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Structural consideration of divalent ion complexes of BPHA for fast renal excretion of ^{99m}Tc-BPHA

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Summary

Protonaton constants of BPHA (N,N′-bis(2-aminoethyl)propane hexaacetic acid) and M(II)-BPHA have been determined. ^{1}H NMR study shows that monoprotonized Zn^{2+}-BPHA at 6.0<pH<9.0 has no uncoordinated amino group, while diprotonized Zn^{2+}-BPHA at pH<6.0 has a N-protonized free imino-diacetate group. By analogy between Zn^{2+}-BPHA and Sn^{2+}-BPHA, it is deduced that Sn^{2+}-BPHA at pH<6 should also have a free iminodiacetate group, but at neutral condition the amine group in the iminodiacetate become coordinated. Different states of the reductant Sn^{2+}-BPHA might influence the structure of ^{99m}Tc-BPHA. Indeed, in contrast to ^{99m}Tc-BPHA formed at pH=4.0, which has a high liver uptake, ^{99m}Tc-BPHA formed at neutral condition has a fast renal excretion similar to ^{99m}Tc-DTPA prepared at pH=6.5.

Key woras: polyaminopolycarboxylic acid, protonation constants, ^{1}H NMR spectra, radiolabelling, technetium, biodistribution.

Introduction

In our previous paper [1], it had been reported that ^{99m}Tc-BPHA prepared at pH=4 had a high liver uptake, although it also highly accumulated in kidneys. The kidney uptake curve versus time was far from that of the typical reno-functional imaging agent ^{99m}Tc-DTPA. We believe that structural factors should be responsible

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for the difference. To clarify the structural influence is not an easy task because the structure of ^{99m}Tc-DTPA in solution is so far not known, even the oxidation state of the central ion is controversial in literature [2]. To elucidate the structures and oxidation states of technetium complexes with amino-carboxylic acids, attempts had been made on macroscopic level, but the formation of primary dimers in +3, +4, and/or +5 oxidation state containing Tc-Tc bond make the system more complicated [3-6]. At tracer level, the concentration of $^{99m}TcO_4^-$ might be too low to form dimers [7]. It is believed that the oxidation state of ^{99m}Tc in ^{99m}Tc-DTPA is lower than +5 based on the observation of Hwang L L-Y et al and Volkert W A et al[8,9].

For little knowledge about the coordination properties of BPHA is acquired, the free BPHA and its complexes of common divalent transition metal ions will be examined first, in a hope of getting some clue about the preparation conditions for ^{99m}Tc-BPHA. We believe the structure of Sn(II)-BPHA will be the same as those of divalent transition metal complexes. The structural difference of Sn(II)-BPHA might affect the formation of ^{99m}Tc-BPHA or its structure, because of the changed reducing ability of Sn^{2+} and coordination availability of BPHA for ^{99m}Tc. The biological behavior of ^{99m}Tc-BPHA will be examined secondly. During the preparation of this manuscript we noticed Verbreggen group [10]are studying another similar ligand TTHA (triethylene tetraamine hexaacetic acid), a fast renal excretion is also reported for ^{99m}Tc-TTHA formed at neutral condition.

Experimental

Materials

Because of slowly losing HCl and absorbing moisture, BPHA·4HCl synthesized two years before had at this time a formula of BPHA·3.60HCl· 3.58H_2O assigned by titration and elemental analysis. The metal salts ($ZnSO_4·7H_2O$, $CuSO_4·5H_2O$, $NiCl_2·6H_2O$, and $CoCl_2·6H_2O$) used in potentiometric measurements were regent grade and standardized before use. The ^{99m}Tc eluate was from ^{99}Mo/^{99m}Tc generator. The determination of radioactivity was fulfilled with a γ ray well-detector.

Potentiometric measurements

The protonation constants of BPHA and its complexes of Co^{2+}, Ni^{2+}, Cu^{2+}, and Zn^{2+} were determined potentiometrically in aqueous solution at 25±0.5°C and ion strength 0.1 ($NaClO_4$). For determination of the protonation constants of BPHA, the concentration of BPHA and the titrant NaOH were 1.000 mmol/l and 0.100 mol/l respectively. For the complexes, equimolar M^{2+} was added before the titration began.

^{1}H NMR spectra determination at different pH

^{1}H NMR spectra were obtained on a Varian 200 nuclear magnetic resonance spectrometer. To obtain the ^{1}H NMR spectra of BPHA, 981 mg BPHA·3.60HCl ·3.58H_2O was dissolved in 15 mL D_2O(>99.6%), then NaOH powder or concentrated D_2O solution was used to adjust the solution to different pD values. At each desired pD, about 1 mL sample solution was transferred to a sample tube, followed by a drop of 0.1 mol/L DSS D_2O solution. For preparation of Zn(II)-BPHA solution, the same procedure was employed except equimolar $ZnSO_4$·7H_2O was added before pD adjustment.

