

## Chemical properties of technetium- $^{99m}\text{Tc}$ -DL-homocysteine, a possible tumor-imaging agent

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The chemical properties of  $^{99m}\text{Tc}$ -DL-homocysteine ( $^{99m}\text{Tc}$ -Hcy) showing high accumulation in several experimental tumors were investigated. The form of tumor-tropic  $^{99m}\text{Tc}$ -Hcy was a polymeric complex which appeared at void volume on Sephadex G-15 by eluting with 5 mM Hcy. This complex changed into smaller complexes of ca. 600 molecular weight in the presence of 150 mM NaCl and 5 mM Hcy, suggesting that  $^{99m}\text{Tc}$ -Hcy was a complex composed of smaller polymers which are weakly bound together by an ionic bond. The complex showed a negative charge. The Hcy/Tc molar ratio in the complex was approximately 2 and no Sn was detected.

**Key words:**  $^{99m}\text{Tc}$ -DL-homocysteine, DL-homocysteine, Chemical property, Tumor affinity, Tumor-imaging agent

### INTRODUCTION

ALTHOUGH  $^{67}\text{Ga}$ -CITRATE has been clinically used as a tumor detecting agent,<sup>1</sup> a new tumor detecting agent needs to be developed because  $^{67}\text{Ga}$ -citrate has some disadvantages such as low specificity for tumors and slow clearance from blood. We have reported that among some  $^{99m}\text{Tc}$ -labeled S-containing amino acids and sugars,  $^{99m}\text{Tc}$ -DL-homocysteine ( $^{99m}\text{Tc}$ -Hcy) was the most promising agent for tumor detection because it accumulated in the tumor within a short time and showed higher affinity for the tumors than for abscesses.<sup>2-4</sup> Furthermore, the behavior on gel chromatography suggested that this compound was a polymeric complex.<sup>3,4</sup> Recently, it has been reported that tumor-tropic  $^{99m}\text{Tc}$  (V)-dimer-captosuccinic acid may be a polymeric form.<sup>5,6</sup> It is of interest to know whether with respect to  $^{99m}\text{Tc}$ -labeled compounds, polymeric complexes generally show high affinity for tumors. However, the chemical properties of these polymeric  $^{99m}\text{Tc}$ -complexes have been little reported.<sup>5,7</sup> This paper deals with the

chemical properties of the tumor-tropic form of  $^{99m}\text{Tc}$ -Hcy.

### MATERIALS AND METHODS

**Chemicals**  $\text{Na } ^{99m}\text{TcO}_4$  was obtained from a  $^{99}\text{Mo}$ - $^{99m}\text{Tc}$  generator (Daiichi Radioisotope Laboratories Ltd., Tokyo) by eluting with physiological saline.  $\text{NH}_4^{99}\text{TcO}_4$  was purchased from Amersham, U.K. DL-homocysteine and ion exchange celluloses [diethylaminoethyl cellulose (DE 52) and carboxymethyl cellulose (CM 52)] were purchased from Sigma Chemical Co., U.S.A. and Whatman International Ltd., U.K., respectively. 4-Phenylspiro [furan-2 (3H), 1'-phthalan]-3,3'-dione (Fluram<sup>®</sup>) was the product of Roche, Switzerland. Other chemicals used were of guaranteed grade.

**Tumor-bearing Mice** Male mice of ddY strain (16-18 g) were purchased from Shizuoka Agricultural Cooperative Association for Laboratory Animals, Hamamatsu. Mice bearing Ehrlich solid tumors were obtained as described previously.<sup>2</sup>

**Preparation of  $^{99m}\text{Tc}$ -Hcy and ( $^{99m}\text{Tc} + ^{99}\text{Tc}$ )-Hcy**  $^{99m}\text{Tc}$ -Hcy was prepared according to the procedure described previously.<sup>2</sup> When Hcy and Sn contents in the labeled compound were determined, ( $^{99m}\text{Tc} + ^{99}\text{Tc}$ )-Hcy was prepared with a mixture of  $\text{Na}^{99m}\text{TcO}_4$  and  $\text{NH}_4^{99}\text{TcO}_4$ .

