation; Leucine, Iron.

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ISSN: 0975-8585

INTRODUCTION

Metal ion complexes with amino acids have been extensively studied because of their possible significance as models for biological process assisted by them. On the ionic strength dependence of formation constants we recently [1-5] reported some interesting features of the function logk=f(I), in particular, all the calculated formation constants seem to follow the same trend as a function of ionic strength for the different types of reaction stochiometry and for the various charges of reactants and products. We suggested relatively strong bond formation between the ions and oxygen and nitrogen donors [1-2].

In determining stability constant at a fixed ionic strength in all cases some uncertainties are present. This fact is mainly due to the uncertainties in numerical values of stability constants. For example when maintaining two different ionic media, 0.1 and 0.5 mol.dm^{-3} sodium perchlorate we observed a difference of about 0.26 log units in the stability constant of iron chelate by leucine.

According to literature, no work has been reported on ionic strength dependence of iron with leucine. The present paper deals with the study of complexes of iron with leucine in an ionic strength range of 0.1-0.9 mol.dm^{-3} sodium perchlorate and 20°C. The parameters which define the dependence on ionic strength were analysed with the aim of obtaining further information with regard to their variation as a function of the charges involved in the complex reaction.

Moreover, a general equation was established for the dependence of formation constants on ionic strength. This equation gives the possibility of estimating a stability constant of a fixed ionic strength when its value is known at another ionic media in the range of 0.1”I”0.9 mol.dm^{-3} and therefore may give a significant contribution to solving many analytical and speciation problems.

Iron compounds

Although its metallurgical role is dominant in terms of amounts, iron compounds are pervasive in industry as well being used in many niche uses. Iron catalysts are traditionally used in the Haber-Bosch Process for the production of ammonia and the Fischer-Tropsch process for conversion of carbon monoxide to hydrocarbons for fuels and lubricants [3]. Powdered iron in an acidic solvent was used in the Bechamp reduction the reduction of nitrobenzene to aniline [4].

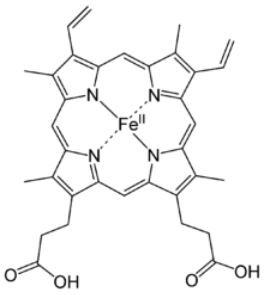
Iron(III) chloride finds use in water purification and sewage treatment, in the dyeing of cloth, as a coloring agent in paints, as an additive in animal feed, and as an etchant for copper in the manufacture of printed circuit boards [5]. It can also be dissolved in alcohol to form tincture of iron. The other halides tend to be limited to laboratory uses.

Iron (II) sulfate is used as a precursor to other iron compounds. It is also used to reduce chromate in cement. It is used to fortify foods and treat iron deficiency anemia. These are its main uses. Iron (III) sulfate is used in settling minute sewage particles in tank water. Iron (II) chloride is used as a reducing flocculating agent, in the formation of iron complexes and magnetic iron oxides, and as a reducing agent in organic synthesis.

Biological role

Iron is abundant in biology [6]. Iron-proteins are found in all living organisms, ranging from the evolutionarily primitive archaea to humans. The color of blood is due to the hemoglobin, an iron-containing protein. As illustrated by hemoglobin, iron is often bound to cofactors, e.g. in hemes. The iron-sulfur clusters are pervasive and include nitrogenase, the enzymes responsible for biological nitrogen fixation. Influential theories of evolution have invoked a role for iron sulfides in the iron-sulfur world theory.

ISSN: 0975-8585



Structure of Heme b, in the protein additional ligand(s) would be attached to Fe.

Iron is a necessary trace element found in nearly all living organisms. Iron-containing enzymes and proteins, often containing heme prosthetic groups, participate in many biological oxidations and in transport. Examples of proteins found in higher organisms include hemoglobin, cytochrome (see high-valent iron), and catalase [7].

Health and diet

Iron is pervasive, but particularly rich sources of dietary iron include red meat, lentils, beans, poultry, fish, leaf vegetables, watercress, tofu, chickpeas, black-eyed peas, blackstrap molasses, fortified bread, and fortified breakfast cereals. Iron in low amounts is found in molasses, teff, and farina. Iron in meat (heme iron) is more easily absorbed than iron in vegetables [8]. Although some studies suggest that heme/hemoglobin from red meat has effects which may increase the likelihood of colorectal cancer [9,10], there is still some controversy [11], and even a few studies suggesting that there is not enough evidence to support such claims [12].

