Ga-Labeling of Immunoglobulin G with High Specific Radioactivity

Takako FURUKAWA,[a] Yasuhisa FUJIBAYASHI,[b] Masao FUKUNAGA[c] and Akira YOKOYAMA*,[b]

Department of Health Chemistry, Kansai Medical University,[a] Moriguchi, Osaka 570, Japan, Department of Radiopharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Kyoto University,[b] Sakyo-ku, Kyoto 606, Japan, and Division of Nuclear Medicine, Kawasaki Medical School,[c] Kurashiki 701–01, Japan. *Received December 7, 1989*

To obtain radiogallium labeled immunoglobulin G with a high specific radioactivity for *in vitro* use, a ^{67}Ga source was purified by extraction from ^{67}Ga-gallium citrate with butyl acetate, and a ^{67}Ga-labeling solution was produced. This solution was then used to label a deferoxamine–immunoglobulin G conjugate. Both a very high specific radioactivity (872±56 MBq/mg) and a high labeling efficiency (94.0%) were achieved.

Keywords ^{67}Ga purification; deferoxamine; ^{67}Ga-deferoxamine–IgG; IgG labeling; high specific radioactivity

Introduction

Recent research in the biological sciences has been directed towards the bio-molecular level, and so now requires highly sensitive radioimmunoassay systems. However, carrier-free iodine-125, the most commonly used protein-labeling nuclide, has a sensitivity limit in the 10^{-16} mol range. Thus, to detect molecules present at lower concentrations than this, new labeling methodology with higher specific radioactivity is required.

Utilization of radionuclides with shorter half-life aroused our interest, since under carrier-free conditions the half-life of a radionuclide is in inverse proportion to its specific radioactivity. From among the various radionuclides with a short half-life, we previously selected radioactive gallium (^{67}Ga, $T_{1/2}=72$ h; ^{68}Ga, $T_{1/2}=68$ min) as a labeling nuclide for immunoglobulin G (IgG), using deferoxamine (DF) as a bifunctional chelating agent.[1-5] When calculated from the specific radioactivity of ^{67}Ga and the conjugation level of DF–IgG (DF/IgG=0.8), the specific radioactivity of ^{67}Ga-DF–IgG should theoretically be 7400—11100 MBq/mg IgG; however, it actually reached only 28 MBq/mg protein,[1,2] a 20-fold lower value than ^{125}I.[6] This was thought possibly to have been caused by the binding of DF–IgG with Fe^{3+} ions, which are non-radioactive impurities in the Ga source.[4] Accordingly, in this study purification of the Ga source was performed by selective extraction of ^{67}Ga to raise the specific radioactivity of Ga-DF–IgG. The ^{67}Ga-labeling solution (^{67}Ga-LS) that we produced enabled the realization of a very high specific radioactivity (872 MBq/mg) for Ga-DF–IgG.

Experimental

Preparation of the DF–IgG Conjugate The DF–IgG conjugate was prepared as previously reported.[5] DF mesylate (10 mg, from Ciba-Geigy, Switzerland) was dissolved in 1 ml of 0.01 M phosphate buffered saline (PBS, pH 7.4), mixed with 63.4 μl of 1% glutaraldehyde, and stirred for 4 min at 4 °C. Then 35.7 μl of this reaction mixture was added to 10 mg of human IgG (Sigma, U.S.A.) in 1 ml of PBS. After stirring for 45 min at 4 °C, 0.1 mg of $NaBH_4$ was added. The reaction mixture was then applied to a Sephadex G-50 column, and the fraction which contained DF–IgG conjugate was collected. The level of conjugation was determined by the method reported by Emery and Hoffer,[7] and was shown to be 0.7 to 0.8. This DF–IgG conjugate was used for the following studies.

Effect of Ascorbic Acid on the Extraction of Fe To 400 μl of 250 ppm Fe^{3+} solution, prepared by dilution of a 1000 ppm Fe^{3+} standard solution (Nacalai Tesque, Tokyo), 100 μl of ascorbic acid solution (10^{-4} to 1 mol/l) was added. HBr was added and the concentration was adjusted to 5 M, using approximately 700 μl of concentrated HBr. Then the solution was extracted with 1.2 ml of butyl acetate. The extract was evaporated by bubbling with nitrogen gas at 50 °C, and the residue was dissolved in 0.1 M Fe-free HCl prepared by the dilution of stock Fe-free HCl (Nacalai Tesque, Tokyo). The Fe concentration was determined by a previously reported method using 2,4,6-tris(2-pyridyl)-*s*-triazine (TPTZ, Dojin Lab. Kumamoto, Japan),[8] and Fe extraction rate was calculated.

