[Chem. Pharm. Bull. 25(12)3163—3171(1997)]

UDC 547.962.3.04 : 546.682.02.113.04.08

Interaction of Albumin, Transferrin, and Human Serum with Indium-113 m Complexes of Ethylenediaminetetraacetic Acid, Penicillamine, and Related Compounds

NAKAO KOJIMA, YUKIO SUGIURA, and HISASHI TANAKA

Faculty of Pharmaceutical Sciences, Kyoto University[1)]

(Received March 17, 1977)

The interaction between the indium complexes and human serum proteins has been investigated by electrophoresis and gel filtration. Excess indium above serum transferrin level bound to albumin and α_1-globulins besides to transferrin. The sulfhydryl-containing ligands and ethylenediaminetetraacetic acid (EDTA) prevented the indium binding by albumin, in contrast with common amino acids. The indium complexes of penicillamine, 2,3-dithiopropanol (BAL), and EDTA resist the transfer of indium from the complexes to transferrin and the ability is related to the stability of the complexes, and to the molecular form and size of the ligands. Thioglycolic acid which prevents the hydrolysis of indium and forms the indium-transferrin-thioglycolate ternary complex, is available for the effective preparation of ^{113m}In- or ^{111}In-transferrin. The binding-affinity of In(III) to ovotransferrin was approximately equal to that of Fe(III). The indium complex of EDTA was present in the form of the dimer at the physiological pH, as well as the ferric complex. On the basis of these results, the usefulness of the inert indium complexes of EDTA, penicillamine, BAL, and the disulfhydryl-containing peptides as imaging agents, has been discussed.

Keywords——indium-113m; radiopharmaceuticals; transferrin; albumin; human serum; EDTA; penicillamine; sulfhydryl-containing peptides; electrophoresis; gel filtration

Since the clinical application of ^{113m}In by Stern, Wagner, and others,[2,3)] many studies have been made for the preparation of suitable ^{113m}In and ^{111}In compounds for the scanning of the lung, blood pool, brain, kidney, bone, and tumor. During the past several years, the following chemicals have been introduced to prepare the effective indium-labelled complexes; diethylenetriaminepentaacetic acid (DTPA), ethylenediaminetetraacetic acid (EDTA),[3,4)] 1-(*p*-benzenediazonium)-ethylenediamine-N,N,N′,N′-tetraacetic acid,[5)] glutathione,[6)] bleomycin,[7)] albumin,[8)] and transferrin.[3,9)]

Lásztity[10)] have showed that In(III) is highly hydrolyzable and that the hydrolysis occurs at pH values above 1 in the In(III) solution of 10^{-5} M. Based on the property, the indium hydroxide particles have been available for the human lung scan. For the distribution to the various organs, however, it is generally desirable for indium to be present in the form of a stable chelate.

1) Location: *Yoshida, Shimoadachi-cho, Sakyo-ku, Kyoto 606, Japan.*
2) H.S. Stern, D.A. Goodwin, H.N. Wagner, Jr., and H.H. Kramer, *Nucleonics*, **24**, 57 (1966).
3) H.S. Stern, D.A. Goodwin, H.N. Wagner, Jr., and H.H. Kramer, *Nucleonics*, **25**, 62 (1967).
4) R.C. Reba, F. Hosain, and H.N. Wagner, Jr., *Radiology*, **90**, 147 (1968).
5) D.A. Goodwin, M.W. Sundberg, C.I. Diamanti, and C.F. Mears, "Radiologic and Other Biophysical Methods in Tumor Diagnosis," Year Book Medical Publishers, Inc., Chicago, 1975, p. 57.
6) K. Tanno, K. Uemura, and Y. Ito, *Pharmacometrics*, **6**, 1155 (1972).
7) M.L. Thakur, *Int. J. Appl. Radiat. and Isot.*, **24**, 357 (1973).
8) S.F. Perez, L.R. Bennett, and J. Larson, *J. Nucl. Med.*, **11**, 639 (1970).
9) D.A. Goodwin, R. Goode, L. Brown, and C.J. Imbornone, *Radiology*, **100**, 175 (1971).
10) S. Lásztity, *Radiochem. Radioanal. Lett.*, **12**, 33 (1972).

Hosain, *et al.*[11] pointed out that excess indium above 4 μg of the metal per ml of normal human plasma bound to α_2-globulins and was not found on the origin in electrophoresis. Whereas, Wochner, *et al.*[12] reported that most of indium migrated with α_1-globulins and the remainder stayed at the origin when transferrin was saturated with iron. Both workers did not confirm the Hartman and Hayes's result[13] that indium forms a weak complex with albumin. In order to develop new indium-labelled radiopharmaceuticals, it is essential to investigate systematically the interaction of the indium complexes with serum proteins, especially transferrin.

In this paper, the reinvestigation of serum protein localization of ^{113m}In and the investigation of the interaction between the biologically important indium complexes and serum protein components, were carried out by electrophoresis and gel filtration. We also estimate the difference between indium and ferric ions with regard to the affinity for ovotransferrin by gel filtration, because the latter is sometimes used as carrier of the former on clinical application in order to decrease the toxicity of indium. The new ^{113m}In-labelled complexes are also discussed with regard to the clinical application.