Biodistribution study of ^{99m}Tc-BPHA prepared at pH=7

113 mg BPHA·3.60HCl·3.58H_2O, 2.25 mg $SnCl_2$·2H_2O were dissolved in 20 mL water, and pH was adjust to 7.5. After filtration through a 0.22 μm Millipore filter, the solution was divided in to 20 vials and lyophilized. 2 mL $^{99m}TcO_4^-$ eluate was added to a lyophilized vial. The labeling efficiency was 98%.

20 Cun-ming small mice (18-22 g) were divided in to 5 groups, Each mouse was injected 0.85 MBq ^{99m}Tc-BPHA in the tail vein. The five groups were sacrificed by exsanguination at 2, 5, 10, 30, 60 min respectively and dissected for blood, liver, kidneys, spleen, lungs, and intestines (containing chyme). The wet organs were weighed (except the intestines) and the activities were determined.

To detect renal excretion, another two groups of four animals, after tail vein administration of 0.85 MBq ^{99m}Tc-BPHA, were sacrificed by dislocation at 10 and 30 min respectively. The urinary bladder, ureters, kidneys, and tail were discarded, and the radioactivity in the remaining body was determined.

To compare the biodistribution of ^{99m}Tc-BPHA and ^{99m}Tc-DTPA, a commercial DTPA kit was used to repeat the same procedure for ^{99m}Tc-BPHA. The kit contained 20 mg DTPA and 1.10 mg $SnCl_2 \cdot 2H_2O$, after reconstitution pH=6.5. The labeling efficiency was 99%.

Results and Discussion

Protonation constants of BPHA and M(II)-BPHA

From the titration curve, six protonation constants were obtained by non-liner regression calculation. The protonation constants of BPHA are listed and compared with those of TTHA in Table 1.

Table 1. Comparison of protonation constants of BPHA and TTHA

	$logk_6$	$logk_5$	$logk_4$	$logk_3$	$logk_2$	$logk_1$	Ref.
BPHA	2.22	3.30	4.82	6.45	9.76	11.07	this paper
TTHA	2.42	2.95	4.16	6.16	9.40	10.19	11

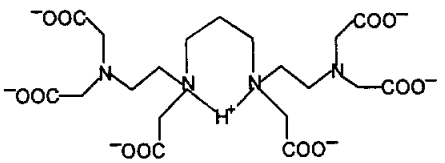


Fig1 The possible six-membered ring of mono-protonized BPHA

The protonation properties of BPHA and TTHA are very similar. Larger differences exist in $logk_1$ and $logk_4$. Because of the stronger basicity of the amine group than the carboxylate group, the protons will, though not completely, first go to the amine sites. The difference in $logk_1$ might be due to the stability of six-membered ring formed by the intra-molecular hydrogen bond (See Fig 1). Larger $logk_4$ value for BPHA might be due to the smaller repulsion force between the positive charges resulting from the incorporation of a propylene group, instead of an ethylene group, into the middle of the molecule.

Non-linear regression treatment with the titration curve of complex M(II)-BPHA can not give out an unique set of stability constants. As long as the initial stability constants are big enough, a good agreement can be reached between the calculated and the experimental curves, but the ratio of the constants are not changed. Because ten coordination sites are available in BPHA and can not be used up in the complex, the complex is just like a polyprotic acid if the complex is stable enough. The titration curve is mainly determined by protonation property. The ratios of the stability constants are the protonation constants. Two protonation constants are obtained as shown in table 2.

Table 2. The protonation constants of M(II)-BPHA

Zn^{2+}-BPHA		Cu^{2+}-BPHA		Ni^{2+}-BPHA		Co^{2+}-BPHA	
$logk_1$	$logk_2$	$logk_1$	$logk_2$	$logk_1$	$logk_2$	$logk_1$	$logk_2$
9.1	5.8	8.8	5.9	9.1	5.5	9.0	5.9

$$MBPHA^{4-} + H^+ = MHBPHA^{3-} \quad k_1=K_1/K_0$$

$$MHBPHA^{3-} + H^+ = MH_2BPHA^{2-} \quad k_2= K_2/K_1$$

$$MH_2BPHA^{4-} + H^+ = MH_3BPHA^{1-} \quad k_3= K_3/K_2$$

$$MH_3BPHA^{-} + H^+ = MH_4BPHA^{3-} \quad k_4= K_4/K_3$$

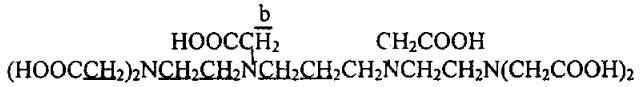
$$M^{2+} + BPHA^{6-} + iH^+ = MH_iBPHA^{(4-i)-} \quad K_i$$

