Nuclear Science and Techniques **22** (2011) 174–177

Study on the reaction kinetics of $^{99}Tc^{m}$-labeled BIDP

LIN Jianguo WANG Yan LUO Shineng* QIU Ling ZHAI Haozhen

Key Laboratory of Nuclear Medicine, Ministry of Health, Jiangsu Key Laboratory of Molecular Nuclear Medicine, Jiangsu Institute of Nuclear Medicine, Wuxi 214063, China

Abstract A novel zoledronic acid derivative, 1-hydroxy-2-(2-butyl-1H-imidazole-1-yl)-ethylidene-1,1-diphosphonic acid (BIDP), was synthesized and labeled with $^{99}Tc^{m}$. The detailed kinetic study on the labeling reaction between BIDP and $^{99}Tc^{m}$ was carried out. The results indicated that the reaction rate constants k were 0.0258, 0.0268, 0.0305, 0.0323, 0.0351 and 0.0384 min^{-1} at 0°C, 5°C, 10°C, 15°C, 20°C and 25°C, respectively. From the Arrhenius equation $k=A\cdot e^{-E_a/(RT)}$, the activation energy E_a of the labeling reaction was calculated to be 10.45 kJ/mol. And the correlation between k and temperature (T) was also deduced as $\ln k=-1258.8\times(1/T)+0.9531$. In addition, it was found that in order to get a high radiolabeling yield (RLY) (> 90%), the reaction temperature must be up to 12°C.

Key words $^{99}Tc^{m}$-BIDP, Labeling reaction kinetics, Reaction rate constant k, Reaction activation energy E_a, Radiolabeling yield (RLY)

1 Introduction

Diphosphonates are useful in treating many disorders, such as metabolic bone disease, Paget's disease, and osteoporosis[1-3]. They are also used as ligands of radiopharmaceuticals for nuclear medicine imaging procedures, and new applications of these drugs are still emerging[4]. On the other hand, owing to the ideal physical properties optimal for diagnostic imaging, $^{99}Tc^{m}$ is the most commonly used radioisotope for diagnostic applications in the nuclear medicine[5]. At present, $^{99}Tc^{m}$-labeled phosphates and phosphonates are becomeing the major radiopharmaceuticals for bone-imaging, and $^{99}Tc^{m}$-methylenediphosphonate ($^{99}Tc^{m}$-MDP) is of the widest clinical applications[6].

Zoledronate[2-(imidazol-1-yl)-hydroxy-ethylid ene-1,1-bisphosphonic acid, ZL], one of the typical third-generation bisphosphonates, is the most potent of the clinically tested compound[7]. It has been labeled with $^{99}Tc^{m}$ in a high yield for SPECT(single photon emission CT) imaging of rabbit bone[8,9]. And a series of $^{99}Tc^{m}$-labeled ZL derivatives have been reported as bone imaging agents[10-15]. To the authors' knowledge, however, no study on reaction kinetics of $^{99}Tc^{m}$ labeling bisphosphonates has been reported up to date, though the kinetic studies on other $^{99}Tc^{m}$-labeled radiopharmaceuticals have been evaluated[16−19]. For example, in exploring the exchange reaction as a common method for preparing $^{99}Tc^{m}$ complexes, the ligand exchange reaction between $^{99}Tc^{m}$-GH (glucoheptonate) and N_2S_2 (BAT) ligands was investigated by Kung *et al*[16]. The results suggested nessessity of studying kinetics and thermodynamics for the radiopharmaceuticals.

In this work, in order to find factors influencing the radiolabeling efficiency and provide a theoretical basis of this kind of complexes for clinical application, kinetics of the labeling reaction between $^{99}Tc^{m}$ and BIDP were studied.