Experimental

Materials——Purified human transferrin and chicken ovotransferrin (Type I) (both iron free) were purchased from Sigma Chemical Company, and human serum albumin (4×crystalline) from Nutritional Biochemicals Corporation. The purity was checked by absorbance and electrophoresis. A molecular weight of 77000 was used for metal-free transferrin[14] and ovotransferrin,[15] and 66500 for albumin.[16] Human serum was obtained by centrifugation of blood sample taken from normal patient. Ionic ^{113m}In was eluted from a ^{113}Sn-^{113m}In generator (Union Carbide) with 0.04 M HCl–saline solution. Indium sulfate (99.9%) was the product of Mitsuwa Chemicals. Thioglycolic acid purchased from Dojin Pharmaceutical Laboratories, was purified by vacuum distillation as described by Leussing and Kolthoff.[17] Other chemicals were of the highest purity available and used without further purification. Distilled water was deionized by the ion exchange resin.

Preparation of Sample Solutions——For the preparation of labelled serum, the In(III) solutions of 10^{-6} or 10^{-4} M containing ^{113m}In generator eluate were added to human serum. Small weighed aliquots of indium (labelled with ^{113m}In) or ferric iron solutions were added to transferrin dissolved in buffer, in order to saturate the protein with the metals. These solutions were equilibrated by careful aeration just before the use. Addition of bicarbonate gave no changes in the results. We concluded that at pH 7.4 our air-equilibrated solutions contain dissolved bicarbonate in excess of our highest transferrin concentrations. Accordingly, metal-protein-bicarbonate ternary complexes were expected to be fully saturated with the metals. The labelled indium complexes were dissolved in buffer. For electrophoresis, 0.1 M veronal buffer (pH 8.6) was used, and for gel filtration 0.1 M Tris buffer (pH 7.4). The pH of the solutions was determined with a Hitachi-Horiba pH meter, model F-5. All the mixtures of the protein and the metal complexes were used after incubation for 20 min at room temperature.

Electrophoretic Measurements——An 1 μl aliquot of the samples was applied on cellulose acetate membranes, 6×5 cm (SELECTA, No. EPI-2, Carl Schleicher and Schüll) in veronal buffer (pH 8.6). Electrophoresis was performed at a constant current of 0.8 mA/cm width of membrane for 45 min. The strips were then removed, air dried and cut in half longitudinally: one half was stained with 0.8% Ponceau 3R, and the remaining half was cut into segments for the estimation of the ^{113m}In content. Radioactivity was measured with a Fujitsu well-type scintillation counter, model ATS-621. Corrections were made on ^{113m}In counts for physical decay. The profile of the protein distribution was obtained by measuring the optical density at 500 nm on the dyed half with a Shimadzu dual-wavelength TLC scanner, model CS-900.

Gel Filtration Technique——Sephadex G-25 was washed and equilibrated with Tris buffer (pH 7.4) and a 2×10 cm column was prepared. The samples of 0.5 ml were placed on the column, and eluted with the same

11) F. Hosain, P.A. McIntyre, K. Poulose, H.S. Stern, and H.N. Wagner, Jr., *Clin. Chim. Acta*, **24**, 69 (1969).
12) R.D. Wochner, M. Adatepe, A. van Amburg, and E.J. Potchen, *J. Lab. Clin. Med.*, **75**, 711 (1970).
13) R.E. Hartman and R.L. Hayes, *Fed. Proc.*, **27**, 838 (1968).
14) K.G. Mann, W.W. Fish, A.C. Cox, and C. Tanford, *Biochemistry*, **9**, 1348 (1970).
15) S.N. Timasheff and I. Tinoco, Jr., *Arch. Biochem. Biophys.*, **66**, 427 (1957).
16) B. Meloun, L. Morávek, and V. Kostka, *FEBS Letters*, **58**, 134 (1975).
17) D.L. Leussing and I.M. Kolthoff, *J. Electrochem. Soc.*, **100**, 334 (1953).

buffer. The radioactivity of each 1 ml fraction collected was determined as described above, and the absorbance was measured at 278.5 nm to determine the concentration of the protein with a Shimadzu spectrophotometer, model QV-50.

Results

Distribution of ^{113m}In in Human Serum

By electrophoresis of human serum containing indium of 10^{-6} M, ^{113m}In migrated with the β-globulin fraction(Fig. 1a), specifically with transferrin as confirmed by immunoelectrophoresis.[11,12] The binding of indium to transferrin was much greater than to any other protein. When indium of 10^{-4} M was added to human serum in order to saturate serum transferrin (*ca.* 5×10^{-5} M), the indium peak shifted to the α_1-globulins and albumin fractions besides the β-globulin fraction but not to the origin (Fig. 1b). Since the radioactivity was found on the origin in electrophoresis of the 10^{-4} M indium solution alone (Fig. 2b), it is considered that excess indium bound to serum protein components. In fact, albumin can bind indium as shown in Fig. 2a. The present result is compatible with the indium binding by albumin[13] and α_1-globulins.[12] Our α_1-globulins may be identical with 9.5*S* α_1-glycoprotein which has been recently characterized.[18] The new α_1-macroglobulin showed a molecular weight of 308000 and the strong affinity for Ca^{2+} and Ni^{2+}.

