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Electrophoretic studies on the chelating tendency of bioactive sulphur-containing amino acids
The metal–methylcysteine–cysteine system

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Abstract

Stability constants of binary Fe(III)–methylcysteine, Cr(III)–methylcysteine and mixed Fe(III)–methylcysteine–cysteine, Cr(III)–methylcysteine–cysteine complexes have been determined by paper electrophoresis at 0.1 *M* ionic strength and a temperature of 35 °C. The stability constants of Fe(III)–methylcysteine–cysteine and Cr(III)–methylcysteine–cysteine mixed complexes were found to be 6.00±0.07 and 5.05±0.15 (logarithm of stability constant values), respectively.

Keywords: Metal complexes; Methylcysteine; Cysteine; Iron; Chromium; Amino acids; Organosulphur compounds

1. Introduction

Data on the complexation of essential metal ions and the bioactive ligands methylcysteine and cysteine give an insight into many physicochemical processes. The significance of these amino acids is enhanced by the fact that they display independent therapeutic activity [1]. Their most valuable use is for the treatment of Wilson's disease, caused by an accumulation of copper. As a consequence of their ability to form stable complexes, they can be employed advantageously for the elimination of other heavy metals (Pb, Hg) from organisms. In recent years they have been utilized in connection with rheumatoid arthritis and neonatal jaundice [2]. Iron and chromium are essential and beneficial metals, respectively [3]. Iron is an important constituent of the blood and tissue of the animal body. Most of the iron in the body is present as iron porphyrin or heme proteins [4]. Chromium(III) is an essential element for man and animals, required as a part of the glucose tolerance factor (GTF), for the initiation of peripheral insulin action [5,6]. The immune system of the body provides protection against foreign substances and pathogenic organisms. The chromium ion is reported to mediate the immune response [7]. The studies on complexation reactions of trivalent iron and chromium is of interest because of their nutrient properties and toxicity [8–15].

Communications [16–23] from our laboratory described a new method for the study of mixed complexes. A search of the literature indicated that no report is available on Fe(III) and Cr(III) binary complexes with methylcysteine and mixed complexes with methylcysteine and cysteine. Hence, attempts

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were made to establish the optimum conditions for metal–methylcysteine and metal–methylcysteine–cysteine complex formation. In addition, the present work describes an electrophoretic method for the determination of the stability constants of these complexes.

2. Experimental

2.1. Instruments

Electrophoresis equipment from Systronic (Naroda, India), model 604, was used. The equipment has a built-in power supply (a.c.–d.c.) that is fed directly to the paper electrophoresis tank. The potential in each experiment was kept at 240 V and electrophoresis was carried out for 60 min.

An Elico (Hyderabad, India) model L_{1-10}, with glass and calomel electrodes assembly and working on 220 V/50 Hz established a.c. mains, was used for pH measurements.

2.2. Chemicals

Solutions of iron(III) and chromium(III) metal perchlorate were prepared by the precipitation of metal carbonates from a 0.1 *M* solution of Fe(III) and Cr(III) sulphates with the solution of sodium carbonate (chemically pure grade, BDH, Poole, UK). The precipitates were thoroughly washed with water and treated with a calculated amount of analytical-reagent grade perchloric acid. These were boiled in a water bath and then filtered to get a stock solution of metal perchlorate $5.0 \cdot 10^{-3}$ *M*.

Metal spots after electrophoresis were detected on the paper using 0.5% solution of potassium ferrocyanide (BDH) for Fe(III) and 1-(2-pyridylazo)-2-naphthol (PAN) (Merck, Darmstadt, Germany) for Cr(III). A saturated aqueous solution (0.9 ml) of silver nitrate was diluted with acetone to 20 ml. Glucose was detected as a black spot by spraying with this solution and then with 2% ethanolic sodium hydroxide.

2.3. Background electrolyte

The background electrolytes (BGEs) used in the study of binary complexes were 0.1 *M* perchloric acid and $1.0 \cdot 10^{-2}$ *M* methylcysteine. For the study of mixed system the BGEs used were 0.1 *M* perchloric acid, $1.0 \cdot 10^{-2}$ *M* methylcysteine and various amounts of 0.01 *M* cysteine. The mixed system was maintained at pH 8.5 by the addition of sodium hydroxide.

