KINETIC STUDY OF LIGAND EXCHANGE REACTION BETWEEN ^{99m}Tc—GH AND ECD

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(Received September 1991)

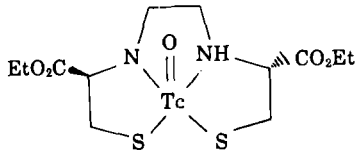
ABSTRACT

In order to explore this type of ^{99m}Tc–GH and N_2S_2 ligands exchange reaction as a common method for the preparation of ^{99m}Tc–N_2S_2 complexes, detailed kinetic study of ligand exchange reaction between ^{99m}Tc–GH and ECD was carried out. This paper presents preliminary results from the study at different ligand concentrations and pH values. The ligand exchange reaction is a second order reaction. Its rate constant being pH dependent were determined as: $k_{pH2}=1.11\times10^4$ l · mol^{-1} · min^{-1}, $k_{pH5}=1.34\times10^4$ l · mol^{-1} · min^{-1}, $k_{pH6}=2.24\times10^4$ l · mol^{-1} · min^{-1}.

Keywords: ^{99m}Tc–Glucoheptonate ^{99m}Tc–ECD Ligand exchange kinetics

1 INTRODUCTION

Recently a series of new technetium chelating agents categorized as N_2S_2 coordinating ligands (bis–aminoethanethiol BAT) have been reported.[1,2] They were confirmed to form neutral lipid–soluble complexes with a TcO(Ⅲ) center core. ^{99m}Tc–ethyl cysteinate dimer (ECD) is one of ^{99m}Tc–N_2S_2 complexes, which can easily passing through the intact blood brain barrier, and by preliminary animal and clinical studies showed to be a valuable regional brain prefusion imaging agent for single photo emission computed tomography (SPECT). The preparation of the ^{99m}Tc–ECD is generally achieved by a simple ligand exchange reaction between ^{99m}Tc–glucoheptonate (GH) and ECD. Due to the fact that ^{99m}Tc–ECD is more stable than the ^{99m}Tc–GH, the exchange reaction proceeds rapidly and gives excellent radiochemical purity and high yield. The chemical structure of ^{99m}Tc–ECD has been characterized by Scheme 1.[3]



Scheme 1

In order to explore this type of ^{99m}Tc–GH and N_2S_2 ligands exchange reaction, detailed kinetic study of ligand exchange reaction between ^{99m}Tc–GH and ECD was

evaluated. This paper presents preliminary results from the study at different ligand concentrations and pH values.

2 MATERIALS AND METHODS

2.1 Materials

Ethyl cysteinate dimer and sodium glucoheptonate were prepared by ourself[4,5], the other chemical agents were of AR grade. Silica gel paper, ITLC silica gel, Xinhua No.1 paper were commercial.

2.2 The radiochemical purity of ^{99m}Tc–GH was determined by thin layer chromatography

ITLC-SG plates, with developing system of 1:1 (v/v) Me_2CO/NaCl (1mol/l), $R_f = 1.0$ for ^{99m}Tc–GH, $^{99m}TcO_4^-$; $R_f = 0.0$ for $^{99m}TcO_2 \cdot xH_2O$. Silica gel paper, with developing system Me_2CO, $R_f = 1.0$ for $^{99m}TcO_4^-$; $R_f = 0.0$ for ^{99m}Tc–GH, $^{99m}TcO_2 \cdot xH_2O$. Both methods gave the radiochemical purity of ^{99m}Tc–GH over 98%. All the TLC samples were counted with a Packard Gamma Counter.

2.3 Separation of ^{99m}Tc–GH and ^{99m}Tc–ECD by TLC

In order to establish a simple and rapid method for the determination of ^{99m}Tc–GH and ^{99m}Tc–ECD, Xinhua No.1 paper, developed by $MeOH/CHCl_3$ (1:9, v/v) was used. The developed paper were cut into 10 fractions and counted in a γ counter. Fraction No.1 was from the origin and fraction No.10 was from the solvent front. Using this system, the R_f value for ^{99m}Tc–GH, $^{99m}TcO_4^-$ and $^{99m}TcO_2 \cdot xH_2O$ are 0.0, while the ^{99m}Tc–ECD shows an R_f value of 1.0. A typical distribution of radioactivity at different TLC fraction is shown in Fig.1.