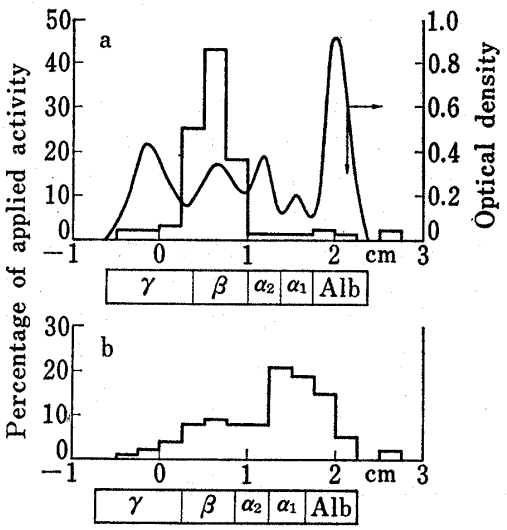

Fig. 1. Distribution of ^{113m}In after Electrophoresis of ^{113m}In-Human Serum System (pH 8.6)

Histogram shows ^{113m}In distribution, and the curve the optical density at 500 nm. The concentrations of In(III)(labelled with ^{113m}In) are 10^{-6} M(a) and 10^{-4} M(b), respectively.

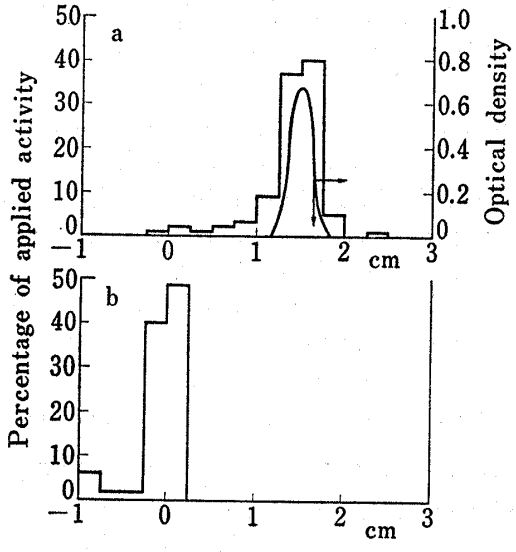

Fig. 2. Distribution of ^{113m}In after Electrophoresis of Albumin containing In(III)(a) and the In(III) Solution alone(b)(pH 8.6)

The curve shows the protein distribution. The concentrations of In(III) and albumin are 10^{-4} and 6×10^{-4} M, respectively.

Interaction of Albumin with ^{113m}In Complexes

Figure 3 shows the distribution of ^{113m}In between albumin (6×10^{-4} M) and the indium complexes (10^{-4} M, In:L=1:50) separated on Sephadex G-25. In the system containing albumin and ^{113m}In, 100% of the applied activity was found on albumin fraction (Fig. 3b). When an indium solution alone was eluted under the same conditions (Fig. 3c), the distribution pattern was somewhat different from that in the presence of albumin. The major activity was assumed to be attributed to colloidal indium having a molecular weight of the same order as

18) H. Haupt, N. Heimburger, T. Kranz, and S. Baudner, *Hoppe-Seyler's Z. Physiol. Chem.*, **353**, 1841 (1972).

the protein, and the minor remainder was slowly eluted. The results indicate that albumin binds indium as well as in Fig. 2a.