Stock solutions of 5.0 *M* perchloric acid (SDS, AnalaR grade), 2.0 *M* sodium hydroxide (AnalaR grade), 0.5 *M* methylcysteine and 0.5 *M* cysteine (BDH) were prepared. Each solution was standardized using the appropriate method.

2.4. Procedure

2.4.1. Binary complexes

The level of the hollow base plate in the instrument was made horizontal using a spirit level and 150 ml of 0.1 *M* perchloric acid was placed in each tank of the electrophoretic apparatus. The levels of two tank solutions were made equal by siphoning. These precautions were taken to prevent any gravitational or hydrodynamic flow. Paper strips (Whatman No. 1) of 30×1 cm in size were soaked in the BGE and the excess of electrolyte solution was blotted by pressing gently within the folds of dry filter paper sheets. Duplicate strips were then spotted in the center with the metal ion solution using a micropipette before being placed on the base plate and sandwiched under the upper hollow metallic plates with the ends of the strips were completely dipped into ligand solution. A 240 V potential difference was then applied between the tank solutions to initiate electrophoresis.

The electrophoresis was carried out for 60 min. In order to maintain a constant temperature of 35 °C water was circulated through the hollow plates by a thermostat. The strips were taken out using a glass rod, dried on a horizontal platform and the spots detected. The observations were repeated for different pH values of the BGE. The differences in the distances recorded in the duplicates were within ±5% and the average distances in the duplicates were noted for calculation. The actual distance the sample spot moved was corrected for the distance travelled by the reference glucose spot. The potential gradient through the strips was found to be 7.5 V/cm. Mobility was calculated by dividing the

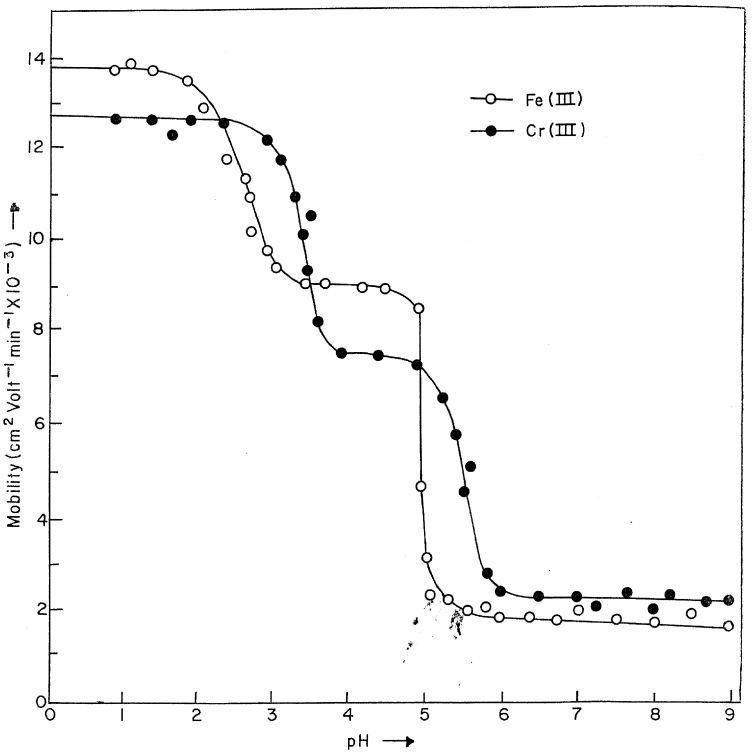

Fig. 1. Mobility curves for the metal(III)–methylcysteine systems. ○, Fe(III)–methylcysteine; ●, Cr(III)–methylcysteine. Background electrolytes: 0.1 *M* perchloric acid and 0.01 *M* methylcysteine. Concentration of Fe(III) and Cr(III) = $5.0 \cdot 10^{-3}$ *M*.

