ON THE $^{67}$Ga-BINDING ACID MUCOPOLYSACCHARIDE IN MALIGNANT TUMOR

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The present study was undertaken to determine the structure of $^{67}$Ga-binding acid mucopolysaccharide in tumor tissues. It was determined from measuring neutral saccharide in the structure that the principal $^{67}$Ga-binding acid mucopolysaccharide in the tumor was keratan sulfate and/or keratan polysulfate. On the other hand, it was clarified from the results of mucopolysaccharase treatment that the main $^{67}$Ga-binding acid mucopolysaccharide in tumor was not keratan sulfate, heparan sulfate, heparin, nor chondroitin sulfate A, B, or C. Based on the present results, it was deduced that the main $^{67}$Ga-binding acid mucopolysaccharide in tumor is keratan polysulfate and that this acid mucopolysaccharide plays the most important role in tumor accumulation of $^{67}$Ga.

Introduction

Since tumor localization of $^{67}$Ga-citrate has been reported by EDWARDS and HAYES, $^{1}$ $^{67}$Ga-binding substances in tumors and other soft tissues have been earnestly investigated. Ferritin, transferrin, glycoprotein of molecular weight 45000, and lactoferrin were reported as substances that bind $^{67}$Ga in the tissues. On the other hand, ANDO $^{2}$ originally determined that $^{67}$Ga, $^{111}$In, and $^{169}$Yb were bound to the acid mucopolysaccharides in two species of tumor tissues (Ehrlich tumor and Yoshida sarcoma). It was reported by ANDO et al. $^{3-5}$ that a $^{67}$Ga-binding acid mucopolysaccharides had been separated by cellulose acetate electrophoresis from tumor tissue and liver lysosome, and that $^{67}$Ga-binding acid mucopolysaccharides in tumor and liver were very similar. It was also reported by us $^{6,7}$ that $^{111}$In, $^{169}$Yb and $^{167}$Tm were bound to the same acid mucopolysaccharide to which $^{67}$Ga was bound. Later, these results of ours were supported by the in vitro study of KOJIMA et al., $^{8}$ and by reports that heparan sulfate (a kind of acid mucopolysaccharide) might be an acceptor for $^{67}$Ga accumulation by SASAKI et al., $^{9}$ KOJIMA et al. $^{10}$ and HAMA et al. $^{11}$ Recently we $^{12}$ determined that $^{67}$Ga was also bound to acid mucopolysaccharide in abscess, kidney, heart, lung and spleen. Concerning the structure of $^{67}$Ga-binding acid mucopolysaccharides, we $^{3,5,12}$ deduced...
that $^{67}$Ga was bound to acid mucopolysaccharide (e.g. keratan sulfate) which con-
tained no uronic acid. Furthermore, we$^{1,3}$ reported that this acid mucopolysaccharide
is keratan polysulfate. The present paper describes the details of $^{67}$Ga-binding acid
mucopolysaccharide in tumor.

Materials and methods

Materials

Male ddY mice (28–36 g) subcutaneously implanted with Ehrlich tumor.

Carrier-free $^{67}$Ga-citrate (100–200 $\mu$Ci/cm$^3$) was prepared from $^{67}$Ga-citrate
(Daiichi Radioisotope Laboratories Ltd., Japan) and 0.08M sodium citrate solution.
Carrier-free sodium sulfate$^{3,5}$S solution, pH 6.0–8.0 (1 cm$^3$ containing 900 $\mu$Ci), was
prepared from $\text{H}_2\text{SO}_4$–$^{3,5}$S in 0.05M HC1 solution (Japan Atomic Energy Research
Institute, Japan) and 0.1N NaOH solution to the osmotic pressure at which this solution
can be injected intraperitoneally into the animals.

Pronase E (Protease from streptomyces griseus, Kaken Chemical Co., Japan).

The following 10 biochemical materials were purchased from Seikagaku Kogyo Co.
Ltd., Japan: Chondroitinase ABC (from Proteus Vulgaris), Heparitinase (from Flavo-
bacterium heparium), Keratanase (from Pseudomonas), Heparinase (from Flavobacterium
heparium), Chondroitin sulfate A, Na-salt (from whale cartilage), Chondroitin sulfate B,
Na-salt (from pig skin), Chondroitin sulfate C, Na-salt (from shark cartilage), Haparan
sulfate, Na-salt (from bovine kidney), Heparin, Na-salt (from pig intestine), Keratan sulfat
Na-salt (from bovine cornea).

Sephadex G-50 (particle size 50-150 $\mu$m), G-100 (particle size 40–120 $\mu$m,
Pharmacia Fine Chemical AB, Sweden).

Dowex 1–X2 (Cl type, anion-exchange resin, The Dow Chemical Co., USA).

Incubation time with pronase E

The above mice were injected intraperitoneally with $^{67}$Ga-citrate (0.4 cm$^3$) and
killed 24 hours later. Tumor was excised and rinsed in 0.9% NaCl solution. All manipula-
tions described below were conducted at 4 $^\circ$C. The tumor was homogenized with 10
volumes of 0.15M KCl containing 0.01M Tris buffer, pH 7.6 in a Potter-Elvehjem
type homogenizer. The homogenates were centrifuged for 15 min at 400 g and the
sediments (cell debris and nuclear fraction) were discarded. The homogenate, from
which the nuclear fraction had been removed, was adjusted to pH 7.8–8.2 with 0.1M
NaOH and divided into 7 cm$^3$ aliquots. The homogenates (7 cm$^3$ each) were then
incubated with 60 mg of pronase E at 37 $^\circ$C.

After digestion of 12 hours, one of the reaction mixtures was centrifuged at
3000 rpm (1500 g) for 20 min, and the sediments were discarded. Five cm$^3$ of