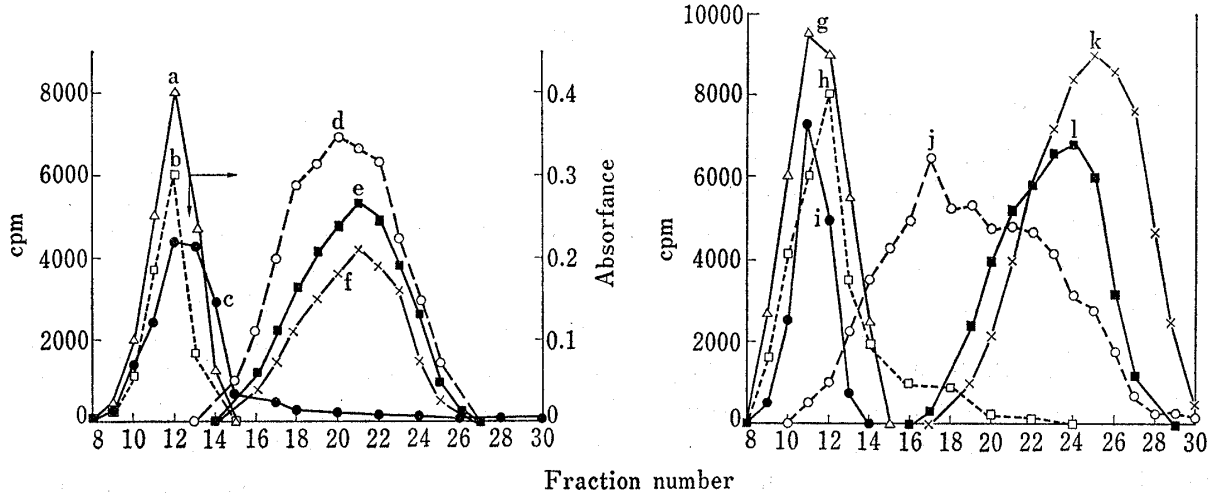

Fig. 3. Elution Pattern of Radioactivity in System containing Human Serum Albumin and the ^{113m}In Complexes with a Sephadex G-25 Column (pH 7.4)

Elution pattern of albumin(a) measured spectrophotometrically is coincident with that of albumin-^{113m}In(b), but not with that of the In(III) solution alone(c). The ligands used are dimercaptosuccinic acid(d), thiomalic acid(e), EDTA(f), glycine(g). citric acid(h), valine(i), thioglycolic acid(j), and penicillamine(k). The curve l represents the elution pattern of the In(III) glycine solution alone. The concentrations of the protein, In(III), and ligands are 6×10^{-4}, 10^{-4}, and 5×10^{-3} M, respectively.

Most of the activity was also detected on the protein fraction even in the presence of common amino acids such as glycine and valine. The activity is not attributed to colloidal indium species but albumin-binding indium complex, because the indium complexes of glycine form nc polymeric species (Fig. 3 l). That is to say, indium-binding capacity of albumin overcomes that of the carboxylic acid and amino acid such as citric acid, glycine, and valine. On the other hand, albumin can not bind the metal in the presence of the compounds such as penicillamine (3-mercaptovaline), thioglycolic acid, thiomalic acid, dimercaptosuccinic acid, and EDTA. These ligands are the sulfhydryl-containing acids and polyamino-polycarboxylic acid. However, glutathione displayed the elution pattern similar to that of citrate.

Interaction of Transferrin with ^{113m}In Complexes

The gel filtration was performed on the system containing transferrin and the ^{113m}In complexes under the similar conditions above-mentioned, except that the concentrations of the protein, ligand, and metal were 5×10^{-5}, 5×10^{-5}, and 10^{-6} M, respectively. The sulfhydryl-containing ligands and EDTA were selected for the experiment, because transferrin binds indium stronger than albumin. The results are shown in Fig. 4. These elution patterns were unchanged over the incubation time range of 20 min—2 hr. In the case of the thioglycolate complex, the indium was incorporated by transferrin. However, the penicillamine, 2,3-dithiopropanol (BAL), and EDTA complexes prevented the indium transfer to transferrin.

Comparison of In(III) and Fe(III) in Affinity for Ovotransferrin

Schwarzenbach, *et al.*[19] reported that the values of the stability constants of the EDTA–In(III) and -Fe(III) complexes were 24.95 and 25.1, respectively. Using these comparable EDTA complexes, incorporation of indium and iron into ovotransferrin was investigated. Ovotransferrin was used instead of transferrin based on the fact that the former has many similar properties to the latter and has been traditionally compared to transferrin with regard to virtually every physico-chemical property. In fact, the use of ovotransferrin gave the

19) G. Schwarzenbach, R. Gut, and G. Anderegg, *Helv. Chim. Acta*, 37, 937 (1954).

similar elution pattern to that in Fig. 4. The concentrations of the metals, EDTA, and ovotransferrin were 10^{-4}, 10^{-4}, and 5×10^{-5} M, respectively. The concentration of the metal–EDTA complex was exactly equal to the metal-binding equivalents of apoovotransferrin. The mixtures of the protein saturated with or without Fe(III)(or In(III)) and the EDTA complexes with or without In(III)(or Fe (III)), were incubated for 20 min at pH 7.4, and gel filtrations were performed. The results are presented in Table I. When EDTA was previously saturated with Fe(III), about 12% of indium moved from ovotransferrin to the EDTA fraction. On the other hand, about 13% of ^{113m}In was transferred from the EDTA-indium complex to ovotransferrin saturated with Fe(III). The percentage of indium or iron removed from ovotransferrin was 12—13% in the presence of EDTA saturated

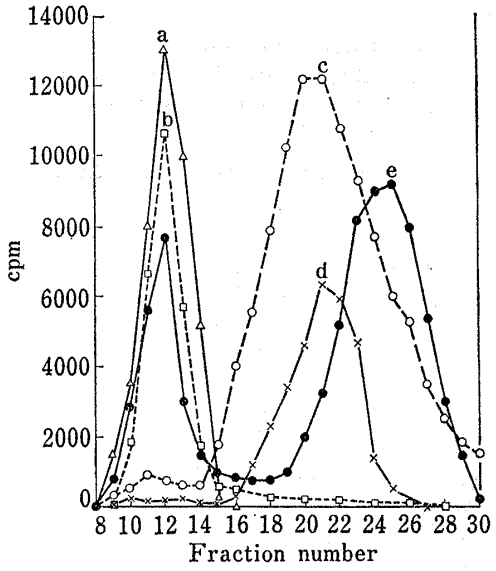

Fig. 4. Elution Pattern of Radioactivity in System containing Transferrin and the ^{113m}In Complexes with a Sephadex G-25 Column (pH 7.4)

The curve a represents the elution pattern of ^{113m}In-transferrin. The ligands used are thioglycolic acid(b), BAL(c), EDTA(d), and penicillamine(e). The concentrations of the protein, In(III), and ligands are 5×10^{-5}, 10^{-6}, and 5×10^{-5} M, respectively.