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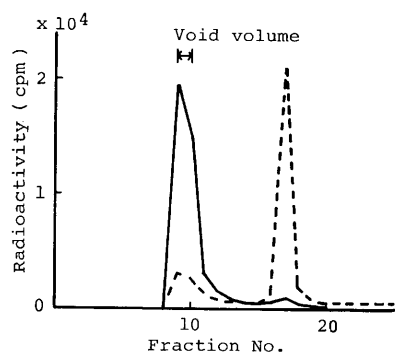
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**Analysis of  $^{99m}\text{Tc}$ -Hcy and ( $^{99m}\text{Tc} + ^{99}\text{Tc}$ )-Hcy** The  $^{99m}\text{Tc}$ -Hcy charge was estimated by adsorption on ion exchange cellulose at neutral pH. Sn was determined by the SATP (salicylideneamino-2-thiophenol)-method.<sup>8</sup> Hcy was assayed as follows.<sup>9</sup> A mixture of 1 ml sample and 0.5 ml borate buffer (0.2 M, pH 9.0) was added to 0.5 ml Fluram® (150  $\mu\text{g}/\text{ml}$ ) with stirring. The fluorescence was measured at 390 nm (excitation wavelength) and 475 nm (emission wavelength). The radioactivity of  $^{99m}\text{Tc}$  and  $^{99}\text{Tc}$  was counted in a gamma counter (Beckman 5500) and a liquid scintillation counter (Aloka 661), respectively.

**In vivo uptake** Four mice bearing Ehrlich solid tumors (approximately 0.5 cm in diameter) were intravenously injected with 0.2 ml/head of  $^{99m}\text{Tc}$ -labeled compound (ca. 1  $\mu\text{Ci}$ ) in physiological saline and, 3 hr later, sacrificed by bleeding under ether inhalation. Tumors and organs were excised and weighed. The radioactivity was counted in a gamma counter. All the counts were corrected for decay.

## RESULTS

**Molecular size and stability of tumor-tropic form of  $^{99m}\text{Tc}$ -Hcy** Elution profiles of  $^{99m}\text{Tc}$ -Hcy on Sephadex G-15 are shown in Fig. 1. The profiles in the presence and absence of Hcy in the eluent ( $\text{H}_2\text{O}$ ) were different. Almost all the radioactivity was eluted in void volume in the presence of 5 mM Hcy, while two peaks were obtained in the absence of Hcy. The radioactive complexes in the first peak at void volume and the second peak were named Complex I and Complex II, respectively. TLC analysis was applied to determine whether both the radioactive complexes were involved in unfractionated  $^{99m}\text{Tc}$ -Hcy containing excess Hcy in the reaction mixture. The unfractionated  $^{99m}\text{Tc}$ -Hcy showed a single peak on TLC (Fig. 2d). However, Complexes I and II had a peak with the same Rf value as  $^{99m}\text{TcO}_4^-$  and

