A New Zinc-62/Copper-62 Generator as a Copper-62 Source for PET Radiopharmaceuticals

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A new $^{62}Zn/^{62}Cu$ generator system was designed to provide a readily available ^{62}Cu source for positron emission tomographic radiopharmaceuticals, based on the differences of complex formation between Zn and Cu. Noncarrier added ^{62}Cu was selectively eluted as a glycine complex from ^{62}Zn-adsorbed cation-exchange resin (CG-120, Amberlite), when a glycine solution (200 mM) was used as the eluant. The elution efficiency and radionuclidic purity of ^{62}Cu were 70% and >99.9%, respectively. Copper in glycine solution showed rapid complex formation with dithiosemicarbazone, which is one of the established Cu-binding agents for bifunctional chelating radiopharmaceutical.

T he zinc-62-copper-62 (^{62}Zn-^{62}Cu) [^{62}Zn: $T_{1/2}$ = 9.2 hr, ^{62}Cu: $T_{1/2}$ = 9.8 min, (*1*)] generator system is considered to be one of the most desirable labeling sources for radiopharmaceuticals (*2*). Copper readily forms complexes with various ligands (*3*), and should be the ideal labeling nuclide of a number of previously reported compounds, such as [^{62}Cu]HSA-DTS (*4*), [^{64}Cu]TPA-DTS (*5*) and [^{67}Cu]PTSM (*6*). Moreover, Cu is well known to be an essential metal in various enzymes (*7*), so that generator-produced ^{62}Cu could potentially also be useful for Cu-related biochemical studies in vivo. Several types of $^{62}Zn/^{62}Cu$ generator systems have been reported (*8–10*), but their elution conditions (low pH, high osmotic pressure, carrier Cu addition, etc.) as well as their complicated labeling procedures limited their clinical application. Therefore, we designed a new $^{62}Zn/$ ^{62}Cu generator system based on the difference between Zn and Cu complex formation, to allow selective ^{62}Cu elution in physiologic as well as noncarrier Cu added conditions. For this purpose, amino acids and their derivatives known to act as ligands forming stable Cu complexes under physiologic conditions (*11*) were selected, and their ability to allow effective Cu elution from Zn-adsorbed resin was studied using nonradioactive Zn/Cu as well as $^{62}Zn/^{62}Cu$.

MATERIALS AND METHODS

Nonradioactive standard Cu and Zn solution for atomic absorption spectrophotometry (1000 ppm, Wako Pure Chemicals Co. Ltd., Japan) were diluted with distilled water. For the column beads, strong cation-exchange resin (CG-120, Amberlite) was conditioned by the conventional method. As elution agents, the following ligands were used: the amino acids glycine, isoleucine, valine, methionine, phenylalanine, alanine, cysteine, glutamic acid, aspartic acid, lysine and histidine were purchased from Wako Pure Chemicals Co. Ltd., Japan. The peptides diglycine, triglycine, glycyl-glycyl-histidine and glycyl-histidyl-lysine were from the Peptide Research Foundation, Japan. The synthetic amino acid derivatives diethylenetriamine-N,N,N',N'',N''-pentaacetic acid, iminodiacetic acid, ethylene diamine-N,N'-diacetic acid and ethylenediamine-N,N,N',N'-tetraacetic acid were from Dojindo Laboratories, Japan.

For ligand exchange studies, kethoxal-bis(N-methylthiosemicarbazone) [KTSM, synthesized according to Petering's method (12)] was used as an example of a Cu-binding radiopharmaceutical compound.

Production of ^{62}Zn

The ^{62}Zn was obtained by reaction of ^{63}Cu(p, 2n)^{62}Zn. A Cu (natural Cu, ^{63}Cu: 69.2%)-electroplated aluminum target

plate was bombarded with 26 MeV protons in a CS-30 cyclotron (The Cyclotron Corporation). The target was left to stand overnight to allow the decay of short-lived radionuclides; subsequently ^{62}Zn purification was performed. The Cu on the target was dissolved with 3 N HCl (25 ml), 10 ml of methanol was added and the solution was applied to an anion exchange resin column (AG 1-X8, 100–200 mesh, Cl form). The column was washed with about 300 ml of eluant (3N HCl:methanol = 7:3), and then ^{62}Zn was eluted with distilled water. The carrier Cu concentration in the ^{62}Zn eluate was < 2 μg/ml (determined colorimetrically using chromazurol S as the chelating agent).