^{1}H NMR spectra of BPHA and Zn(II)-BPHA

There are six peaks in the spectra of BPHA, a high sharp single peak *a* , a sharp single peak *b* with half height of peak *a*, two partially overlapped hump peaks c_1 and c_2, a triple low peak *d*, and a low hump peak *e*, as shown in table 3. The chemical shift of each peak goes to the upfield when pD value is raised. At two pD values, *a* and *b* as well as c_1 and c_2 are superposed into one peak. The chemical shift of *a* is smaller than that of *b* in the pD range < 3.0 and >7.0, but bigger in the range 3.0-7.0.

This particular interchanging property is only for BPHA in contrast to other polyaminopolycarboxylic acids. The same phenomenon is observed for the two methyl groups in the ethylene group, so c_1 and c_2 can be assigned.

Table 3. The chemical shifts of various protons in BPHA at different pD versus DSS



pD	0.80	2.02	3.09	3.99	5.15	6.06	7.06	8.01	8.95	10.07	10.93	12.40
δ_a	4.037	3.879	3.909	3.904	3.826	3.688	3.548	3.510	3.429	3.224	3.130	3.096
δ_b	4.293	3.981	3.909	3.881	3.708	3.608	3.548	3.548	3.505	3.338	3.167	3.096
δ_{c_1}	3.615	3.534	3.652	3.654	3.527	3.346	3.178	3.140	3.055	2.794	2.632	2.562
δ_{c_2}	3.692	3.555	3.652	3.654	3.454	3.297	3.178	3.140	3.055	2.817	2.632	2.562
δ_d	3.506	3.444	3.377	3.343	3.203	3.121	3.080	3.067	3.010	2.780	2.564	2.463
δ_e	2.355	2.264	2.252	2.214	2.067	1.990	1.956	1.948	1.917	1.795	1.680	1.630

To understand the structure of M(II)-BPHA, diamagnetic divalent ion Zn^{2+} of full *d* electrons is chosen as M(II). The spectra of Zn^{2+}-BPHA are very complicated in comparison with those of free ligand BPHA. Four types of spectra were observed. (1) pD<3.0, hump-like, broadened spectra. (2) 3.0≤pD<6, there is a sharp peak, corresponding to four protons. A typical spectrum at pD=5.05 is shown in Fig 2a. Because the sharp peak is of the biggest δ value, it can be definitely assigned to the methyl group in a carboxymethyl group. The δ value 3.85 ppm is almost the same as those of the amino-protonized nitrilotriacetate(3.83 ppm)[12] and the uncoordinated iminodiacetate moiety in $H[Co(H_2DTPA)NO_2]$(3.83 ppm)[13], showing it is a pendant N-protonized iminodiacetate group. (3) 6≤pD≤9, the absence of sharp single peak indicates that the iminodiacetate moiety has joined the coordination system. A typical spectra at pD=7.97 is shown in Fig 2b. (4) pD>9, a sharp single peak appears again, its δ value is almost unchanged with the raising pD from 10.32 (3.15 ppm) to 11.50 (3.14 ppm). It is close to, but a little bigger than, the δ value of unprotonized

iminodiacetate group (3.10 ppm in NTA, EDTA, TTHA, and BPHA). A typical spectra at pD=11.05 is shown in Fig 2c.

The abrupt pattern change from pD=5.0 to pD=6.0 and that from pD=9.0 to pD=10.3 are consistent to the logarithms of the protonation constants, $logk_2$=5.8 and $log\ k_1$=9.0. Because the ^{99m}Tc-BPHA was prepared at pH=4.0 in the previous paper and the pH of blood is 7.4, what we are interested in is the pattern change from pD=5.0 to pD=6.0, from the structure of diprotonized form to monoprotonized form. If the coordination system is assumed an octahedron the structural change can be expressed as the scheme in Fig 3.