Uptake and storage

Iron acquisition poses a problem for aerobic organisms, because ferric iron is poorly soluble near neutral pH. Thus, bacteria have evolved high-affinity sequestering agents called siderophores [13-15].

After uptake, in cells, iron storage is carefully regulated; "free" iron ions do not exist as such. A major component of this regulation is the protein transferrin, which binds iron ions absorbed from the duodenum and carries it in the blood to cells [16].

Amino acid of Leucine

Leucine (abbreviated as Leu or L) is a branched-chain α-amino acid with the chemical formula $HO_2CCH(NH_2)CH_2CH(CH_3)_2$. Leucine is classified as a hydrophobic amino acid due to its aliphaticisobutyl side chain. It is encoded by six codons (UUA, UUG, CUU, CUC, CUA, and CUG) and is a major component of the subunits in ferritin, astacin, and other 'buffer' proteins. Leucine is anessential amino acid, meaning that the human body cannot synthesize it, and it therefore must be ingested.

Biosynthesis

As an essential amino acid, leucine cannot be synthesized by animals. Consequently, it must be ingested, usually as a component of proteins. In plants and microorganisms, leucine is synthesised from pyruvic acid by a series of enzymes [17]:

- Acetolactate synthase
- Acetohydroxy acid isomeroreductase
- Dihydroxyacid dehydratase
- α-Isopropylmalate synthase
- α-Isopropylmalate isomerase
- Leucine aminotransferase

Synthesis of the small, hydrophobic amino acid valine also includes the initial part of this pathway.

Biology

Leucine is utilized in the liver, adipose tissue, and muscle tissue. In adipose and muscle tissue, leucine is used in the formation of sterols, and the combined usage of leucine in these two tissues is seven times greater than its use in the liver [18].

Leucine is the only dietary amino acid that has the capacity to stimulate muscle protein synthesis (19). As a dietary supplement, leucine has been found to slow the degradation of muscle tissue by increasing the synthesis of muscle proteins in aged rats [20]. However, results of comparative studies are conflicted. Long-term leucine supplementation does not increase muscle mass or strength in healthy elderly men [21].

EXPERIMENTAL SECTION

Reagents leucine (E.Merck, analytical reagent grade) was recrystallized from hot water, washed with ethanol and dried over P_2O_5. Equivalent weights were checked by titration against standard alkali. The NaOH solution was prepared from titrisol solution (E.Merck) and its concentration was determined by several titrations with standard HCl. Sodium perchlorate and perchloric acid were supplied from E.Merck and ferro nitrate was from fluka. All three were used without further purification. All dilute solutions were −1 −1 prepared from bouble-distilled water with specific conductance equal to1.3±0.1 μΩ *cm.*

Measurements

Measurement were performed by using an apparatus consisting of a potentiometer, Eyela model PHM 2000, equipped with an Ingold 3236 calomel electrode and an Ingold 3234 glass electrode for measuring the hydrogen ion concentration. The pH-meter was calibrated for the relevant H^+ concentration with a solution of 0.01mol.dm^{-3} perchloric acid solution containing0.09mol.dm^{-3} sodium perchlorate(For adjusting the ionic strength on 0.1*mol dm*$^{-3}$). The same procedure was performed for the other ionic strengths. For these standard solution, we set -log $[H^+]$=2.00 [22]. All titrations were carried out in a double-jacketed glass cell. The temperature (20°C) was maintained by circulating water from the thermostat through the outer jacket of the cell. The thermostat was constant to 0.1°C. All titrations were performed by stirring magnetically and by bubbling purified nitrogen through the solution in order to exclude O_2 and CO_2 inside. For each experiment an acidic solution of iron (2.5×10^{-4}mol.dm^{-3}) was titrated with an alkali solution of leucine (containing a large excess of leucine, 8×10^{-2}mol.dm^{-3}), both in the same ionic media.

The -log$[H^+]$ and the absorbance were measured after addition of a few drops of titrant and this procedure extended up to the required -log$[H^+]$. In all cases the procedure was repeated at least three times and the resulting average values.