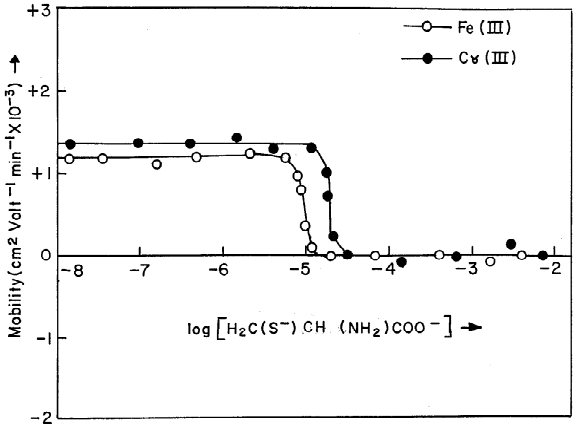

Fig. 2. Mobility curves for the metal(III)–methylcysteine–cysteine systems. ○, Fe(III)–methylcysteine–cysteine; ●, Cr(III)–methylcysteine–cysteine. Background electrolytes: 0.1 *M* perchloric acid, 0.01 *M* methylcysteine and 0.01 *M* cysteine, pH 8.5 (maintained by addition of sodium hydroxide). Concentration of Fe(III) and Cr(III) = $5.0 \cdot 10^{-3}$ *M*.

movement by the potential gradient and time. A plot of mobility against pH is shown in Fig. 1.

2.4.2. Mixed complexes

Paper strips in duplicate were marked with metal ions along with an additional one marked with glucose. After drenching the strips with BGE, electrophoresis was carried out on for 1 h at 240 V potential difference as in case of binary complexes. For subsequent observations cysteine solution, maintained at pH 8.5, was recorded. A plot of mobility against −log [cysteine] was made and is shown in Fig. 2.

3. Results and discussion

3.1. Metal–methylcysteine binary system

The plot of the overall electrophoretic mobility of

the metal spot against pH is shown in Fig. 1. The first plateau corresponds to a region in which metal ions are uncomplexed. It is obvious that protonated ionic species of the methylcysteine, which exist in large numbers in low pH ranges are noncomplexing. The second plateau indicates the formation of a 1:1 complex of cationic nature, the ligand being the methylcysteine anion. A further increase in pH also gives rise to a third plateau with positive mobility from the anionic species of methylcysteine [$H_2C(SCH_3)CH(NH_2)COO^-$] with each trivalent metal ions. The ligating properties of the unprotonated anionic species of methylcysteine rules out any such property to zwitterions [24,25]. The complexation of metal ions with the methylcysteine anion [L^-] may be represented as

$$M^{3+} + L^- \overset{K_1}{\rightleftharpoons} ML^{2+} \quad (1)$$

$$ML^{2+} + L^- \overset{K_2}{\rightleftharpoons} ML_2^+ \quad (2)$$

where M^{3+} is trivalent iron or chromium ions; [L^-] is the methylcysteine ligand; ML^{2+} and ML_2^+ are the 1:1 and 1:2 metal complexes; K_1, K_2 are the first and second stability constants, respectively.

For the calculation of the first stability constant K_1, the region between the first and second plateau is pertinent. The overall mobility U is equal to the arithmetic mean of mobility of uncomplexed metal ion, u_o, and that of first complex, u_1, at pH where $K_1 = 1/[H_2C(SCH_3)CH(NH_2)COO^-]$. With the help of protonation constants of methylcysteine (electrophoretically obtained value, $pK_1 = 2.25$, $pK_2 = 8.55$), the concentration of the methylcysteine anion [L^-] is determined for the pH, from which K_1 can be calculated. The concentration of complexing methylcysteine [L^-] is calculated with the help of equation

$$[L^-] = \frac{[L_T]}{1 + \frac{[H]}{k_2} + \frac{[H]^2}{k_1 k_2}} \quad (3)$$

where [L_T] is the total concentration methylcysteine and k_1, k_2 are the first and second dissociation constants, respectively, of pure methylcysteine.

The stability constant K_2 of second complex can be calculated by taking into consideration the region between second and third plateau of the mobility curve. The calculated values of K_1 and K_2 are given in Table 1.