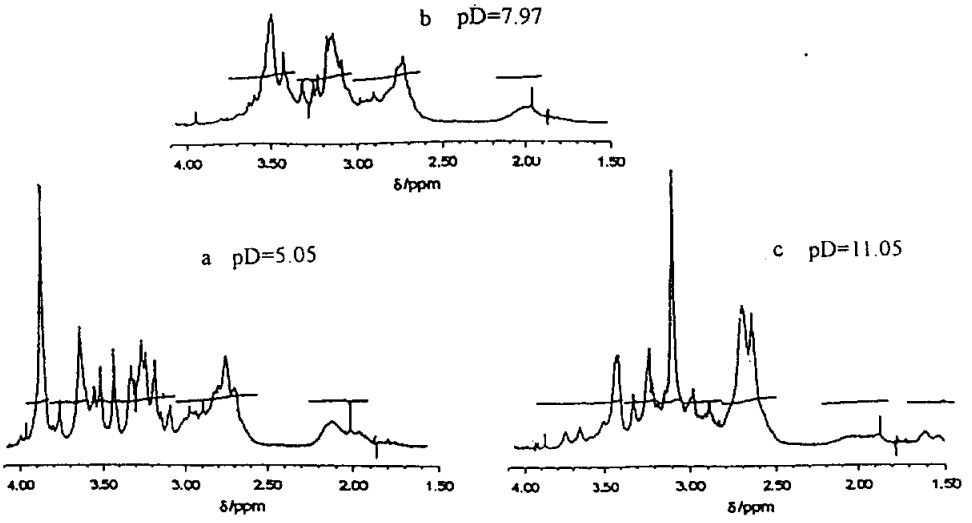


Fig 2 The ^{1}H NMR spectra of Zn^{2+}-BPHA at different pD

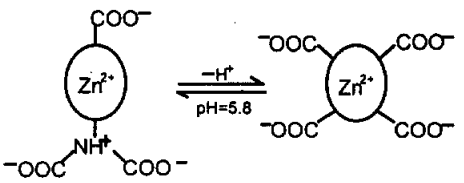
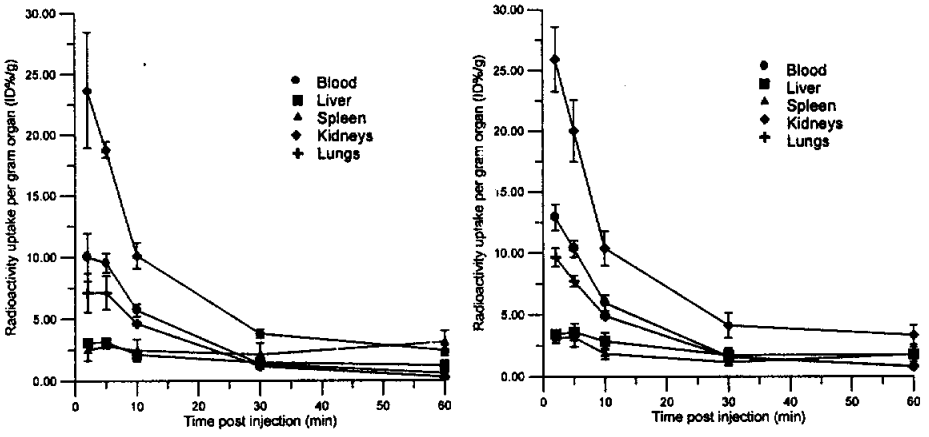


Fig 3. The structural change at pH=5.8

Biodistribution results of ^{99m}Tc-BPHA and ^{99m}Tc-DTPA

For the structure of Zn^{2+}-BPHA at pH=4.0 is different from that at 6.0<pH<9.0, the same case may occur for Sn^{2+}-BPHA, and may further influence the structure of ^{99m}Tc-BPHA. The biodistribution results of ^{99m}Tc-BPHA (pH=7.6) and ^{99m}Tc-DTPA are almost the same as shown in Table 4, Table 5, and Fig 4. The biodistribution of ^{99m}Tc-BPHA (pH=7.6) is really quite different from that of ^{99m}Tc-BPHA (pH=4.0)[1]. The blood radioactivity and the radioactivity of other organs listed in Table 4 and 5 for ^{99m}Tc-BPHA are slightly higher than those for ^{99m}Tc-DTPA. These results might result from the higher hydrophilicity of ^{99m}Tc-BPHA.



(a) ^{99m}Tc-DTPA (b) ^{99m}Tc-BPHA

Fig 4. The change of radioactivity uptake per gram organ versus time (n=4, $\bar{x}\pm$sd)

To accurately compare the urinary excretion of ^{99m}Tc-BPHA and ^{99m}Tc-DTPA, the radioactivity in the remaining body apart from the urinary bladder, ureters, kidneys, and the tail is determined at 10 and 30 min. The tail is discarded to get rid of any radioactivity left at the administration site. The urinary system is not included because we believe that the radioactivity in it will forever leave the circulation system of the body. To exclude the possible urine contamination on the skin, a piece of it on the back is cut off , weighed, and the activity is determined. Other part of the skin is weighed and the radioactivity can be calculated by assuming the same uptake

as the sample skin. The radioactivity of the remaining body (including skin) and the skin activity uptake are shown in Table 6.

