COMPLEXES OF TECHNETIUM III AND IV WITH PYROPHOSPHATE: PREPARATION, ANALYSIS AND METABOLIC BREAKDOWN STUDIES.

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SUMMARY

The oxidation state of technetium-pyrophosphate (99m-Tc PYP) reduced by $SnCl_2 .2H_2O$ was determined by radiochromatography. Two different complexes were formed (complex I and complex II). The oxidation states of Tc to be III or IV or both in complex II or hydrated technetium dioxide in complex I were investigated. Formation of Tc (III)/Tc(IV)-PYP and hydrated technetium dioxide-PYP complexes were studied by radiochromatography and by biodistribution in mice. Experiments in animals helped to clarify the behaviour of these complexes. Thus urine obtained from one group of mice injected with 99m-Tc-PYP complexes wase reinjected to another group of mice. It was found that complex II excreted in the urine was not broken down while cleavage and deposition of 99m-Tc-species were found with complex I excreted in urine.

INTRODUCTION

The practical importance of pyrophosphate complexes lies in their medical use for radionuclide bone scanning and acute myocardial infarct (1). They are prepared by reducing the tracer (nanomolar) amount of $99m\text{-}TcO_4^-$ with stannous ions in the presence of PYP. There are limited information on the oxidation states in various chelates (2). Most experiments have been carried out using 99-Tc at carrier concentration about 10^4 times larger than 99m-Tc concentration. The important consideration is the method used to determine the oxidation state of 99m-Tc at the nanomolar level (3).

In this paper we tried by means of chromatography as it is simple, rapid and precise technique to determine the valence states of 99m-Tc in 99m-Tc-PYP. Biodistribution studies in animals clarify the behaviour of these complexes and throw light on the metabolic breakdown (4).

Metarials and Methods

Materials

99m-Tc pertechnetate was eluted from 99-Mo $\longrightarrow$ 99m-Tc-generator (Amersham, England). $Na_4P_2O_7 . 10H_2O$ was obtained from BDH chemical pool, England. Hydrated stannous chloride was obtained from Hean AG, seelze, Hannover. PYP kits were obtained from NRC, Baghdad (5).

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Complex Formation and Chemical Analysis

The 99m-Tc PYP (Sn) was prepared as previously described by Shafiq et al (6). The radiopharmaceutical was prepared after the addition of 1ml 99m-Tc eluate to 3ml of each Sn (II)-PYP complex. The conditions of preparation of Sn (II)-PYP complexes were as follows.

Complexes	Molar ratios Sn: PYP	Final concentration (mg/ml) PYP	Sn (II)	pH
Complex I	2:1	0.54	0.55	3.2
Complex II	1:31	33.3	0.54	6.4

Chromatographic Analysis

Paper chromatography was applied to find the valence state of 99m-Tc and its labelling yield in the 99m-Tc-PYP complexes using the method of cifka (7). A sample was taken with a 5 µl capillary and applied on whatman No.3 MM paper strips, prewashed with 0.6 NHCl and then with dist-water. The 3MM paper strips were developed in 0.3 N HCl and after drying scanned with berthold thin-layer scanner II. W.Germany.

Gel chromatography Column Scanning technique (GCS)

Gel filtration using Sephadex G-50 superfine (AB pharmacia Fine Chemicals, Sweeden) proved to be a reliable and simple method for studying the chemical state of 99m-Tc in labelled compounds and radiopharmaceuticals (8,9). A column with 17mm i.d. and 330mm length was filled to 300mm with Sephadex G-50 superfine. A sample of 0.2ml was applied at the top of the column. The elution was carried out with a volume of 25ml of the eluant (0.67mg PYP in 100ml of 0.9% NaCl adjusted to pH 8.0). The column was sealed and scanned with a slit-(1mm)-collimated NaI (Tl)-crystal. Reduced hydrolyzed 99m-Tc was found at the top of the column within a zone of 25mm, 99m-Tc-pertechnetate in a zone of 25-60mm and 99m-Tc-PYP complexes in a zone of 60-130mm for complex I and 60-160mm for complex II.

