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論 文

Tissue Distribution and Binding Proteins of Radionuclides in Bivalve, *Gomphina melanaegis*

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=Abstract=

Radioisotope tracer experiments on the distribution and the binding of radionuclides to proteins in bivalve were carried out in order to gain further information on biochemical behavior of radionuclides in marine bivalve, *Gomphina melanaegis*.

The radioactivities (cpm/g) of ^{65}Zn and ^{54}Mn after 7 days exposure were highly concentrated in liver and kidney in comparison to soft parts. The gel filtration profile of ^{65}Zn in liver and kidney showed three elution peaks, while ^{54}Mn showed two peaks in liver and three peaks in kidney. On the gel filtration of ^{137}Cs in liver and kidney, most of ^{137}Cs were eluted on one peak.

Thus, it was considered that each radionuclide was bound to different proteins in liver and kidney of bivalve.

1. Introduction

Radionuclides released from nuclear power plants into marine environment are accumulated into marine organisms through seawater and food organisms. The investigations on radionuclides in marine organisms have been carried out quantitatively for bioconcentration and tissue distribution. It is, however, important to obtain more information on the biochemical behavior of radionuclides that entered into marine organisms for eluicidation of the mechanisms of radionuclide metabolism, and also for more accurate estimation of radiation effect on man who takes marine organisms as foods.

The experiments reported here were designed to examine the distribution of radionuclides and the binding of radionuclides to proteins and/or peptides in liver and kidney of marine bivalve, *Gomphina melanaegis*.

2. Materials and Methods

The chemical forms of radionuclides used in the experiments were $^{137}CsCl$ (The Radiochemical Centre, U.K.), $^{54}MnCl_2$ (Carrier free, New England Nuclear, U.S.A.) and $^{65}ZnCl_2$ (Specific Activity: 4.34 mCi/mg, New England Nuclear, U.S.A.) in 0.5 M HCl solution.

Marine bivalves used for this experiment were *Gomphina melanaegis* (average weight: 81.7 g). These bivalves were divided into three groups and reared separately in three aquariums with 1 liter of sea water containing ^{65}Zn, ^{54}Mn and ^{137}Cs, respectively. The radioactivity in sea water was kept at 3,000

cpm/ml and the temperature of rearing sea water was kept at 15°C. After 7 days exposure, the bivalves were transferred into non-radioactive sea water and dissected into shell and soft parts. Furthermore, the soft parts were divided into liver, kidney and remainder. The radioactivity in shell and tissues was measured with the whole body counter (Packard 446).

For the comparison of the gel filtration profile, samples of liver and kidney combined with 4 individuals were prepared by the method of Ueda et al.[1] The sample (0.5 g) was homogenized with 10 ml of 0.025 M Tris acetate buffer solution (pH 8.4) by a high speed homogenizer (20,000 rpm) and centrifuged at 10,000 rpm for 30 minutes. The supernatant was applied on a Sephadex G-75 colum (75×2 cm). The gel filtration was performed at a flow rate of 40 ml/hour using the same buffer. Each 5 ml fraction was measured on the radioactivity with a well-type gamma ray counter (Aloka auto-well gamma system, JDC-752). The recovery of the radioactivity in the gel filtration was over 90%. After radioactivity was measured, the protein content in each fraction was monitored at 280 nm with a spectrophotometer (Hitachi 124 DB).

3. Results and Discussion

Table 1 shows the distribution of ^{65}Zn in the shell and tissues of *G. melanaegis* after 7 days exposure. Most of ^{65}Zn (52.9%) was accumulated in soft parts. Radioactivities in liver (5.6%) and kidney (3.2%) were particularly high, although the weights of liver and kidney were only 0.4 and 0.2% of total weight of whole body, respectively. The radioactivity of ^{54}Mn in shell was 64.9% to total radioactivity of whole body (Table 2). Radioactivity (cpm/g) of ^{54}Mn in kidney was much more than that in liver. Table 3 shows the distribution of ^{137}Cs in *G. melanaegis*. As shown in Table 3, most of radioactivity (80.6 %) was found in soft parts. The difference in the distribution patterns of ^{65}Zn, ^{54}Mn and ^{137}Cs suggested different metabolism due to radionuclides in the organs of marine bivalve[2].

Table 1. Distribution of ^{65}Zn in *Gomphina melanaegis* after 7 days exposure

	Weight		Activity	
	g	%	cpm/g	%
Whole body	82.32	100	7,223	100
Shell	59.46	72.2	2,010	20.1
Soft parts	11.52	14.0	27,292	52.9
Liver	0.35	0.4	95,363	5.6
Kidney	0.13	0.2	147,651	3.2

Table 2. Distribution of ^{54}Mn in *Gomphina melanaegis* after 7 days exposure

	Weight		Activity	
	g	%	cpm/g	%
Whole body	102.10	100	23,425	100
Shell	73.10	71.6	21,221	64.9
Soft parts	15.15	14.8	45,107	28.6
Liver	0.39	0.5	53,903	0.9
Kidney	0.14	0.1	172,698	1.0

Table 3. Distribution of ^{137}Cs in *Gomphina melanaegis* after 7 days exposure

	Weight		Activity	
	g	%	cpm/g	%
Whole body	61.71	100	4,807	100
Shell	45.40	73.6	839	12.9
Soft parts	9.92	16.1	24,098	80.6
Liver	0.26	0.4	50,808	4.5
Kidney	0.11	0.1	87,903	3.3