2 Experimental

2.1 Materials and Instruments

BIDP was prepared according to the method reported by Widler *et al*[7]. All analytical chemical reagents employed were purchased from commercial sources and used without further purification. $Na^{99}Tc^{m}O_4$ was

Supported by National Natural Science Foundation of China (Nos. 20801024 and 21001055), Natural Science Foundation of Jiangsu Province (No.BK2009077), Science Foundation of Health Department of Jiangsu Province (No.H200963)

* Corresponding author. *E-mail address:* shineng914@yahoo.com.cn

Received date: 2010-11-23

supplied by Jiangsu Institute of Nuclear Medicine. Packard-multi-prias γ counter was used.

2.2 Preparation of $^{99}Tc^{m}$-BIDP

$^{99}Tc^{m}$-BIDP was prepared according to the following reaction (Fig.1).

At different temperatures, from 0°C to 25°C, 100 μL BIDP aqueous solution (containing 5 mg BIDP), 100 μL freshly prepared solution of stannous chloride dehydrate (containing 100 μg $SnCl_2{\cdot}2H_2O$) and 1.8 mL 0.2 $mol{\cdot}L^{-1}$ phosphate buffered solution (PBS) (pH=6) were added into cylindriod vials and vortexed adequately. Then, 37.0 MBq freshly eluated $Na^{99}Tc^{m}O_4$ was added.

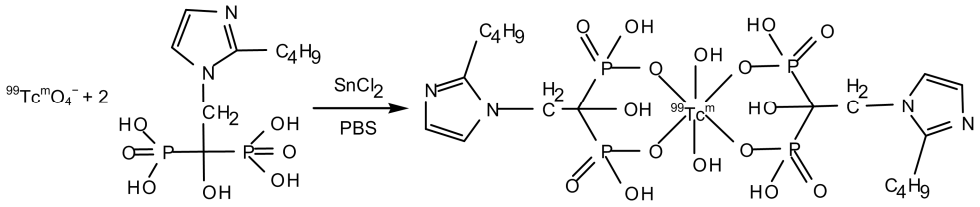

Fig.1 Synthesis of $^{99}Tc^{m}$-BIDP.

2.3 Determination of RLY

The radiolabeling yield (RLY) of $^{99}Tc^{m}$-BIDP was determined by paper chromatography at different reaction time from 5 s to 30 min, with developing systems of (1) purified water and (2) acetone. In the first system, $^{99}Tc^{m}$-colloidal impurities remain at the origin of the paper strip, while free pertechnetate and $^{99}Tc^{m}$-BIDP both migrate with the solve front. In the second system, the $^{99}Tc^{m}$-colloidal impurities and $^{99}Tc^{m}$-BIDP remain at the origin of the paper strip, while free pertechnetate moves with the solve front.

2.4 Deduction of the reaction rate constant

The radiolabeling reaction can be expressed as follows:

$$^{99}Tc^{m}O_4^{-} + 2BIDP \underset{SnCl_2}{\overset{k}{\rightleftharpoons}} {}^{99}Tc^{m}(BIDP)_2(OH)_2$$

t=0	a	b	0
t=t	a-x	b-2x	x

Assuming the reaction is a second order reaction[16]:

$$dx/dt=k(a–x)(b–2x) \quad (1)$$

where a=[$^{99}Tc^{m}O_4^{-}$], b=[BIDP], x=[$^{99}Tc^{m}(BIDP)_2(OH)_2$] and k is rate constant of the reaction.

To calculate the integral of Eq.(1), Eq.(2) can be obtained,

$$\ln[(a/(a–x)\ ] + \ln[(b–2x)/b] = k\ (b–2x)t \quad (2)$$

Since $a \approx x \approx 10^{-8}$–$10^{-9}$ $mol{\cdot}L^{-1}$ and $b \approx 10^{-3}$–10^{-5} $mol{\cdot}L^{-1}$, Eq.(2) can be simplified as

$$\ln[1–(x/a)]^{-1}=k{\cdot}b{\cdot}t \quad (3)$$

where, $x/a=RLY$. Then, k can be calculated through the least square multivariate linear regression between $\ln[1–RLY]^{-1}$ and reaction time (t). And the conclusion can be obtained from Eq.(3) that the labeling reaction between BIDP and $^{99}Tc^{m}O_4^{-}$ was a first order reaction.