Table I. Distribution of ^{113m}In between Ovotransferrin (OTf) and EDTA separated on a Sephadex G-25 in the Various Incubation Systems with or without Fe(III)[a)]

Incubation systems	Percentage of applied activity	
	OTf–^{113m}In fraction	EDTA–^{113m}In fraction
(1) OTf–^{113m}In+EDTA–Fe	87.6	12.4
(2) OTf–^{113m}In+EDTA	73.4	26.6
(3) OTf–Fe +EDTA–^{113m}In	13.4	86.6
(4) OTf +EDTA–^{113m}In	3.5	96.5

a) The concentrations of the protein, EDTA, and metals are 5×10^{-5}M, 10^{-4}M, and $10\times^{-4}$M, respectively.

with the another metal. Consequently, the affinity of indium for the protein is comparable to that of the ferric iron. When ovotransferrin was free from the metals, the transfer of indium from the EDTA-indium complex to the protein was negligibly small.

Interaction between Stable Indium Complexes and Human Serum

The indium complexes of BAL, penicillamine, and EDTA were expected to be stable even in human serum. The interaction of the penicillamine- and EDTA-indium complexes with human serum was investigated by electrophoresis in the same concentration of the complexes as that in gel filtration system.

Figure 5a shows the distribution of ^{113m}In after electrophoresis of the solution containing human serum and the penicillamine-indium complex. The two indium peaks were found on the β-globulin and albumin fractions. In the electrophoresis of the complex solution alone, the maximum activity was located at about 1.8 cm from the origin, corresponding to the albumin location (Fig. 5b). As the penicillamine-indium complex is inactive to albumin, however, the activity is not attributed to indium-binding albumin, but the penicillamine complex itself. The distribution pattern after electrophoresis is quite similar to that after gel filtration (see Fig. 4).

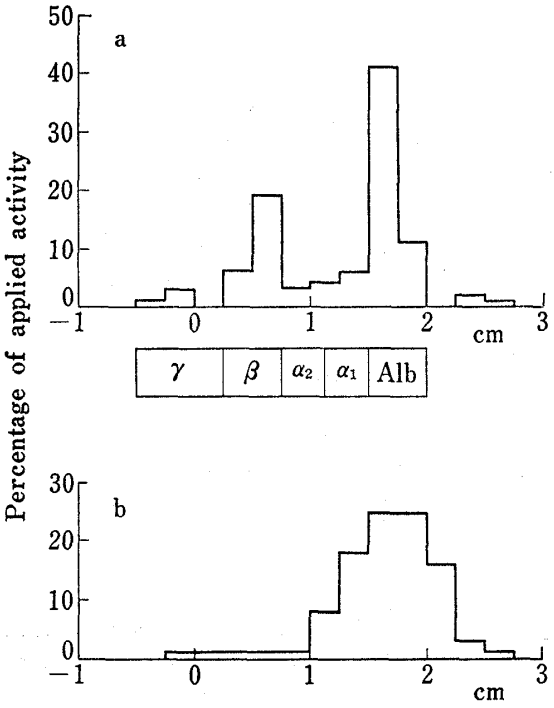

Fig. 5. Distribution of ^{113m}In after Electrophoresis of Human Serum containing the ^{113m}In-Penicillamine Complex(a) and of the ^{113m}In-Penicillamine Complex alone (b)

The concentrations of In(III) and penicillamine are 10^{-6} and 5×10^{-5} M, respectively.

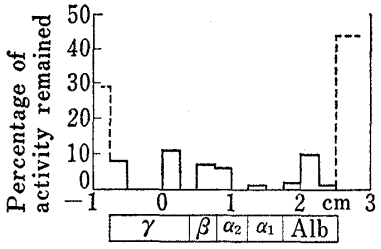

Fig. 6. Distribution of ^{113m}In remained on Membrane after Electrophoresis of Human Serum containing the ^{113m}In-EDTA Complex

The concentrations of In(III) and EDTA are 10^{-5} and 2×10^{-5} M, respectively.