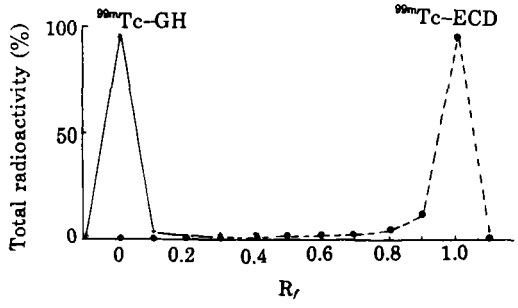


Fig.1 Distribution of radioactivity for ^{99m}Tc–GH and ^{99m}Tc–ECD on Xinhua No.1 paper TLC plate

2.4 Ligand exchange reaction

a. Ligand exchange reactions between ^{99m}Tc–GH (1 drop) and ECD were performed (pH 6.0, 15°C) at different final ligand concentrations. The pH of the solution of ECD were adjusted to 6.0 by adding 0.1 mol/l sodium hydroxide solution. At different time intervals, samples were removed and analyzed by the TLC method described above.

b. Ligand exchange reactions between ^{99m}Tc–GH (1 drop) and ECD (final ligand concentration, 6.29×10^{-5} mol/l) were performed at different pH values. Then proceeded the way similar to a.

3 RESULTS AND DISCUSSIONS

3.1 Determination of rate constant of the ligand exchange reaction between ^{99m}Tc–GH

and ECD

This reaction seems likely to be a second order reaction. Therefore it can be expressed as follows.

$$^{99m}\text{Tc-GH} + \text{ECD} \underset{}{\overset{k}{\rightleftharpoons}} {}^{99m}\text{Tc-ECD} + \text{GH}$$

$t=0$	a	b	0
$t=t$	$a-x$	$b-x$	x

where k is rate constant of formation for ^{99m}Tc-ECD. Assuming the reaction is a second order reaction:

$$dx/dt = k\,(a-x)\,(b-x) \tag{1}$$

Converting Eq. (1) to an other form

$$k \cdot (a-b) \cdot dt = [d(a-x)/(a-x)] - [d(b-x)/(b-x)] \tag{2}$$

Then the integrating of Eq. (2) between $t=0$, $x=0$ and $t=t$, $x=x$ gives

$$k \cdot t \cdot (a-b) = \ln[b(a-x)/a(b-x)] \tag{3}$$

Where $a=[^{99m}\text{Tc-GH}]_0$, $b=[\text{ECD}]_0$, $x=[^{99m}\text{Tc-ECD}]_t$. Since $a \approx x = 10^{-9} \sim 10^{-8}$ mol/l, $b = 10^{-5} \sim^{-4}$ mol/l, so $b \gg a \approx x$. Eq. (3) becomes

$$k \cdot b \cdot t = \ln\,[a/(a-x)] \tag{4}$$

$$a/(a-x) = 1/(1-x/a) = 1/(1-P),\ P = x/a$$

where P is the labelling yield of ^{99m}Tc-ECD, it can be determined by TLC.

$$k \cdot b \cdot t = \ln[1/(1-P)] \tag{5}$$

Plot $\ln[1/(1-P)]$ vs t shows a linear relationship. And the rate constant k for the formation of ^{99m}Tc-ECD can be determined from the slope.