Animal experiments

The organ distribution of 99m-Tc-PYP complexes was evaluated in Swiss albino mice, having an average weight of 20-25g. After an intravenous injections (via tail vein) of 20 µCi of 99m-Tc radioactivity in 0.2ml containg 0.1mg PYP, the animals under study were sacrified for tissue radioassay at certain time intervals after injection.

Blood, urine, liver, spleen, kidneys, stomach, muscle and bone were removed and weighed. The % I.D/1g tissue in each organ was determined by counting, using a well scintillation counter. The extent to which the radioactive material in urine retains bone-seeking properties was investigated as follows:- 99m-Tc-PYP complexes were injected into two groups of mice (3 mice per group). The mice were sacrified 2hr.after i.v. injection and their organ activity investigated. The urine collected was intravenously injected into another group of mice, and the 2hr distribution measured in three animals of two groups. The 1g bone: 1ml blood, 1g bone : 1g liver and 1g bone: 1g tissue ratios which are important for scintigraphy were compared for different preparations of 99m-Tc-PYP complexes. Perecentages of 99m-Tc-PYP complexes in solution and urine were determined using GCS technique as described above.

The organ distribution of 99m-Tc-PYP complexes were also studied in white rats of 250g. 0.2ml sample of 200 μCi was given intravenously through the tail vein. Urine and faeces were collected over the period of 24hr and measured using a well-type scintillation counter.

RESULTS AND DISCUSSION

Complex Formation

The Gelchromatography Column Scanning technique (GCS) is a particularly useful technique for the detection of different 99m-Tc-PYP complexes (8,9). Table I summerized the fraction of complex I and II as a function of total PYP concentration and at constant pH value (6.4). Complex II is formed in 93% yield at high PYP concentration (33.3 mg/ml), a decrease in the yield of complex II (76%) was observed with increase in the yield of complex I (20%). The formation of complex II at high PYP concentration was found to be strongly dependent on the pH of the solution (10) as shown in Fig.1. The data obtained from GCS profiles show that more than 95% of complex II is obtained at pH value 6.4. These results are in agreement with those obtained by Schumichen et al (10). Differencesin the efficiency of compound formation strongly suggest that more than one valence state of 99m-Tc can bind to the same chelate, namely Tc (III) and Tc (IV), (3). Fig.1. shows the fraction of complex I and II as a function of pH and at low PYP concentration (0.54mg/ml). The data obtained from GCS profiles show that complex I is formed in 34% at pH 3.2 whereas 18% of complex I is obtained at pH 4.0 . Schumichen et al (10) reported that only one complex was formed at low PYP concentration (hydrogen ion independent) which shows no bone affinity. However, we found two complexes of PYP at low PYP concentration.

Table I. The effect of high concentration of PYP (molar ratio 1:31 Sn:PYP) at a fixed pH value (6.4) on the formation of 99m-Tc-PYP complexes as obtained from GCS technique.

% of 99m-Tc-activity atarea correspondingto:99m-Tc-PYP					
PYP mg/ml	$SnCl_2 2H_2O$ mg/ml	red.hyd.99m-Tc	99m-TcO_4^-	complex I	complex II
6.66	0.53	0.53±0.31	14.3±0.98	14.0±0.47	70.9±0.7
13.33	0.53	0.67±0.23	7.0±0.47	13.2±1.16	79.2±0.7
20.00	0.53	0.67±0.20	3.0±0.47	19.7±0.72	76.4±0.5
26.66	0.53	0.40±0.14	3.6±0.72	15.3±0.72	80.6±1.3
33.33	0.53	0.83±0.34	3.0±0.23	3.3±0.27	92.7±0.1
40.00	0.53	0.37±0.07	1.5±0.23	6.4±0.32	91.7±0.5
50.00	0.53	0.37±0.07	1.2±0.12	6.0±0.23	92.7±0.2

Valence state of 99m-Tc in 99m-Tc-PYP complexes

In order to find the valence state of technetium in the 99m-Tc-PYP complexes, an investigation by paper chromatography was performed using 3 MM paper strips. Different oxidation states were identified according to the method of Cifka (7). Table II shows the standards used to find the lower oxidation state of 99m-Tc. The percent of activity distributed in different zones were determined.