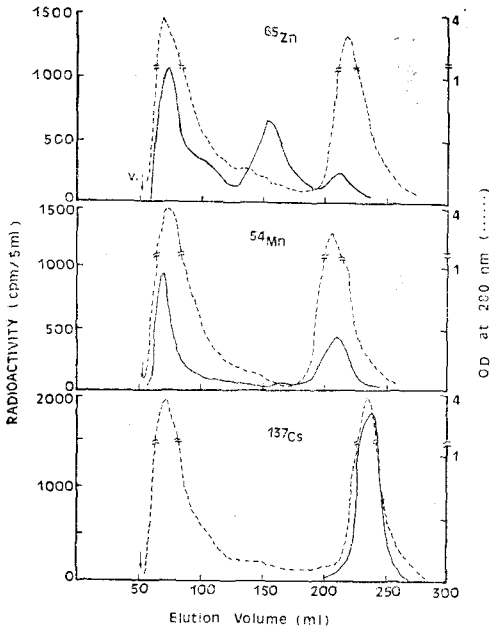

Fig. 1. Sephadex G-75 gel filtration profiles of ^{65}Zn, ^{54}Mn and ^{137}Cs in liver of bivalves.

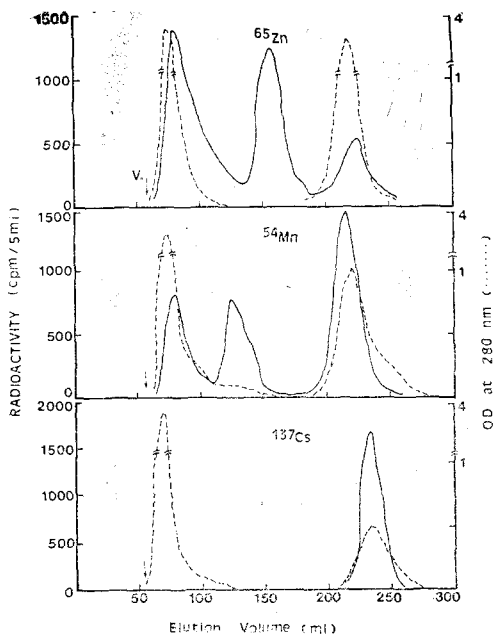

Fig. 2. Sephadex G-75 gel filtration profiles of ^{65}Zn, ^{54}Mn and ^{137}Cs in kidney of bivalves.

The gel filtration profiles of ^{65}Zn, ^{54}Mn and ^{137}Cs in liver of *G. melanaegis* are shown in Fig. 1, accompanied with an optical density curve at 280 nm. After 7 days exposure, the profile of ^{65}Zn showed three peaks, eluted between 60~120, 120~180 and 200~230 ml of elution volume and radioactivities of each peak were 51, 36 and 12% to total applied radioactivity, respectively. ^{54}Mn accumulated in liver was combined with two constituents eluted between 60~100 and 190~230 ml of elution volume and radioactivities in them were 52 and 38% to total radioactivity, respectively. The radioactivity peak eluted 120~180 ml in ^{65}Zn was not shown in ^{54}Mn. Fig. 2 shows the gel, filtration profiles of ^{65}Zn, ^{54}Mn and ^{137}Cs in kidney. The profile of ^{65}Zn in kidney was similar to that of liver, but the profile of ^{54}Mn in kidney showed three peaks eluted between 60~110, 110~150 and 190~230 ml of elution volume, although that of liver showed two peaks. Most of ^{137}Cs in samples of liver and kidney applied on Sephadex G-75 column was eluted between 210~260 ml of elution volume as shown in Fig. 1 and 2. The shape of these curves was almost same as those reported so far.[3~7] This constituents which has strong affinity for ^{137}Cs seems to be a kind of peptide which was conformed from the measurement of absorbancy at 280 nm. The radioactivity peak seen around 150 ml of elution volme was not found ^{54}Mn and ^{137}Cs, but clearly observed in ^{65}Zn. This fraction was seemed to be metallothionec one.[8~10]

Thus, it is considered that there exist some rules particular to each radionuclide in the binding to proteins in bivalve, and it is one of the important subjects to elucidate the rules of metabolism of radionuclides in marine organisms.

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민들조개 (*Gomphina melanaegis*)에서 방사성 동위원소의 조직내 분포와 결합단백질에 관한 연구

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=요　　약=

민들조개에 의해 흡수된 방사성 동위원소의 조직내 분포와 생화학적 거동을 알아 보기 위해 민들조개를 ^{65}Zn, ^{54}Mn, ^{137}Cs 용액에 7일간 노출시킨 후 조직을 잘라내서 방사능을 측정하고 간과 콩팥을 균질화시켜서 Sephadex G-75 유리관을 통한 겔투석 실험을 하였다.

방사성 동위원소의 조직내 분포는 방사성 동위원소에 따라 서로 다른 조직내 축적경향을 나타냈으며 특히 다른 조직에 비해 간과 콩팥에 아주 높게 농축되었다.

방사능이 농축된 간과 콩팥에서 방사성 동위원소와 결합하고 있는 단백질을 조사하기 위해서 간과 콩팥을 균질화시킨 상등액을 Sephadex G-75 column chromatography로 분리한 결과 ^{65}Zn, ^{54}Mn, ^{137}Cs가 서로 다른 유출 패턴을 나타냈고 또한 ^{65}Zn과 ^{54}Mn은 간과 콩팥에서 서로 다르게 유출되었다.

본 실험의 결과 방사성 동위원소의 종류와 조직에 따라서 결합하고 있는 단백질의 종류가 서로 다른 것을 알 수 있었다.