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Biological reduction and intracellular trapping differences of ^{188}Re(VII) and ^{99}Tc^{m}(VII) in *Escherichia coli*

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Abstract ^{99}Tc^{m} is the most commonly used diagnostic radioisotope. And ^{188}Re is an excellent therapeutic radioisotope with a gamma photon for imaging. Both locate in the same group and have very similar properties. ^{99}Tc^{m}(VII) can be partially reduced to a black insoluble precipitate by aerobic cultures of *Escherichia coli*, and trapped intracellularly. However, ^{188}Re(VII) cannot be reduced and trapped，because of its lower oxidizing power of perrhenate as compared with pertechnetate or other reasons.

Key words ^{99}Tc^{m}, ^{188}Re, *Escherichia coli*, Biological reduction, Intracellular trapping

1 Introduction

^{99}Tc^{m} is the most commonly used radioisotope for diagnostic applications in nuclear medicine because of its ideal physical properties (140 keV γ-ray, 89%), optimal for diagnostic imaging, convenient availability from an inexpensive ^{99}Mo/^{99}Tc^{m} generator, and the half-life of $t_{1/2}$=6 h suitable for most applications[1].

Lloyd and Macaskie have monitored the enzymatic reduction of Tc(VII) by resting cells of the Fe(III)-reducing bacteria *Shewanella putrefaciens* and *Geobacter metallireducens*[2]. Subsequent studies have shown, except for the above Fe(III)-reducing bacteria, a range of microorganisms can reduce and remove Tc(VII) from solution including the sulfate-reducing bacterium *Desulfovibrio desulfuricans*[3], and the facultative anaerobe *Escherichia coli* (*E.coli*)[4,5]. Especially, the enzyme system responsible for Tc(VII) reduction in *E.coli* has been studied most intensively. In *E.coli*, the Tc(VII) reductase activity has been identified as hydrogenase 3, a component of the formate hydrogenlyase complex (FHL), encoded by the hycE and hycG genes in the hyc operon[6]. *E.coli* reduces Tc(VII) with either the fermentation product formate (long recognized as the source of electrons for H_2 evolution by enteric bacteria) or hydrogen itself as electron donors[4,7].

Electron microscopy, in combination with energy-dispersive X-ray analysis, showed that Tc(VII) was reduced and precipitated inside. Proton-induced X-ray emission analysis supported the hypothesis that the radionuclide was precipitated as a low valence insoluble oxide[4], such as TcO, TcO_2, and Tc_2O_5, all of which form insoluble precipitates at neutral pH[8,9]. Then they reacted non-specifically with intracellular proteins and nucleic acids[10].

The trapped radionuclide can be detected through emitting radiation by radioscintigraphy, such as PET and SPECT. At the same time, it can be useful for therapy due to its cytotoxicity to the target cell by radiation emission and disruption cellular function through nonspecific binding[11].

^{188}Re, which emits high energy β particles (2.12 MeV, 79% and 1.97 MeV, 20%) and 155 keV γ -ray (15%), with half-life of 16.9 h, is an excellent radioisotope for both therapy and imaging. It can be obtained as carrier-free sodium perrhenate by saline elution of ^{188}W/^{188}Re generator system. Rhenium is in the Group VIIb of the periodic table, directly below technetium. Their chemical properties are similar but

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not identical. In particular, with lower redox potential than Tc, Re is more difficult to be reduced[15-17].

Based on these concepts, biological reduction and intracellular trapping of ^{188}Re(VII) ($^{188}ReO_4^-$) in *E.coli* were investigated and compared with that of $^{99}Tc^m$(VII) ($^{99}Tc^mO_4^-$) in this paper.

2 Materials and methods

2.1 Organisms and culture conditions

Escherichia coli strains (DH5a) were maintained aerobically at 37℃ in LB culture medium. Cells were shaken aerobically overnight (250 rpm) at 37℃. K562 cells as a control were maintained at 37℃ in 1640 culture medium.

Phosphate buffer solution (PBS) to wash the cells contains 8.0 g/L NaCl, 0.2 g/L KCl, 1.56 g/L $Na_2HPO_4{\cdot}H_2O$ and 0.2 g/L KH_2PO_4. The pH was adjusted to 7.3 with hydrochloric acid.

Each tube was added with 2 mL culture medium (~6×10^7Cells). The mixture was incubated at 37℃ for certain time after adding 50 μL (~2 μCi or 7.4×10^4 Bq) $Na^{99}Tc^mO_4$ or $Na^{188}ReO_4$ to each tube. The cells were washed with PBS twice and centrifuged. Radioactivity of the cells was counted by γ radioscintigraphy.

2.2 Radionuclides

$Na^{99}Tc^mO_4$ was purchased by Shanghai Yuanpu Isotope Technology Co., Ltd. Carrier-free $Na^{188}ReO_4$ was freshly eluted with saline from $^{188}W/^{188}Re$ generator (Shanghai Institute of Applied Physics).

3 Results and discussion

The radioactivity retained in the cells after rinsing away residual soluble technetium or rhenium was recorded in counts per minute (Fig.1). For technetium, radioactivity of the cells was far higher than the control and increased with time. For rhenium, however, radioactivity of the cells remained almost the same over time and had no difference at all with the control or background.