Sn-PYP reduced the pertechnetate ion to Tc (III), Tc (IV) or Tc (V) depending on the concentration of the solution, and pH in the process of labelling of Sn-PYP with 99m-Tc. The

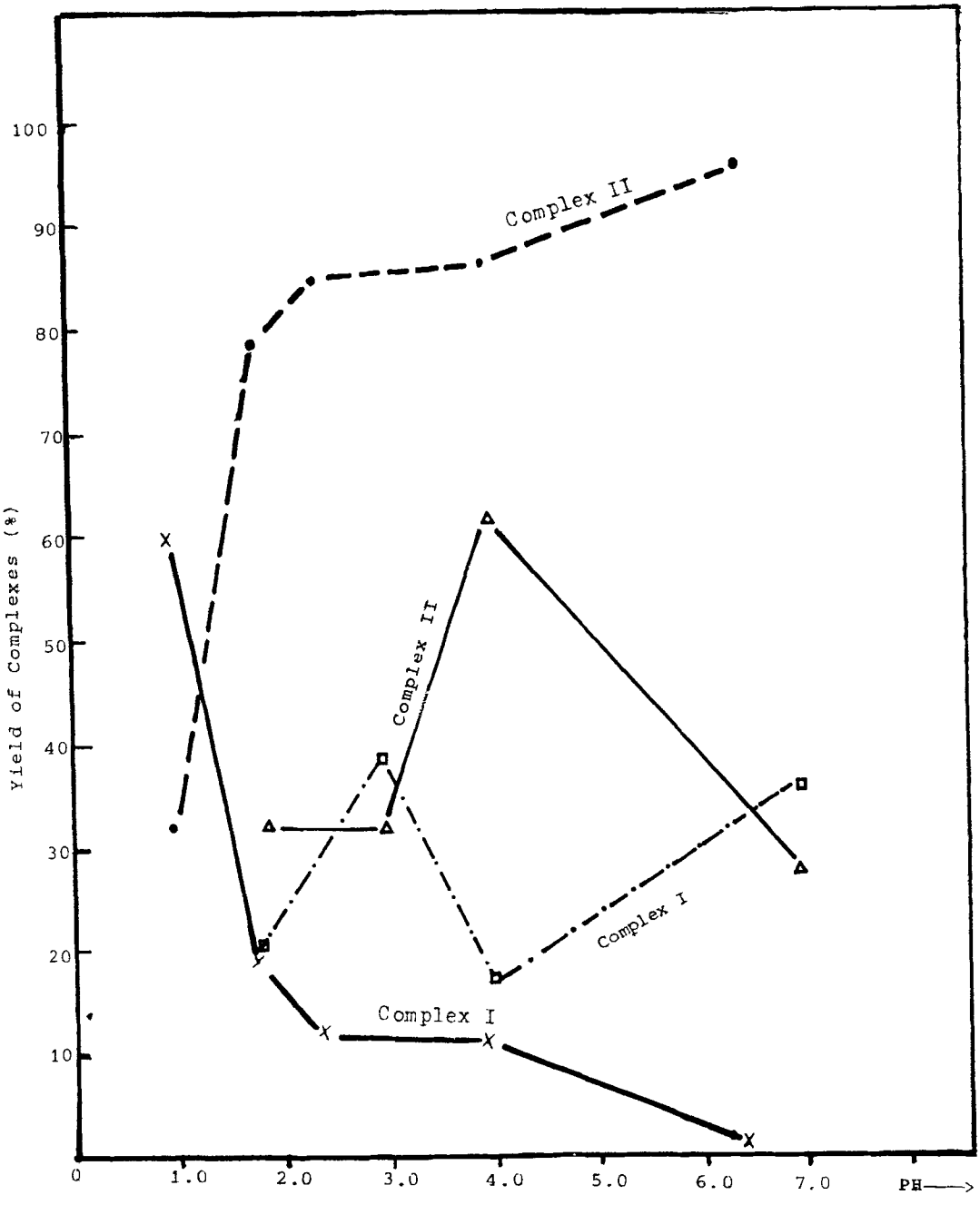

Fig.2 Yield of 99m Tc-Complexes as a function of PH.
At high PYP concentration (33.3 mg/ml) complex I(x—x), complex II (● —●) and at low PYP concentration (0.54 mg/ml) complex I (□—□), complex II (△—△).

radiochromatogram of complex II shows that higher technetium fraction appears in the form of Tc (IV) while Tc (III) is much less. This is due to reoxidation of Tc (III) to Tc (IV). Russel et al (11) confirmed by polaragraphy that the oxidation state of 99m-Tc reduced by Sn (II) ion in 0.1 M pyrophosphate was (III) and Tc (IV). Tc (III) could be reoxidized to Tc (IV). Our results are in good agreement with those found by Russel et al (11). The data obtained from the radiochromotogram of complex I shows that the valence states of technetium were Tc (IV), Tc (V) and Tc (VII). These results are summarized in Table III.

In vivo behaviour of Tc (III) and Tc (IV) .
To determine whether the intact technetium PYP complex is accumulated in the target organ or whether cleavage and deposition of technetium alone take place. The results of animal experiments helped to clarify the behaviour of these complexes. We have therefore undertaken to characterize by experimental animals the extent to which the urine retains bone-seeking properties , by injecting comparable complexes (complex I and complex II) in mice. The urine of mice which received 99m-Tc-PYP complexes were injected into second group of mice (Table IV). The organ distribution of complex II showed that in the second group (group B), bone uptake was only reduced by about 10% compared with the first group, while negligible increase in the soft tissue uptake was observed (Table IV B) these results confirmed that complex II excreted in urine was not broken down. Formation of high affinity bone seeking properties (complex II) was due to the Tc (III) and Tc (IV) formed as found by 3MM paper strips above. Our results are in good agreement with those found by Steligman et al (12). Formation of complex I leads to the formation of poor bone affinity. Lose of bone affinity was accompanied by a corresponding increase of uptake in kidneys, and soft tissue (Table IV group B). Formation of complex I leads to a poor bone seeking agent due to the formation of $^{99m}TcO_2 . 2H_2O$ microcolloid (13). This observation is in agreement with the finding that in the preparation of complex I, about 14% of I.D/1g tissue was taken by liver and spleen, suggesting uptake

Table II the oxidation state of 99m-Tc and the percentage of activity distributed of $^{99m}TcO_4^-$ as determined from the profile using 3MM paper strips developed with 0.3 N HCl.

	% activity distributed corresponding to:		
	Tc(IV) R_f(0.78-0.85)	Tc(V) R_f(0-0.2)	Tc(VII) R_f(0.68-0.70)
$^{99m}TcO_4^-$ in 0.3 NHCl incubated at room temp.	-	11.7±2.7	88.5±2.6
$^{99m}TcO_4^-$ in 6 NHCl incubated at 100 C for 30 min.	95.2±1.23-		3.80±1.17

Table III. The oxidation state and the percentage of activity distributed for each complex as determined from the profile using 3MM paper strips developed by 0.3 NHCl .