2.5 Calculation of the reaction activation energy

The activation energy E_a of the radiolabeling reaction was calculated according to the relationship between k and temperature, i.e., Arrhenius equation.

$$k=A{\cdot}e^{-E_a/(RT)} \quad (4)$$

By logarithmic transformation of Eq.(4), one gets

$$\ln k=-E_a/(RT)+\ln A \quad (5)$$

where E_a, R, T and A are the reaction activation energy, the gas constant, the absolute temperature and the frequency factor, respectively.

3 Results and discussion

Since the freshly prepared complexes were used without further purification, the radiochemical purity (RCP) was identical with the radio labeling yield (*RLY*). This meets clinical requirements for other diphosphonates such as $^{99}Tc^{m}$-MDP[20]. The *RLY* determined for the labeling reactions at different temperature systems are shown in Fig.2(a). One can see that the labeling reaction between BIDP and $^{99}Tc^{m}O_4^-$ is rapid and it reaches the equilibrium in 30 s when the temperature of the reaction system is over 5°C. Moreover, the reaction temperature has significant influence on the *RLY* of the target complex. Obviously, the *RLY* increases monotonically with increasing temperature and it will be larger than 90% and 93% in the system of 15°C and 25°C at 30 s, respectively. At room temperature, the high RLY can be easily obtained in less than 30 s. Compared with other $^{99}Tc^{m}$-labeled complexes, $^{99}Tc^{m}$-BIDP is more convenient to get high *RLY* for the clinical application. Taking $^{99}Tc^{m}$-MIBI as example, the reaction time is 3 min in the boiling water[18], while that for $^{99}Tc^{m}$-BIDP is only a few seconds at room temperature.

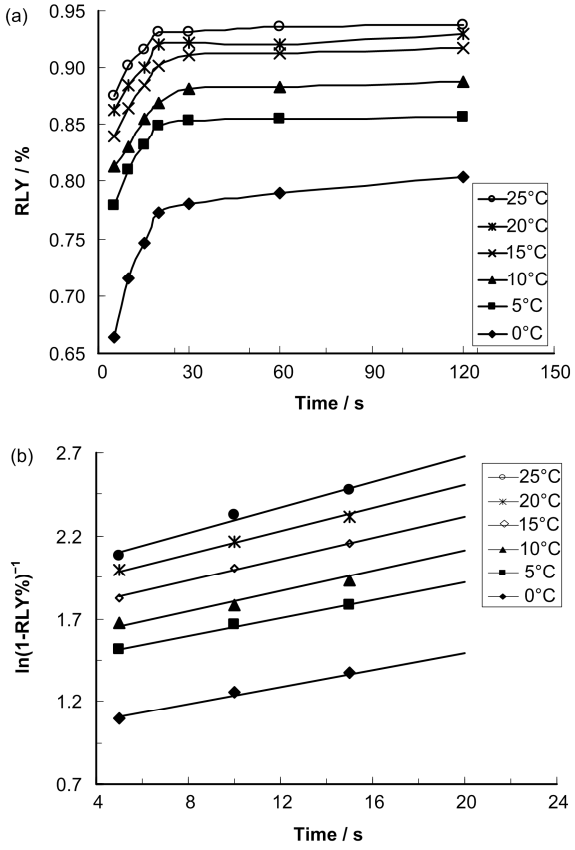

Fig.2 *RLY* (a) and $\ln(1-RLY)^{-1}$ (b) as a function of reaction time (*t*).