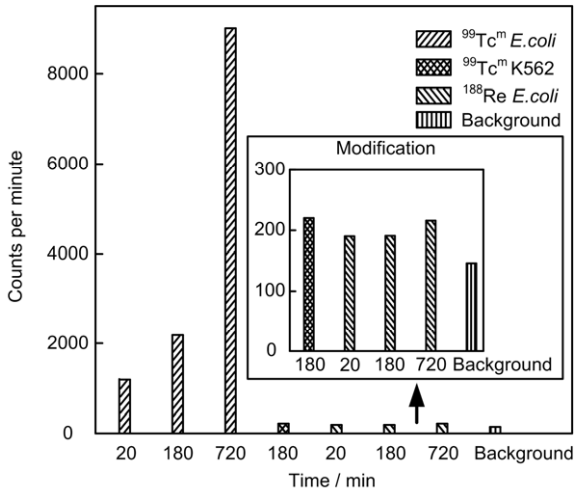

Fig.1 Amount of radioactivity retained in the cells.

The results of intracellular trapping of $Na^{99}Tc^mO_4$ and $Na^{188}ReO_4$ by *E.coli* (DH5a) and K562 are summarized in Table 1.

Table 1 Results of intracellular trapping experiments

Samples		20 min	3 h	12 h
$Na^{99}Tc^mO_4$	*E.coli* (DH5a)	Trapping (0.00085)	Trapping (0.00156)	Trapping (0.00642)
	K562	No trapping	No trapping	No trapping
$Na^{188}ReO_4$	*E.coli* (DH5a)	No trapping	No trapping	No trapping
	K562	No trapping	No trapping	No trapping

*The data within parentheses presents that the amount of radioactivity is divided by the total number of counts added in the cultures.

From Table 1, *E.coli* with hydrogenase 3 can reduce $^{99}Tc^mO_4^-$ to low valence insoluble oxide accumulating in cells aerobically, and as the extension of the incubating time, the amount of $^{99}Tc^m$ intracellular trapping rises, although the amount of intracellular $^{99}Tc^m$ is lower than expected. As a control, K562 cells without corresponding hydrogenase cannot reduce the $^{99}Tc^m$ pertechnetate.

However, ^{188}Re has no intracellular trapping of *E.coli* with hydrogenase 3. This means that the enzyme cannot reduce ^{188}Re perrhenate aerobically. The oxidizing power of perrhenate (standard redox

potential for $ReO_4^- \rightarrow ReO_2$: –0.59V) is lower than pertechnetate ($TcO_4^- \rightarrow TcO_2$: –0.31V). This makes it difficult to reduced rhenium into lower oxidation states but easily re-oxidized. This may be the main reason why $^{99}Tc^m$ pertechnetate can be reduced and trapped intracellularly in *E.coli*, ^{188}Re perrhenate, however, cannot.

The tendency of reaction occurs spontaneously in the direction of decreased Gibbs function (G), that is ΔG ($\Delta G = -nFE$)<0, or E>0, and the following results can be obtained according to Nernst equation.

$$E = E^{\theta} + \frac{RT}{nF} \ln \frac{\alpha_C{}^c \alpha_D{}^d}{\alpha_G{}^g \alpha_H{}^h} \quad \text{(Reaction: } c\text{C}+d\text{D}=g\text{G}+h\text{H)}$$

where F is Faraday constant, n is the amount of transferable electrons in the redox and R is Gas constant.

^{188}Re perrhenate may be reduced by the same amount of H_2, only when the concentration of ^{188}Re perrhenate is far higher than that of $^{99}Tc^m$ pertechnetate. For example, calculating with the Nernst equation, $Na^{99}Tc^mO_4$ could be reduced by 1 mol/L H_2 only under the condition that its concentration is higher than 2.64×10^{-13} mol/L. For $Na^{188}ReO_4$, however, the concentration shall be over 37.15 mol/L.

The fermentative glucose metabolism can hardly occur under aerobic condition, so the electron donor H_2 or formate is very little. In addition, the activity of hydrogenase 3 is low aerobically. These may be other reasons why *E.coli* with hydrogenase 3 cannot reduce and trap ^{188}Re and the amount of reduced and trapped $^{99}Tc^m$ is not as much as expected in this experiment. More experiments are needed to further study whether ^{188}Re perrhenate can be reduced and trapped in the cell by *E.coli* with hydrogenase 3 anaerobically, other microbes with similar hydrogenase, high-activity strains such as the use of an *E. coli HycA* mutant (upregulating FHL activities by approx. 50%) which had an enhancing effect on the rate of Tc reduction[7], or the use of the inducer such as IPTG (Isopropyl-beta-D-thiogalactopyranoside), at the aerobic or anaerobic conditions.

Microbial hydrogenase mediated reduction of radionuclides, except for the use as a biotechnological method of treating radionuclide contaminated effluents[18,19], will mainly be utilized to this aim-By transfecting the target cell, such as mammalian cell, with a transgenic vector encoding a microbial hydrogenase expressible in the target cell, the target cell can possess the ability of enzyme-mediated intracellular trapping of a radionuclide. From this point of view, the suitable microbial hydrogenase should be oxygen stable, such as the hydrogenases of *D.vulgaris* and *D. desulfuricans*, more accessible and periplasmic. Otherwise, it should remain catalytically active from room temperature or 37℃. This is why in this experiment the enzyme-mediated reduction of radionuclides by *E.coli* was conducted aerobically at 37℃.

4 Conclusion

Either in the organism or in the chemical reaction, perrhenate is more difficult to be reduced than pertechnetate due to its lower oxidizing power. In this experiment, $^{99}Tc^m$ pertechnetate can be partially reduced and trapped intracellularly aerobically at 37℃ by *E.coli* with hydrogenase 3. However, at the same condition, ^{188}Re perrhenate cannot be reduced and trapped.

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