Table 1
Stability constants of binary and mixed complexes of Fe(III) and Cr(III)

Metal ion	Complex	Log K
Fe(III)	ML	8.37±0.05
	ML_2	13.92±0.01
	MLL′	6.00±0.07
Cr(III)	ML	7.09±0.11
	ML_2	12.14±0.03
	MLL′	5.05±0.15

Ionic strength, 0.1 *M*; temperature, 35 °C; methylcysteine anion, [$H_2C(SCH_3)CH(NH_2)COO^-$]; cysteine anion, [$H_2C(S^-)CH(NH_2)COO^-$]. M, Metal cations; L, primary ligand (methylcysteine); L′, secondary ligand (cysteine).

3.2. Metal–methylcysteine–cysteine mixed system

It was observed from the mobility curves of the metal–methylcysteine system that binary complexes are formed at pH<8.5. Therefore, it was considered necessary to study the transformation of the metal–methylcysteine binary complexes into the metal–methylcysteine–cysteine mixed complexes at pH 8.5 to avoid any side interaction.

The plot of mobility against the logarithm of concentration of added cysteine gives a curve (Fig. 2) containing two plateaus. The mobility in the range of the first plateau is in agreement with mobility of the metal–methylcysteine complex. The mobility of the last plateau is more negative than that of the first plateau. This indicates the formation of more negatively charged complex. Further, since the mobility in the last plateau does not tally with the mobility of 1:1 and 1:2 metal–methylcysteine complexes, it is inferred that the moiety in the last plateau is due to co-ordination of the cysteine anion to 1:1 metal–methylcysteine moiety resulting in the formation of 1:1:1 mixed complexes metal–methylcysteine–cysteine as

$$ML + L' \overset{K_3}{\rightleftharpoons} MLL' \quad (4)$$

where L′ is the cysteine anion and K_3 is the stability constant of the mixed complex. The zero mobility of

the last plateau shows the neutral nature of the metal–methylcysteine–cysteine mixed complexes.

In the present electrophoretic study transformation of a simple complex into mixed complex takes place, hence the overall mobility U of the complex is given by

$$U = u_0 f_{[\text{M–methylcysteine}]} + u_1 f_{[\text{M–methylcysteine–cysteine}]} \quad (5)$$

where u_0, u_1 and $f_{[\text{M–methylcysteine}]}$, $f_{[\text{M–methylcysteine–cysteine}]}$ are the mobility and mole fractions of the metal–methylcysteine and metal–methylcysteine–cysteine complexes, respectively. On adding the value of the mole fraction, Eq. (5) becomes

$$U = \frac{u_0 + u_1 K_3 [\text{H}_2\text{C}(\text{S}^-)\text{CH}(\text{NH}_2)\text{COO}^-]}{1 + K_3 [\text{H}_2\text{C}(\text{S}^-)\text{CH}(\text{NH}_2)\text{COO}^-]} \quad (6)$$

From the concentration (Fig. 2) of cysteine at which the overall mobility occurs, the mean of the mobilities of two plateau is determined. The concentration of the cysteine anion at pH 8.5 (for this cysteine concentration) is calculated. K_3 is equal to $1/[\text{H}_2\text{C}(\text{S}^-)\text{CH}(\text{NH}_2)\text{COO}^-]$. All the calculated values of K_3 are given in Table 1.

A perusal of Table 1 reveals that the order of stability constants, viz. Fe(III)>Cr(III), is the same for metal–methylcysteine binary and metal–methylcysteine–cysteine mixed complexes. The stability of binary and mixed complexes follows the order: $\log K_1 > \log K_2 > \log K_3$. Since these metal complexes are being reported for the first time, no comparison can be made for the values of the stability constants. Since an uncertainty of ±5% attends the measurements of the mobility of metal spots the results reported here are fairly reliable.

To examine the possibility of hydrolysis of iron(III) and chromium(III), experiments have been performed at two concentrations (0.01 M and 0.001 M) of ligands. The mobility plots show that the plateau at the lower ligand concentration is shifted towards a higher pH range but the calculated stability constants are found to be same in both cases. Thus, the stability constants obtained were found to be independent of pH, indicating that hydrolysis of iron(III) and chromium(III) can be ignored here.

It can be concluded from these studies that methylcysteine and cysteine can be used to reduce the levels of iron(III) and chromium(III) in biological systems.

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