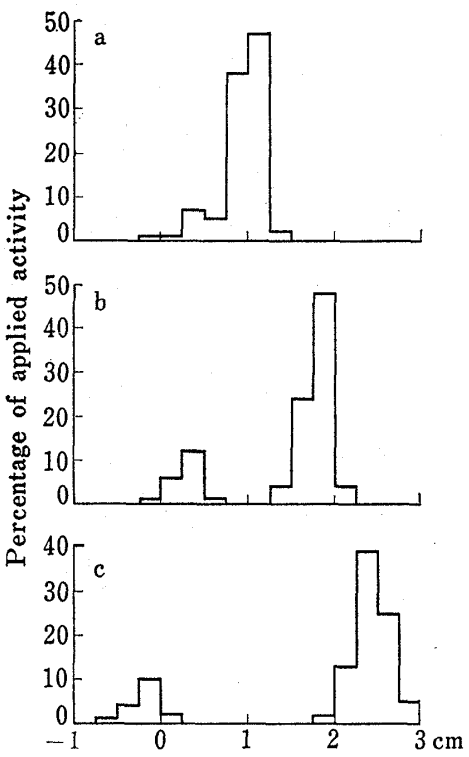

Fig. 7. Electrophoretic Migration of the ^{113m}In-EDTA Complex after 10 min (a), 20 min (b) and 30 min (c)

The concentrations of In(III) and EDTA are 10^{-5} and 2×10^{-5} M, respectively.

The result with EDTA is shown in Fig. 6. Only 12% of the applied activity was detected on cellulose acetate membrane, and the majority disappeared from the membrane. No significant difference in the distribution of the detected activity among each protein fractions was apparent. The EDTA-indium complex migrated rapidly on the membrane, as demonstrated by electrophoreses of the EDTA complex alone at various times (Fig. 7). These results indicate that the EDTA complex hardly interacts with human serum.

Discussion

Biedermann[20] reported that In(III) was hydrolyzed at the pKa$_1$ of 4.42 and polymerized as follows.

$$(n+1)\mathrm{In}^{3+} + 2n\mathrm{H_2O} \rightleftharpoons \mathrm{In}((\mathrm{OH})_2\mathrm{In})_n^{(3+n)+} + 2n\mathrm{H}^+$$

At the concentration of 10^{-3} M, n value is already greater than 2. In the physiological pH, therefore, In(III) is almost hydrolyzed and polymerized (Figs. 2a and 3c). The common amino acids are able to prevent this hydrolytic polymerization of indium by the weak coordi-

20) G. Biedermann, *Arkiv Kemi*, **9**, 277 (1956).

nation to the metal (Fig. 3 l), but not to occupy the six coordination sites on In(III).[21] Because of the instability of the amino acid complexes, indium was taken off even by albumin (Fig. 3).

Contrary to the amino acid complexes, the indium complexes with the sulfhydryl-containing ligands resisted the metal binding by albumin. The result is closely related to the stability of these complexes (Table II). The In–S bond is significantly stabilized by the $d\pi$ back-donation from In(III)($4d^{10}$) to the vacant 3d orbitals of sulfur. The disulfhydryl-containing peptides, such as 2,3-dimercaptopropionylglycine and 2-mercaptopropionyl-L-cysteine, prevent the hydrolytic reaction of indium by the strong coordination of the two SH groups.[22] Glutathione containing one SH group is impossible to keep indium in the competitive reaction with albumin. From this reason, its clinical availability[6] based on the property of the complex itself is considered to be very restricted.

Table II. Stability Constants (log K_1) of the Indium(III) Complexes

	Donor set	log K_1	I (Medium)	Temp. (°C)	Ref.
Glycolic acid	OO	2.91	0.2($NaClO_4$)	25	47
Glycine	NO	2.39	0.2($NaClO_4$)	25	47
Thioglycolic acid	SO	12.10	0.2($NaClO_4$)	25	47
Thiomalic acid	SOO	14.95	0.1($NaClO_4$)	25	48
Penicillamine	SNO	15.330	0.1(KNO_3)	21	26
BAL	SSO	16.83	0.1(KNO_3)	21	49
2-Mercaptopropionyl-L-cysteine	SSO	16.41	0.1(KNO_3)	20	22
2,3-Dimercaptopropionylglycine	SSO	17.60	0.1(KNO_3)	20	22
EDTA	2(NOO)	24.95	0.1(KNO_3)	15	19

The sulfhydryl-containing ligand readily forms the sulfur-bridged and polymeric complex.[23] The polymeric species is most likely found in the metal ion which contains a filled or almost filled d electron shell. Actually, we[24] have clarified the presence of the sulfur-bridged trimeric In(III) complex with cysteine. BAL also forms the polymeric complex. Furthermore, in the In(III) solution containing thioglycolic acid, the trinuclear In_3L_6 species was formed, as suggested by the computer calculation of the potentiometric data with the non-linear least-squares program.[25] In Figs. 3 and 4, accordingly, the difference of the elution volumes of each complexes is assumed to be attributed to the degree of such polymerization. In the case of penicillamine,[26] the formation of the polymeric species was sterically prevented by the β,β-dimethyl groups, and various protonated species were formed. The various complex formation may contribute toward the somewhat broad distribution spectrum in the electrophoresis of the penicillamine-indium complex alone (Fig. 5b). It is probably due to the polymeric tendency of the citrate-indium complex[27] that citrate resists the binding of indium by albumin. Citric acid has been known to form the polymeric species with Ga(III)[28] and with Fe(III).[29,30] For example, the ferric citrate polymer of 2×10^5 molecu-