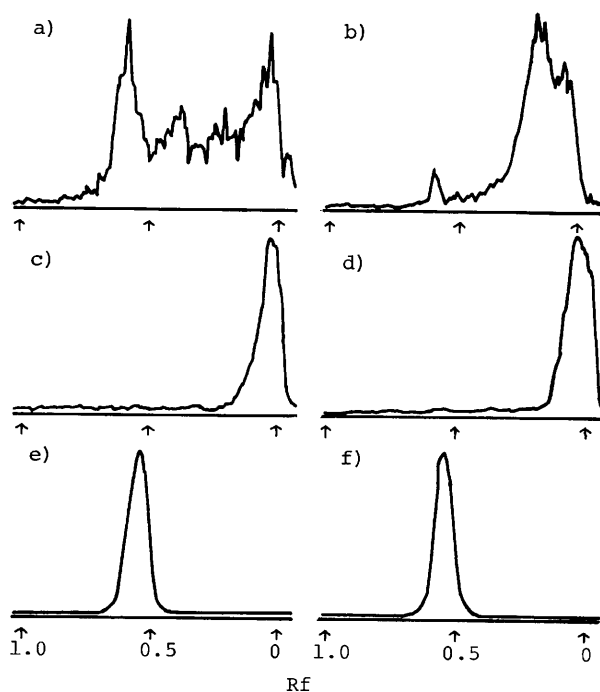


**Fig. 1** Elution profiles of  $^{99m}\text{Tc}$ -Hcy on Sephadex G-15. -----: eluted with  $\text{H}_2\text{O}$ , —: eluted with 5 mM Hcy, Column:  $1.0 \times 33.5$  cm.

some undefined peaks besides that of unfractionated  $^{99m}\text{Tc}$ -Hcy (Fig. 2a, b). These TLC profiles indicated that Complexes I and II were unstable in Hcy-free  $\text{H}_2\text{O}$ .

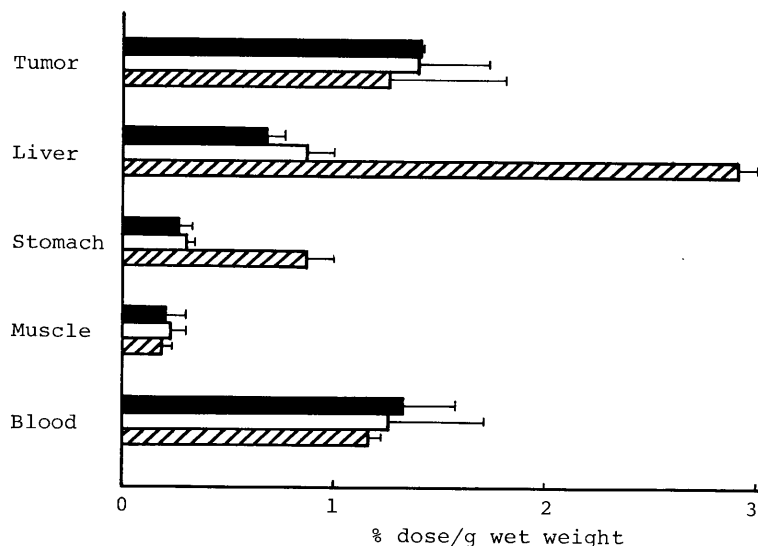
On the other hand, the fact that most of the radioactivity appeared at void volume in the presence of 5 mM Hcy suggests that  $^{99m}\text{Tc}$ -Hcy mainly consisted of Complex I. This was confirmed by the finding that Complex I eluted with 5 mM Hcy had the same TLC profile as unfractionated  $^{99m}\text{Tc}$ -Hcy (Fig. 2c).  $^{99m}\text{TcO}_4^-$  in 5 mM Hcy had the same Rf value as  $^{99m}\text{TcO}_4^-$  (Fig. 2e, f), indicating that  $^{99m}\text{TcO}_4^-$ , without a reducing agent such as  $\text{SnCl}_2$ , did not react with Hcy.

In order to confirm that Complex I was the tumor-tropic form of  $^{99m}\text{Tc}$ -Hcy, the distribution of these complexes in mice bearing Ehrlich solid tumors was compared with that of unfractionated  $^{99m}\text{Tc}$ -Hcy (Fig. 3). Complex I had almost the same distribution pattern as unfractionated  $^{99m}\text{Tc}$ -Hcy and had higher affinity for tumors than for normal tissues, while Complex II, which might be artificially formed by eluting with  $\text{H}_2\text{O}$ , showed the highest affinity for liver, different from that of  $^{99m}\text{Tc}$ -Hcy.