Fig.2 shows that at three different ligand concentrations the exchange reaction is a second order reaction as expected. The rate constant k for the formation of ^{99m}Tc-ECD can be calculated:

7.54×10^{-5} mol/l: $k = 1.675/7.54\times10^{-5} = 2.22\times10^{4}$ l · mol^{-1} · min^{-1},

5.03×10^{-5} mol/l: $k = 1.135/5.03\times10^{-5} = 2.26\times10^{4}$ l · mol^{-1} · min^{-1},

2.51×10^{-5} mol/l: $k = 0.610/2.51\times10^{-5} = 2.42\times10^{4}$ l · mol^{-1} · min^{-1},

$k_{avg} = 2.30\times10^{4}$ l · mol^{-1} · min^{-1}

The slopes of the three experiments were different, however, the formation constants k measured were the same.

Fig.3 shows the ligand exchange reactions at different pH values (ECD 6.29×10^{-5} mol/l). $\ln[1/(1-P)]$ vs time also shows a linear relationship. This was the further evidence supporting the validity of assumptions on the kinetics of this exchange reaction. The rate constant k for the formation of ^{99m}Tc-ECD can be calculated as

follows: $k_{pH2}=0.701/6.29\times10^{-5}=1.11\times10^{4}$ l · mol^{-1} · min^{-1}, $k_{pH5}=0.841/6.29\times10^{-5}=1.34\times10^{4}$ l · mol^{-1} · min^{-1}, $k_{pH6}=1.405/6.29\times10^{-5}=2.24\times10^{4}$ l · mol^{-1} · min^{-1}.

Keeping concentration of ECD (6.29×10^{-5} mol/l) the same at different pH values different slopes appeared, and these different k were obtained. It showed that the rate constant of the ligand exchange reaction is pH dependent.

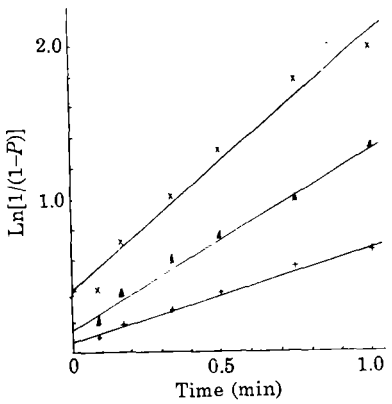


Fig.2 Exchange reaction at different ligand concentrations (pH 6.0, 15°C)

× $y=1.675x+0.405$, $r=0.985$, 7.54×10^{-5} mol/l
▲ $y=1.135x+0.169$, $r=0.996$, 5.03×10^{-5} mol/l
\+ $y=0.610x-0.067$, $r=0.997$, 2.51×10^{-5} mol/l

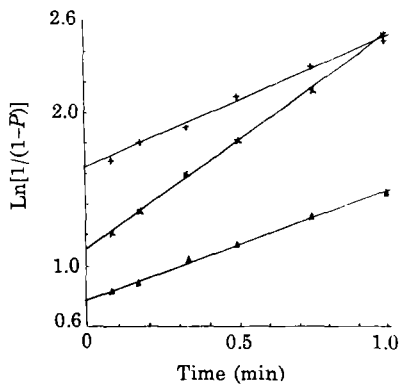


Fig.3 Exchange reaction at different pH values (ECD 6.29×10^{-5} mol/l)

▲ pH2: $y=0.701x+0.779$, $r=0.999$
\+ pH5: $y=0.841x+1.637$, $r=0.999$
× pH6: $y=1.405x+1.103$, $r=0.991$

3.2 Conclusion

The ligand exchange reaction between ^{99m}Tc-GH and a stronger N_2S_2 ligand-ECD is a reaction of second order. The rate constant of this ligand exchange reaction at different pH values were determined: $k_{pH2}=1.11\times10^{4}$ l · mol^{-1} · min^{-1}, $k_{pH5}=1.34\times10^{4}$ l · mol^{-1} · min^{-1}, $k_{pH6}=2.24\times10^{4}$ l · mol^{-1} · min^{-1}.

ACKNOWLEDGEMENTS

The authors extend thanks to Mr Pan Shangren, Mr Xu Xijie and Mr Qian Yuxin for their kind helps.

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