Preparation of ^{67}Ga-LS ^{67}Ga-Citrate (Nihon Medi-Physics Co., Ltd., Takarazuka) was used as the ^{67}Ga source in this study, because it is the only source routinely available commercially. The ^{67}Ga-citrate solution, 0.1 mol/l ascorbic acid, and concentrated HBr were mixed at the volume ratio of 4:1:7. Then the mixture was extracted with butyl acetate by vigorous mixing for 30 s. The butyl acetate layer was separated off and its radioactivity was measured for determination of the extraction ratio. After butyl acetate was evaporated, the residue was dissolved in 20 to 30 μl of 0.05 M Fe-free HCl to make the ^{67}Ga-LS.

It has been reported that ascorbic acid partly prevents the binding of Fe to DF,[4] and for this reason ^{67}Ga-labeling of DF–IgG was performed in the presence of ascorbic acid. The optimum ascorbic acid concentration (50 mmol/l) was determined in our preliminary work using a DF–human serum albumin (HSA) conjugate (data not shown).

The lack of Fe contamination in the ^{67}Ga-LS was confirmed by the TPTZ method mentioned above.

Labeling of DF–IgG Conjugate with ^{67}Ga Two hundred microliters of DF–IgG conjugate solution (50 to 1000 μg/ml in PBS, pH 7.4) was mixed with 20 μl of ^{67}Ga-LS (37—10000 MBq/ml) and was left to stand for 1 h at room temperature. An aliquot of the labeled solution was applied to a Sephadex G-50 column, the eluate was fractionated, and the radioactivity was measured. Radiochemical purity (%) was calculated as follows:

$$\text{radiochemical purity (\%)} = \frac{\text{radioactivity in the conjugate fraction}}{\text{radioactivity in the total eluate}} \times 100$$

To provide a reference level, non-purified $^{67}GaCl_3$ was also used to label the DF–IgG conjugate.

Stability of ^{67}Ga Labeled IgG The ^{67}Ga-DF–IgG conjugate (500 μg/ml, labeled with ^{67}Ga-LS) was diluted 10000-fold with PBS containing 200 μg/ml of IgG and left to stand for 24 h at 4 °C. The radiochemical purity was measured before and after standing, as described above.

Results and Discussion

The method of selective Ga^{3+} extraction from an Fe containing solution was originally developed for the chemical analysis of Ga^{3+} ion. The most likely impurity in the radiogallium solution affecting Ga-labeling of DF–IgG was considered to be Fe^{3+},[4] so this separation method was of special interest. In fact, as shown in Table I, for an Fe solution prepared according to the same protocol as that for ^{67}Ga extraction, the Fe extraction rate was lower than 1%, when the ascorbic acid concentration was above 10^{-1} mol/l. This was because Fe^{2+} was produced by the ascorbic acid reduction of Fe^{3+}, and its extraction ratio with butyl acetate was low. Fe contamination increased with the decrease of ascorbic acid concentration from 10^{-2} to 10^{-4} mol/l. Thus, the optimum concentration of ascorbic acid to prevent Fe contamination was determined to be 100 mM. Using this concentration, Fe contamination of the

TABLE I. Effect of Changes in the Concentration of Added Ascorbic Acid on the Iron Extraction Rate from 5 N HBr Solution under the Conditions Used in Preparation of ^{67}Ga-LS

	Concentration of added ascorbic acid (mol/l)				
	1	10^{-1}	10^{-2}	10^{-3}	10^{-4}
Extraction rate (%)	0.69 ±0.057	0.60 ±0.117	1.05 ±0.119	13.7 ±0.990	68.7 ±0.045

Mean ± 1 S.D., $n=5$.

TABLE II. Effect of ^{67}Ga Purification on Its Labeling Efficiency for the DF–IgG Conjugate

DF–IgG Concentration (μg/ml)	^{67}Ga-LS	$^{67}GaCl_3$
50	58.5±1.27	25.2±3.58
100	85.8±3.99	55.7±2.04
200	95.6±0.88	85.8±4.15
500	96.5±0.47	93.2±1.28
1000	96.6±0.59	93.9±0.59

Mean ± 1 S.D., $n=5$.