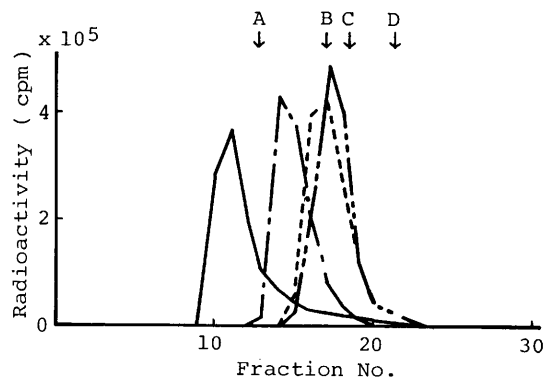
**Fig. 2** Radio TLC profiles of  $^{99m}\text{Tc}$ -compounds. The sample spotted on TLC plate (AVICEL® SF,  $20 \times 20$  cm) was developed with 1 N AcOH-saturated n-BuOH. The radioactivity was measured with a TLC scanner (Aloka JTC-203).

a) Complex I (eluted with  $\text{H}_2\text{O}$ ), b) Complex II (eluted with  $\text{H}_2\text{O}$ ), c) Complex I (eluted with 5 mM Hcy), d) unfractionated  $^{99m}\text{Tc}$ -Hcy, e)  $^{99m}\text{TcO}_4^-$ , f)  $^{99m}\text{TcO}_4^-$  in 5 mM Hcy (allowed to stand for 1 hr).



**Fig. 3** Tumor accumulation of Complex I, II, and  $^{99m}\text{Tc}$ -Hcy. Each peak fraction of Complexes I and II shown in Fig. 1 was used in the experiments. Four mice were intravenously injected with ca.  $1\ \mu\text{Ci}$  of the sample in saline and then treated as described in the text.

■: unfractionated  $^{99m}\text{Tc}$ -Hcy, □: Complex I (eluted with 5 mM Hcy), ▨: Complex II (eluted with  $\text{H}_2\text{O}$ ). Each bar and line indicate the mean  $\pm$  S.D. ( $n=4$ ).



**Fig. 4** Effect of NaCl on elution profiles of Complex I on Sephadex G-25.

Arrows show the elution positions of (A) gastrin (M.W. 2098.5), (B) oxidized glutathione (M.W. 612.6), (C) glutathione (M.W. 307.3), and (D) homocysteine (M.W. 135.2).

—: eluted with 5 mM Hcy, —·—: eluted with 5 mM NaCl+5 mM Hcy, —••—: eluted with 50 mM NaCl+5 mM Hcy, ———: eluted with 150 mM NaCl+5 mM Hcy, Column:  $1.2 \times 42$  cm.

**Chemical properties of  $^{99m}\text{Tc}$ -Hcy** Since it was found that the tumor-tropic form of  $^{99m}\text{Tc}$ -Hcy was Complex I, its chemical properties were investigated. When Complex I was eluted with a mixture of 5 mM Hcy and 5, 50, or 150 mM NaCl on Sephadex G-25, it changed into compounds of smaller molecular weight; in the case of 5 mM Hcy and 150 mM NaCl it was eluted at a position corresponding to a molecular weight of about 600 (Fig. 4).

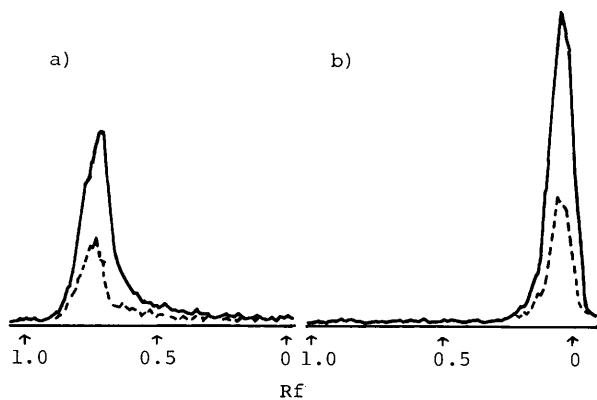
**Table 1** Adsorption of Complex I on ion exchange cellulose

Resin	Buffer	Adsorbed radioactivity (%)
CM-52	2 mM Acetate (pH 6.0)	2.8
DE-52	2 mM Borate (pH 7.6)	94.6
	2 mM Borate (pH 7.6)+0.1 M NaCl	24.9
	2 mM Borate (pH 7.6)+0.2 M NaCl	6.6

A mixture of Complex I and cellulose suspended in each buffer was stirred for 10 min at room temperature and then centrifuged at 2,700 rpm for 15 min. The resin was washed once with the same buffer. The radioactivity was counted in a gamma counter.