Although no significant differences of radioactivity uptakes are there in the blood, liver, kidneys, spleen, lungs, and intestines, the skin radioactivity is lower and the radioactivity excretion rate is considerable faster for ^{99m}Tc-BPHA than for ^{99m}Tc-DTPA. These results might be also related to the stronger hydrophilicity of ^{99m}Tc-BPHA. There is a possible explanation for the different biological behaviors at pH=4.0 and at pH=7.6. At pH=4.0, because there is a free iminodiacetate group in the Sn^{2+}-BPHA molecule, it is similar to HIDA molecule as shown in Fig 5. When $^{99m}TcO_4^-$ is reduced, some similar ^{99m}Tc complex to ^{99m}Tc-HIDA may be formed, so high liver radioactivity is observed.

Table 4. Biodistribution of ^{99m}Tc-DTPA in mice (ID%)*

Organs \ Time	2min	5min	10min	30min	60min
Blood	13.97 ±2.70	13.33 ±1.10	7.98 ±0.71	1.88 ±0.21	0.83 ±0.14
Liver	3.00 ±0.61	3.00 ±0.07	1.80 ±0.18	1.44 ±0.34	1.21 ±0.44
Kidneys	5.79 ±1.54	4.32 ±0.18	2.53 ±0.30	0.93 ±0.10	0.54 ±0.04
Spleen	0.19 ±0.03	0.20 ±0.01	0.15 ±0.04	0.16 ±0.06	0.19 ±0.03
Lung	1.39 ±0.36	1.30 ±0.27	1.07 ±0.32	0.30 ±0.07	0.09 ±0.03
Intestines	3.02 ±0.73	3.69 ±0.44	2.18 ±0.29	0.88 ±0.18	0.66 ±0.05

*Mean ±sd of four animals.

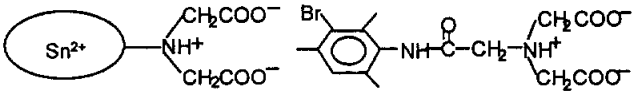
Table 5. Biodistribution of ^{99m}Tc-BPHA in mice (ID%)*

Organs \ Time	2min	5min	10min	30min	60min
Blood	18.06 ±1.51	14.46 ±0.95	8.33 ±0.91	2.25 ±0.98	1.09 ±0.15
Liver	3.67 ±0.27	3.94 ±0.78	2.67 ±0.51	1.76 ±0.50	1.94 ±0.68
Kidneys	6.14 ±0.56	4.46 ±0.31	2.49 ±0.36	1.00 ±0.20	0.79 ±0.16
Spleen	0.23 ±0.05	0.26 ±0.06	0.12 ±0.03	0.06 ±0.01	0.14 ±0.08
Lung	1.54 ±0.06	1.31 ±0.16	0.97 ±0.18	0.25 ±0.11	0.13 ±0.02
Intestines	5.04 ±0.67	4.51 ±0.37	3.26 ±0.64	1.12 ±0.30	0.91 ±0.17

* Mean ±S. D. of four animals.

Table 6. The activity in remaining body and the skin activity uptake

	Activity in remain body (ID%)		Activity in per gram skin (ID%/g)	
Time/min	10	30	10	30
^{99m}Tc-DTPA	38.09±4.20	13.87±1.65	3.70±0.65	1.17±0.25
^{99m}Tc-BPHA	31.71±2.80	8.71±0.39	2.79±0.33	0.53±0.04



Sn(II)-BPHA HIDA(Mebrofenin)

Fig 5. Similarity between Sn(II)-BPHA and HIDA ligand

Conclusion

The protonation constants of free BPHA and complexes M(II)-BPHA are determined. The ^{1}H NMR spectra shows that the diprotonized Zn^{2+}-BPHA exists at pH<5.8 and has a free iminodiacetate group, while the four amino groups in the monoprotonized form of Zn^{2+}-BPHA at 6.0<pH<9.0 are all coordinated to the metal ion. By assuming the structure of Sn^{2+}-BPHA is similar to that of Zn^{2+}-BPHA, it is deduced that different states of Sn^{2+}-BPHA might result in different ^{99m}Tc-BPHA. The biodistribution of ^{99m}Tc-BPHA made at pH=7.6 are really much different from that at pH=4, but very similar to that of the commercial ^{99m}Tc-DTPA.

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