There is a better linear relationship between $\ln(1-RLY)^{-1}$ and the reaction time (*t*) at different temperatures, which is depicted in Fig.2(b). They can also be expressed as Eqs. (6) to (11).

$$T = 273\ \text{K},\ \ln[1-RLY)]^{-1} = 0.0258t + 0.9783 \quad R^2 = 0.9906 \tag{6}$$

$$T = 278\ \text{K},\ \ln[1-RLY)]^{-1}\ln = 0.0268t + 1.3822 \quad R^2 = 0.9988 \tag{7}$$

$$T = 283\ \text{K},\ \ln[1-RLY)]^{-1} = 0.0305t + 1.4978 \quad R^2 = 0.9760 \tag{8}$$

$$T = 288\ \text{K},\ \ln[1-RLY)]^{-1} = 0.0323t + 1.6703 \quad R^2 = 0.9995 \tag{9}$$

$$T = 293\ \text{K},\ \ln[1-RLY)]^{-1} = 0.0351t + 1.8110 \quad R^2 = 0.9939 \tag{10}$$

$$T = 298\ \text{K},\ \ln[1-RLY)]^{-1} = 0.0384t + 1.9070 \quad R^2 = 0.9927 \tag{11}$$

From the slope of every line, the rate constant *k* can be estimated at 0.0258, 0.0268, 0.0305, 0.0323, 0.0351 and 0.0384 for the radiolabeling reaction at 0°C, 5°C, 10°C, 15°C, 20°C and 25°C, respectively. However, the increment for the rate constant *k* at low temperature was slightly larger than that at a higher temperature. For instance, it increased by 13.8% from 5°C to 10°C and 5.9% from 10°C to 15°C, respectively. In a word, the increase of temperature will affect significantly the reaction rate constant *k*, which increases more at the low temperature than that at the higher temperature.

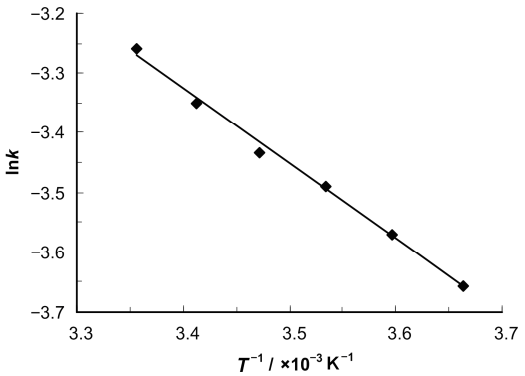

Fig.3 Relationship between ln *k* and T^{-1}.

According to Eq.(5), the activation energy of the present labeling reaction can be deduced. Fig.3

shows a good linearity in the Arrhenius plot, i.e., ln$k$ *vs.* T^{-1}. This indicates a single reaction mechanism for the formation of $^{99}Tc^{m}$-BIDP in the temperature range considered. The activation energy E_a was calculated from the slope: E_a=−1258.8×(−8.3)=10.45 kJ·mol^{-1}. And the correlation between the rate constant k and temperature T was deduced to be ln$k$=−1258.8/T+0.9531 from the least square multivariate linear regression between in k and 1/T (Fig.3). In fact, the derived E_a value for $^{99}Tc^{m}$-BIDP is lower than those reported for other radiopharmaceuticals[18]. This may be attributed to the fact that $^{99}Tc^{m}$-BIDP carries a relatively quick reaction rate. As well known, the labeling reactions of radiopharmaceuticals are affected by factors of the reaction temperature, pH value, ligand concentration, reductant concentration, etc[11,15,17,21,22], among which the reaction temperature is the most important. As stated in the preparation of $^{99}Tc^{m}$-BIDP, the increase of labeling reaction temperature can lead to the increase of RLY.

Based on the equation between k and temperature, a worthy conclusion can be drawn that the overall RLY for $^{99}Tc^{m}$-BIDP can reach over 90% when the reaction temperature was up to 12°C. Compared with 100°C for $^{99}Tc^{m}$-MIBI[14], the reaction temperature at 25°C is of great convenience for further investigation and application of $^{99}Tc^{m}$-BIDP, with just a slight heating in winter. Therefore, we conclude that as a potential radiopharmaceutical, $^{99}Tc^{m}$-BIDP might be more convenient for the clinical use.