^{67}Ga-LS was not detectable, when 0.1 M HCl prepared from Fe-free HCl was used as a reference standard. The ^{67}Ga extraction ratio from ^{67}Ga citrate solution was 95.2±0.43% ($n=5$).

Using the ^{67}Ga-LS, DF–IgG labeling studies were performed. Table II shows the radiochemical purity of ^{67}Ga-DF–IgG conjugates prepared with the ^{67}Ga-LS and the non-purified $^{67}GaCl_3$. The ^{67}Ga-LS showed a higher radiochemical purity than the non-purified $^{67}GaCl_3$, especially at lower DF–IgG concentrations. A ^{67}Ga-DF–IgG conjugate with a specific radioactivity of 872± 56 MBq/mg and a high radiochemical purity of 94.0± 2.0% was formed when 20 μl of ^{67}Ga-LS containing 9287±705 MBq/ml ^{67}Ga was mixed with 200 μl of IgG solution (1000 μg/ml IgG) and allowed to stand for 1 h (mean ± 1 S.D. of 5 experiments). This is a higher specific radioactivity than that of ^{125}I (540 MBq/mg),[6] and it reached one-tenth of the theoretical maximum value calculated from the specific radioactivity of ^{67}Ga and the DF–IgG conjugation level (DF/IgG=0.8).

The effect of dilution on the radiochemical purity of the ^{67}Ga-LS was also studied; the purity of ^{67}Ga-DF–IgG before and after 10000-fold dilution and standing for 24 h was 96.0±0.15% and 94.4±0.15%, respectively ($n=3$). Thus, dilution and standing induced no significant changes in the radiochemical purity of ^{67}Ga-DF–IgG. Our previous *in vivo* study showed a higher stability of ^{67}Ga-DF–protein (HSA, fibrinogen) than of ^{125}I-protein. Thus, the stability of ^{67}Ga-DF–IgG appears to be sufficient for *in vitro* application also.

The present study showed that IgG could be labeled with the metal radionuclide ^{67}Ga with a higher specific radioactivity than that achieved using ^{125}I. Unlike the situation when using ^{125}I, labeling could be completed by the simple mixing of the Ga-LS and DF–IgG conjugate solutions, without the need for further purification. Further studies using generator-produced ^{68}Ga ($T_{1/2}=68$ min) should provide on-site labeling as well as an even higher specific radioactivity (55.5 GBq/mg IgG, estimated from the present data and half life ratio of ^{68}Ga/^{67}Ga). In addition, the use of a radionuclide with a shorter half-life will contribute to the reduction of radioactive waste disposal problems. The method presented should provide a new radioimmunoassay system with a higher sensitivity which is also easier to handle. Further applications in the labeling of biologically active proteins also appear to be promising areas for investigation.

References

1) A. Yokoyama, Y. Ohmomo, K. Horiuchi, H. Tanaka, K. Yamamoto, Y. Ishii and K. Torizuka, *J. Nucl. Med.*, **23**, 909 (1982).
2) Y. Ohmomo, A. Yokoyama, J. Suzuki, H. Tanaka, K. Yamamoto, K. Horiuchi, Y. Ishii and K. Torizuka, *Eur. J. Nucl. Med.*, **7**, 458 (1982).
3) Y. Ohmomo, A. Yokoyama, Y. Yamauchi, K. Horiuchi, H. Saji, C. Tanaka and K. Torizuka, *Int. J. Nucl. Med. Biol.*, **12**, 47 (1985).
4) K. Takahashi, N. Ueda, M. Hazue, Y. Ohmomo, A. Yokoyama, T. Suzuki and K. Torizuka, "Radiopharmaceuticals and Labeled Compounds," IAEA, Vienna, 1985, pp. 471—482.
5) T. Furukawa, K. Endo, Y. Ohmomo, H. Sakahara, M. Koizumi, T. Nakashima, M. Fukunaga, R. Morita, H. Hirai, A. Yokoyama and K. Torizuka, *Kakuigaku*, **23**, 337 (1986).
6) W. C. Eckelman, C. H. Paik and R. C. Reba, *Cancer Res.*, **40**, 3036 (1980).
7) T. Emery and P. B. Hoffer, *J. Nucl. Med.*, **21**, 935 (1980).
8) P. Collins and H. Diehl, *Anal. Chim. Acta*, **22**, 125 (1960).