Complexes	% activity distributed corresponding to: Tc(III) R_f (0.95-0.99)	Tc(IV) R_f (0.78-0.85)	Tc(V) R_f (0-0.02)	Tc(VII) R_f (0.68-0.7)
Complex I	-	63.3±2.2	19.60±1.22	16.7±3.1
Complex II	13.0±1.7	76.2±1.1	1.55±0.12	9.2±1.3

Table IV. Organ distribution of 99m-Tc-PYP in mice
A= Mice 2hr after i.v injection of 99m-Tc-PYP complexes
B= Mice 2hr after i.v injection of urine of mice A

Organs	Group A		Group B	
	Complex I	Complex II	Complex I	Complex I
	% I.D/1g tissue			
Blood	2.68±0.16	0.54±0.3	3.23±0.1	2.00±0.17
Liver	11.83±0.58	0.32±0.03	1.40±0.13	0.84±0.07
Spleen	2.44±0.26	0.05±0.006	0.78±0.3	0.50±0.17
Kidneys	5.24±0.22	1.64±0.35	6.20±0.17	2.51±0.22
Lungs	0.75±0.09	0.28±0.06	0.24±0.05	0.75±0.17
Stomach	1.00±0.55	0.40±0.08	1.41±0.37	1.31±0.08
Muscle	0.59±0.04	0.31±0.02	1.00±0.39	0.70±0.12
Bone	3.62±0.62	7.93±0.31	1.91±0.18	4.67±0.21
Urine	23.13%	29.49/0.1ml		
1g Bone / 1ml Blood	1.36±0.28	14.6±0.30	0.6±0.03	2.41±0.31
1g Bone / 1g Liver	0.31±0.06	25.6±0.2	1.44±0.29	5.84±0.74
1g Bone / 1g tissue	6.22±1.95	23.5±0.17	6.13±1.3	7.40±0.94
Paper chromatography in methanol (%99m-Tc-PYP)	70.8±1.2	98.0±1.2	30±0.7	97.50±1.5

of colloidal material by the reticulo-endothelial system and 5% of I.D/1g tissue was taken in the kidneys might be due to formation of 99m-TcO_4^- , and about 23% of dose was excreted in the urine and only about 4% of I.D/1g bone was found in the bone as shown in Table IV group A. These findings confirmed that complex I excreted in urine was broken down and cleavage and deposition of technetium species take place. This was confirmed by the analysis using paper chromatography (Table IV). The percentage of the dose of 99m-Tc-excreted in faeces

and urine during the first 24hr after i.v.injection in rat shows that the radiopharmaceutical was not excreted in large amount in faeces (1.0% and 1.23%) but about 25% and 23% appears in urine at 24 hr after injection of complex I and II respectively as shown in Table V. Analysis of urine of complex II isolated from the bladder by GCS, shows that 95% of the technetium activity was still as 99m-Tc-PYP , the remaining 2% was 99m-TcO_4^-, while of complex I , 51% appeared in the form of reduced hydrolyzed 99m-Tc.

CONCLUSION

At least two different 99m-Tc-PYP complexes were determined (complex I and complex II) using GCS technique. The chromatographic method shows that the oxidation state of Tc might be III and IV in complex II and hydrated $TcO_2 .2H_2 O$ microcolloid in complex I. Complex II was a stable one with ^{99m}Tc (III/IV) with a yield dependent mainly on pH and PYP concentration, while complex I was unstable with hydrated 99m-$TcO_2.2H_2O$. The in vivo behaviour of these complexes emphasizes the superior stability of the complex II.

Table V. Percentage dose of 99m-Tc-excreted from rats 24hr after intravenous injection for different preparations of 99m-Tc-PYP and the relative amount of 99m-Tc-species.

99m-Tc-PYP	% I.D.	
Preparation	Urine	Faeses
Complex I	24.97±2.5	1.03±0.40
Complex II	22.50±2.63	1.23±0.34

Relative amount of 99m-Tc-species in urine as obtained from GCS.

99m-Tc-PYP in urine	% activity coresponding to:		
	red.hyd.99m-Tc	99m-TcO_4^-	99m-Tc-PYP
Complex I	50.6±2.9	6.20±0.40	43.00±3.10
Complex II	0.48±1.0	5.23±1.55	94.75±1.55

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