21) R. Sarin and K.N. Munshi, *Aust. J. Chem.*, **25**, 929 (1972).
22) Y. Sugiura, N. Kojima, and H. Tanaka, *Chem. Pharm. Bull.* (Tokyo), **25**, 2263 (1977).
23) S.E. Livingstone, *Quart. Rev.*, **19**, 386 (1965).
24) N. Kojima, Y. Sugiura, and H. Tanaka, *Bull. Chem. Soc. Jpn.*, **49**, 3023 (1976).
25) N. Kojima, Y. Sugiura, and H. Tanaka, the 96th Annual Meeting of Pharmaceutical Society of Japan, Nagoya, April 1976.
26) N. Kojima, Y. Sugiura, and H. Tanaka, *Bull. Chem. Soc. Jpn.*, **49**, 1294 (1976).
27) A. Adin, P. Klotz, and L. Newman, *Inorg. Chem.*, **9**, 2499 (1970).
28) J.D. Glickson, T.P. Pitner, J. Webb, and R.A. Gams, *J. Am. Chem. Soc.*, **97**, 1679 (1975).
29) T.G. Spiro, L. Pape, and P. Saltman, *J. Am. Chem. Soc.*, **89**, 5555 (1967).
30) T.G. Spiro, G. Bates, and P. Saltman, *J. Am. Chem. Soc.*, **89**, 5559 (1967).

lar weight appears to consist of an iron hydroxide core with citrate ions bound to the surface. The polymeric citrate complex resists the iron binding by transferrin.[31]

The stable thioglycolic acid-indium complex does not interact with albumin, but with transferrin. It has been corroborated that transferrin does not bind iron at the special metal binding sites in the absence of carbonate or synergistic anions.[32] According to "the interlocking hypothesis,"[33] the anion molecule in Fe(III)-transferrin-anion must possess a carboxylic acid group and a proximal functional group in order to achieve a "carbonate-like" configuration. The synergistic anions are accommodated in a site at least 3 Å deep, approximately 6 Å wide, and between 4 and 6 Å or more in length. A site size requirement of thioglycolic acid is estimated at 6.3 Å, based on a study of Corey-Pauling-Koltun molecular model. Thus, the activity on the protein fraction in Fig. 4b is considered to originate from the ^{113m}In-transferrin-thioglycolate ternary complex, and the anion could be slowly displaced by HCO_3^- equilibrated in the solution. In contrast, BAL and penicillamine are expected to be ineffective as synergistic anions, because the former has no carboxylic group and the latter has two β-methyl substituents unaccommodated by the anion binding site.

The ESR results[34] have shown that ovotransferrin and transferrin form the high spin Fe(III)($S=5/2$) complexes. Table I suggests that the affinity toward ovotransferrin is similar between Fe(III) and In(III). The result is consistent with the fact that the crystal field stabilization energies of $3d^5$(Fe(III)) and $4d^{10}$(In(III)) in the weak crystal field are theoretically zero. Ga(III) also binds to the proteins in a similar fashion to Fe(III).[35,36] In addition, the indium migration from transferrin to red cells has been observed.[37] These results reveal that In(III) binds to transferrin in a similar manner to Fe(III). The EDTA-Fe(III) complex, as well as the indium complex(Table I), showed the markedly slow iron transfer toward transferrin.[38] Gustafson and Martell[39] have pointed out that the ferric complex of EDTA is hydrolyzed by alkali to give equilibrium mixtures of monohydroxy monomer and binuclear complex species. X-ray analysis[40] of the Fe(III) complex with N-(hydroxyethyl)ethylenediamine-N,N′,N′-triacetic acid(HEDTA) revealed the structure of $[(HEDTA)_2Fe_2O]$ with an Fe-O-Fe bond angle of about 165°. The complex is identical to the EDTA-Fe(III) complex with regard to the spectroscopic and magnetic properties.[41] On the other hand, the EDTA-In(III) complex also displayed the dissociation of proton from the coordinated water in weakly basic solution.[42] Figure 4 suggests the existence of the polymeric EDTA-In(III) complex. In the case of penicillamine, the predominant species InL_2 at pH 7.4 has a molecular weight of 409, which corresponds to the Kd value of 0.8 in Fig. 8. The figure was constructed from the gel filtration data[43] of dextran-2(number average molecular weight $\bar{M}n=1800$) with a Sephadex of water regain 2.3. Using the Kd value of the penicillamine-In(III) complex, the value of 0.55 for the EDTA-In(III) complex was estimated from Fig. 4. The molecular weight ($\bar{M}n$) corresponded to Kd=0.55 in Fig. 8 is about 900, indicating the presence of the