The charge of Complex I was examined by means of adsorption on ion exchange cellulose (Table 1). Complex I adsorbed on DE 52, but not on CM 52, and was released from DE 52 by adding 0.2 M NaCl, indicating that it had a weak negative charge at neutral pH.

To determine the molar ratio of Hcy to Tc in Complex I, Hcy was labeled with a relatively large amount of  $^{99}\text{Tc}$  in addition to  $^{99m}\text{Tc}$  because the amount of  $^{99m}\text{Tc}$ -Hcy alone was too small to analyze. ( $^{99m}\text{Tc}+^{99}\text{Tc}$ )-Hcy exhibited the same Rf value as  $^{99m}\text{Tc}$ -Hcy (Fig. 5). Complex I was fractionated on Sephadex G-15 using  $\text{H}_2\text{O}$  as an eluent in order to



**Fig. 5** Radio TLC profiles of  $^{99m}\text{Tc}$ -Hcy and ( $^{99m}\text{Tc} + ^{99}\text{Tc}$ )-Hcy.

The procedure was as described in the legend to Fig. 2, developing solvent: a) 50% EtOH, b) 1 N AcOH-saturated n-BuOH.

—:  $^{99m}\text{Tc}$ -Hcy, ----: ( $^{99m}\text{Tc} + ^{99}\text{Tc}$ )-Hcy.

**Table 2** Molar ratio of Hcy to Tc in Complex I

	Hcy/ $^{99}\text{Tc}$	
	Untreated	H <sub>2</sub> O <sub>2</sub> -treated
Mean $\pm$ SD	2.13 $\pm$ 0.35	2.12 $\pm$ 0.48

Tc and Hcy were determined according to the procedure described in the text. In H<sub>2</sub>O<sub>2</sub>-treatment, the sample (1.0 ml) was treated with H<sub>2</sub>O<sub>2</sub> (0.1 ml) for 48 hr at room temperature and freeze-dried to remove H<sub>2</sub>O<sub>2</sub>.

remove free Hcy completely. Hydrogen peroxide was used to dissociate Complex I to TcO<sub>4</sub><sup>-</sup> and Hcy for the determination of Hcy with a fluorescent agent, Fluram®, that reacted with primary amine. Tc was measured by the radioactivity of  $^{99}\text{Tc}$  after the decay of  $^{99m}\text{Tc}$ . As shown in Table 2, the binding ratio was approximately 2 both before and after the H<sub>2</sub>O<sub>2</sub>-treatment. No detectable amount of Sn was found in Complex I by the SATP method.

## DISCUSSION

$^{99m}\text{Tc}$ -Hcy was a polymeric complex which eluted with 5 mM Hcy at void volume on Sephadex G-15. This polymer (Complex I) was a tumor-tropic form. In the presence of 5–150 mM NaCl, Complex I changed into smaller complexes. This suggests that Complex I in 5 mM Hcy was a polymer in which smaller complexes were bound together by a weak

ionic bond. In a recent study, we found that  $^{99m}\text{Tc}$ -cysteine also contained a polymeric complex and the polymer had relatively high affinity for tumors as well as  $^{99m}\text{Tc}$ -Hcy (unpublished data). Glickson et al.<sup>10</sup> reported that  $^{67}\text{Ga}$ -citrate formed polymeric complexes. Recently it was reported that  $^{99m}\text{Tc(V)}$ -dimercaptosuccinic acid of which a considerable amount accumulated in tumors was assumed to be a polymer.<sup>5,6</sup> The form of the polymeric complex might be one of the important properties in tumor-imaging agents.

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