RESULTS AND DISCUSSION

The proton-ligand and the metal ion-ligand stability constants were determined by carrying out leucine as ligand and Fe^{2+}, titrations by keeping the temperature constant at 20±0.1°C and varying the ionic strength (0.1"I"0.9mol.dm^{-3}). The protonation constants of leucine have been extensively studied in different kinds of background electrolytes and the results are reported in literature. The following equilibrium was studied.

$$H^+ + L^- = HL \qquad K_p=[HL]/[H^+][L^-] \qquad (1)$$

Where L represents the fully dissociated amino acid anion. The protonation constant of the amino group, Kp have been determined using potentiometric techniques and calculated using a computer program that employs a nonlinear-least-squares method [23]. The

protonation constants at different ionic strengths expressed as logKp, are collected in

Table.1 together with the values reported in the literature which are in good agreement with those reported before.

Table1: Protonation constant of the amino group of L-asparagine, Kp at different temperatures and various ionic strengths, I, of NaClO4

I(mol.dm^{-3})	logKp
0.1	7.29±0.10
0.3	7.18±0.10
0.5	7.11±0.10
0.7	7.12±0.10
0.9	7.25±0.10

Considering the protonation constant of the amino acid in acidic pH the predominant species for complexation is HL. In this case data were analysed by using the absorbance of Fe-Lu at wavelengths in the UV range that is given by:

$A= \varepsilon_0 [Fe^{2+}]+ \varepsilon_1 [complex]$ (2)

Where ε_0 and ε_1 are the molar absorptivities of $Fe^{2+}$and the formed complex respectively.

For the mass balance:

$[Fe^{2+}]=C_M-[complex]$ (3)

$[HL]=C_L-[complex]$ (4)

Where C_M and C_L are the total concentration of $Fe^{2+}$and the ligand respectively.

The following equilibriums were studied for determination of the formation constant

$$Fe^{2+} + HL = FeL^{+} + H^{+} \qquad K_1 = [FeL^{+}][H^{+}]/[FeL^{+}][HL] \quad (5)$$

$$FeL^{+} + HL = FeL_2 + H^{+} \qquad K_2 = [FeL_2][H]/[FeL^{+}][HL] \quad (6)$$

+

Substituting Eq. (3), (4) and (5) into Eq. (2) and canceling like terms and rearranging gives

$$A = \varepsilon_0 C_m + 0.5(\varepsilon_1 - \varepsilon_0)(C_m + C_L + [H^{+}]/K_1) \pm 0.5(\varepsilon_1 - \varepsilon_0) \times \{(C_M + C_L + [H^{+}]/K_1)^2 - 4C_M C_L\}^{0.5} \quad (7)$$

The values ε_0 at different wavelengths are determined in this work using a suitable computer program [12] the data were fitted to Eq. (7) for estimating K_1. We define

$$\epsilon_1 = \epsilon_0 X_M + \epsilon_1 X_{ML} \quad (8)$$

Through the rearrangement of Eq. (8) the average ligand number *n* can be calculated directly from

$$n_1 = (\epsilon_1 - \epsilon_0)/(\epsilon_1 - \epsilon_0) \quad (9)$$

The second complex is apparently attributed to Eq.(6) and its formation constant K_2 was determined in usual manner using Eq. (10)

$$A = \epsilon_1 C_M + 0.5(\epsilon_2 - \epsilon_1)\left(C_L + \frac{[H^+]}{K_2}\right) \pm 0.5(\epsilon_2 - \epsilon_1) \times \left\{\left(C_L + \frac{[H^+]}{K_2}\right)2 - 4C_M C_L + 4C_M^2\right\}0.5 \quad (10)$$

Where ε_2 is the molar absorptivity of FeL_2^+ through the rearrangement of Eq.(8) for the species FeL_2^+ the average ligand number n_2 can be calculated from Eq.(11) [14]

$$n_2 = (\varepsilon_2 + \varepsilon_2 - 2\varepsilon_1)/\varepsilon_2 - \varepsilon_1 \quad (11)$$

Where ε_2 is similar to ε_2 but for the species FeL_2^+.Calculation has shown that n_2 equals to 1.998 at pH=6.44. so at this pH complexation is completed and asparagines forms a mononuclear 1:2 complex with FeL^+ and the average of their stability constants β_2 at different wavelengths are listed in Table II. The dependence of the protonation constant on ionic strength for the species $H_J L$ can be described by semi-empirical Eq.

$\log K(I^*) = \log K(I^*) - f(I) + CI$ (12)

Where

$$f(I) = Z^* AI^{1/2}/(I + BI)^{1/2} \quad (13)$$

and $\log K_j(I)$ and $\log K_j(I^*)$ are the protonation constants of the actual and the reference ionic strength at the minimum dilution respectively.