31) G.W. Bates, C. Billups, and P. Saltman, *J. Biol. Chem.*, **242**, 2810 (1967).
32) E.M. Price and J.F. Gibson, *Biochem. Biophys. Res. Commun.*, **46**, 646 (1972).
33) M.R. Schlabach and G.W. Bates, *J. Biol. Chem.*, **250**, 2182 (1975).
34) R. Aasa, B.G. Malmström, P. Saltman, and T. Vänngård, *Biochem. Biophys. Acta*, **75**, 203 (1963).
35) R.C. Woodworth, K.G. Morallee, and R.J.P. Williams, *Biochemistry*, **9**, 839 (1970).
36) P. Aisen, G. Lang, and R.C. Woodworth, *J. Biol. Chem.*, **248**, 649 (1973).
37) M.H. Adatepe, P. Penkoske, A. van Amberg, T. Wharton, R.G. Evens, and E.J. Potchen, *Int. J. Appl. Radiat. Isot.*, **22**, 498 (1971).
38) G.W. Bates, C. Billups, and P. Saltman, *J. Biol. Chem.*, **242**, 2816 (1967).
39) R.L. Gustafson and A.E. Martell, *J. Phys. Chem.*, **67**, 576 (1963).
40) S.J. Lippard, H. Schugar, and C. Walling, *Inorg. Chem.*, **6**, 1825 (1967).
41) H. Schugar, C. Walling, R.B. Jones, and H.B. Gray, *J. Am. Chem. Soc.*, **89**, 3712 (1967).
42) K. Saito and H. Terrey, *J. Chem. Soc.*, **1956**, 4701.
43) K.A. Granath and P. Flodin, *Makromol. Chem.*, **48**, 160 (1961).

dimeric EDTA-In(III) species. Being considered these observations and the similarity with iron,[44] the EDTA–In(III) equilibria (pH >7) may be expressed as follows:

$$[(EDTA)InOH_2] \rightleftarrows [(EDTA)InOH]^- + H^+$$

$$2[(EDTA)InOH]^- \rightleftarrows [(EDTA)_2In_2O]^{2-} + H_2O$$

The characteristic similarity of the EDTA–In(III) complex to the corresponding ferric complex may be attributed to the slow transfer of indium from the complex to transferrin.

In view of the clinical application, it is required that the indium complexes are protected from the hydrolysis in the physiological pH region. The sulfhydryl-containing ligand satisfies this requirement. In addition, it is desirable that the ligand has an adequate bulky size which does not accommodate in the anion binding sites of transferrin. Penicillamine and BAL are much preferable and the indium complexes are expected to be metabolized effectively in the human body. For example, the ^{99m}Tc-penicillamine complex is clinically available as a cholescintigraphic agent.[45,46] Furthermore, the disulfhydryl-containing peptides, such as 2,3-dimercaptopropionylglycine and 2-mercaptopropionyl-L-cysteine, stand ready for hereafter investigation of their potential usefulness. On the other hand, thioglycolic acid is preferable for the instantaneous and effective preparation of ^{113m}In or ^{111}In labelling transferrin, since the ligand prevents the metal hydrolysis which occurs prior to the metal-protein binding. The innertness of the EDTA–indium complex for transferrin and its rapid kidney excretion give the clinical basis on its choice for brain tumor imaging.

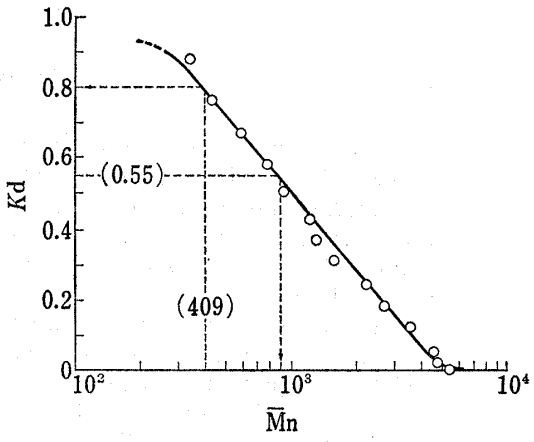

Fig. 8. Relationship between Distribution Coefficient (Kd) and Number Average Weight ($\bar{M}$n)

Circles are obtained from Ref. 43. The penicillamine-In(III) complex has molecular weight of 409 and the EDTA complex Kd=0.55.

44) H.B. Gray, *Rec. Chem. Prog.*, **29**, 163 (1968).
45) M. Tubis, G.T. Krishnamurthy, J.S. Endow, and W.H. Blahd, *J. Nucl. Med.*, **13**, 652 (1972).
46) A. Yokoyama, H. Saji, H. Tanaka, T. Odori, R. Morita, T. Mori, and K. Torizuka, *J. Nucl. Med.*, **17**, 810 (1976).
47) R. Sarin and K.N. Munshi, *J. Inorg. Nucl. Chem.*, **35**, 201 (1973).
48) R. Sarin and K.N. Munshi, *J. Inorg. Nucl. Chem.*, **34**, 581 (1972).
49) N. Kojima, Y. Sugiura, and H. Tanaka, the 95th Annual Meeting of Pharmaceutical Society of Japan, Nishinomiya, April, 1975.