4 Conclusion

The labeling reaction kinetics for the preparation of $^{99}Tc^{m}$-BIDP from BIDP and $^{99}Tc^{m}O_4^-$ was investigated. The results indicate that it is a first order reaction and the activation energy E_a is 10.45 kJ·mol^{-1}. The radiolabeling yield increases obviously as the temperature increases, and to get a high yield (>90%), the reaction temperature must be up to 12°C. The results also indicate that this radiopharmaceuticals can be applied directly and conveniently to the clinic since the ligand can react with the radioisotope at the room temperature (or they are heated slightly in the winter) for only a few seconds. In summary, mild reaction conditions and easy operation make this kind of radiopharmaceuticals be worthy of further investigation and application in the clinic.

References

1 EI-Mabhouh A A, Mercer J R. Eur J Nucl Med Mol Imaging, 2008, **35:** 1240–1248.

2 Green J R. J Org Chem, 2005, **690:** 2439–2448.

3 Ross J R, Saunders Y, Edmonds P M, *et al*. Health Technol Asses, 2004, **4:** 1–16.

4 Ogawa K, Mukai T, Arano Y, *et al.* Nucl Med Biol, 2006, **33:** 513–520.

5 Garcia E，Schibli R，Schubiger P A. Nucl Sci Tech, 2007, **18:** 88–100.

6 Shalaby-Rana E, Majd M. J Nucl Med, 2001, **42:** 878–883.

7 Widler L, Jaeggi K A, Glatt M, *et al*. J Med Chem, 2002, **45:** 3721–3738.

8 Wang H Y, Luo S N, Xie M H, *et al*. Nucl Tech, 2006, **29:** 438–441.

9 Asikoglu A, Durak F G. Appl Radiat Isot, 2009, **67:** 1616–1621.

10 Luo S N, Wang H Y, Xie M H, *et al*. Chin J Nucl Med, 2005, **25:** 341–344.

11 Guo X H, Luo S N, Wang H Y, *et al*. Nucl Sci Tech, 2006, **17:** 285–288.

12 Yan X H, Luo S N, Niu G S, *et al*. Nucl Sci Tech, 2008, **19:** 165–168.

13 Niu G S, Luo S N, Yan X H, *et al*. Nucl Tech, 2008, **31:** 698–701.

14 Chen C Q, Luo S N, Lin J G, *et al*. Nucl Sci Tech, 2009, **20:** 302–306.

15 Lin J G, Luo S N, Chen C Q, *et al*. Appl Radiat Isot, 2010, **68:** 1616–1622.

16 Kung H F, Liu B L, Pan S R. Appl Radiat Isot, 1989, **40:** 677–681.

17 Wei Y, Wang X B, Liu B L, *et al*. Nucl Tech, 1990, **13:** 445–448.

18 Luo S N, Xie M H, Fang P, *et al*. Nucl Tech, 1992, **15:** 296–299.

19 Liu S, Edwards D S, Harris A R, *et al*. Appl Radiat Isot, 1997, **48:** 1103–1111.

20 EI-Mabhouh A A, Angelov C A, Cavell R, *et al*. Nucl Med Biol, 2006, **33:** 715–722.

21 Zhu J Q, Wu C Y, Lu C X. Nucl Sci Tech, 2003, **14:** 135–137.

22 DjokićD D, JankovićD L, NikolićN S. Bioorgan Med Chem, 2008, **16:** 4457–4465.

Supported by National Natural Science Foundation of China (Nos. 20801024 and 21001055), Natural Science Foundation of Jiangsu Province (No.BK2009077), Science Foundation of Health Department of Jiangsu Province (No.H200963)

* Corresponding author. *E-mail address:* shineng914@yahoo.com.cn

Received date: 2010-11-23