A is the parameter of the extended Debye-Huckel equation (A=0.51115 at 25C),

$Z^* = 1 + Z_L^2 - JZ_j$ (z_L and Z_J are the charges on the anion and the species $H_J L$ respectively),

C is an empirical parameter that its value is considered and B is set equal to 1.5[1] (a small error in fixing B is absorbed in the linear term C). If an approximate value of C is known the protonation constant can be determined for the variation of ionic strength from I^* to I by the equation

$\log K_j(I) = \log K_j(I^*) - f(I, I^*) + C(I - I^*)$ (14)

Where

ISSN: 0975-8585

$$f(I,I^*) = Z^* A\left[I^{1/2}/(1+1.5I^{1/2}) - I^{*1/2}(I+1.5I^{*1/2})\right] \quad (15$$

Where I and I^* are the ionic strength of the solution by NaCl.

The values of $\log K_j$ are reported in Table 1. The dependence of the protonation constant on ionic strength was then studied. A preliminary analysis of the data showed that if a fixed value is assigned to C the fit with Eq.(12) is not always good over the whole range of ionic strength from 0.1 to 1.0mol.dm^{-3}. This equation may be useful for small changes of ionic strength but a better fit is obtained by adding a further term of the form $DI^{3/2}$ (D is another adjustable parameter). Therefore the data were fitted to the equation

$$\log K_j(I) = \log K_j(I^*) - f(I,I^*) + D(I - I) \quad (16)$$

The value obtained for C and D are collected in Table-III. It is noticeable that the

Introduction of the term $D(I^{3/2} - I^{3/2})$ very often improves the fit of the data. For example for the protonation constant of leucine K_j(j=1) from Eq.(16) we obtained two sets of values depending on whether or not we take into account the term in D:

C=0.36,D=0.0,U=4.01×10^{-3} [for Eq.(17)]

C=0.12,D=-0.02,U=1.87×10^{-4} [for Eq.(17)]

The square sum, U, show that there is a significant improvement in the fit when D term is introduced.

We used the Gauss-Newton nonlinear least-squares method in a computer program [15] to refine the parameter C and D by minimizing the squares sum from Eq.

$$U = \sum (a_i - b_i)^2 \rightarrow i = 1,2,3 \quad (17)$$

Where a_i is a quasi-experimental quatity and b_i is a calculated one.

As can be seen from Table 1 the parameters which characterize the dependence of protonation constants on ionic strength show a regular trend and seem to be a function of the approximated value of Z'

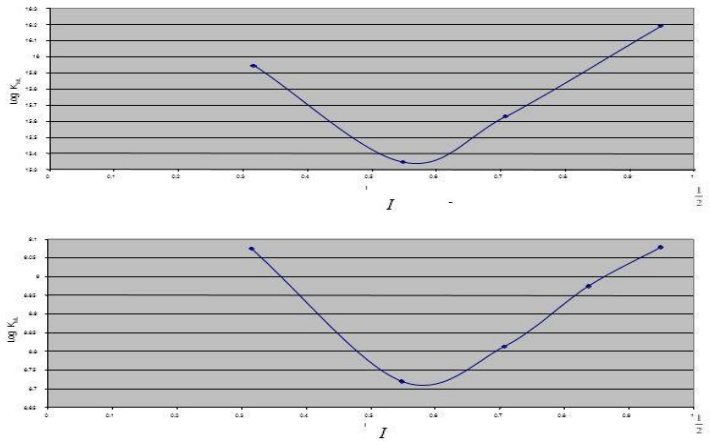


Fig.1: Plots of logß$_1$ and logß$_2$ versus the square root of the ionic strength $NaClO_4$ at 20°C

The same procedure was applied for determining the dependence of the stability constants of Fe^{2+}- leucine complexes on ionic strength where in this case

$$z^* = Z_M^2 + JZ_J^2 - Z_C^2 - 1(ForFeL) and Z^* = JZ_L^2 + Z_C^{*2} - 1(\ forFeL_2)$$

Where Z_M, Z_C and z_c^* are the changes on the metal ion, FeL^+ and FeL_2 respectively.

The semi-empirical parameters were determined as before and are shown in Table III. The dependence of $\log \beta_1, \log \beta_2$ and protonation constant have a large values at I=0.1 and a minimum when the ionic strength approaches 0.3mol.dm-3.

Table 3: parameters for dependence on ionic strength of protonation constant β_1 and β_2

species	C	D
β_1	-3.01	2.97
β_2	-2.92	2.25

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