Determination of Nonradioactive Cu and Zn

Cu and Zn concentrations were measured by atomic absorption spectrophotometry (AA-630-01, Shimadzu, Japan) using an acetylene-air flame.

Ligand Survey Studies Using Nonradioactive Cu and Zn (Batch Method)

Cu and Zn (1 μg/ml each, 5 ml) solutions were added to 500 mg of CG-120 resin and incubated for 30 min at room temperature. Filtration of the resin was performed on a glass filter, followed by washing with water, washing with distilled ethanol and drying. The Cu and Zn concentrations of the filtrate were measured to confirm the complete adsorption of Cu and Zn. Five hundred milligrams of the Cu,Zn-adsorbed resin was mixed with 5 ml of ligand solution for 30 min. The eluate of this mixture was collected, and the Cu and Zn concentrations were measured using atomic absorption spectrophotometry.

^{62}Zn/^{62}Cu Column Studies

CG-120 resin was packed into a plastic column (0.8 × 0.5 cm). A ^{62}Zn solution (3.7–11.1 MBq, in 2 ml water, carrier Cu derived from the target: < 2 μg/ml, pH 5.0) was loaded to adsorb ^{62}Zn and the column was then washed with water. The radioactivity of the eluate was measured to confirm the complete adsorption of ^{62}Zn. The column was next washed with ~10 ml of ligand solution to change the solvent and to wash out the carrier Cu derived from the target. Following this, it was left for 30 min to allow radio-equilibration of ^{62}Zn/^{62}Cu. The washings were collected (2 ml × 5 fractions) and tested by atomic absorption spectrophotometry to confirm complete washout of the carrier Cu. Eluate was collected (2 ml) and the radioactivity was measured, as described below. As a control, a previously reported generator system (9) was also evaluated using the same method.

Measurement of Radioactivity

The radioactivity of the eluate was measured immediately after elution using a Radioisotope Calibrator (Capintec Co. Ltd. CRC-7, Ramsey, NJ) and the elution efficiency was calculated from the theoretical ^{62}Cu content in the column. Part of the eluate was diluted properly and its radioactivity was measured with a well-type scintillation counter (ARC-301, Aloka Co. Ltd., Japan, 511-keV range). Measurements were performed both immediately (as ^{62}Cu) and 1 day after elution (as ^{62}Cu equilibrated with leaked ^{62}Zn) to calculate the radionuclidic purity of ^{62}Cu.

Ligand Exchange Studies

The ligand exchange ability of Cu in glycine solution was studied by spectrophotometry, using nonradioactive Cu. Glycine solution containing 1 μg/ml of Cu was mixed with KTSM solution (final KTSM conc: 0.1 mM). Cu-KTSM complex formation was detected spectrophotometrically (330-S, Hitachi Co. Ltd., Japan). As controls, reaction mixtures without Cu, glycine or KTSM were prepared and measured. Based on these results, noncarrier added ^{62}Cu-labeling of KTSM was performed. Copper-62 eluate (in 200 mM glycine) was added to 0.1 ml of KTSM (2.0 mM in DMSO) and mixed. The labeling efficiency was determined using reversed-phase HPLC [Cosmosil C18 column: 4.6 × 50 mm + 4.6 × 150 mm (Nakarai Tesque, Japan), acetonitrile:water = 1:1, 1 ml/min] having both a uv detector and an NaI-scintillation detector.

RESULTS AND DISCUSSION

Elution Ligand Survey Studies Using Nonradioactive Cu and Zn

Ligand structure and concentration are important factors affecting Cu elution as well as Zn leakage. Therefore, using the batch method, we investigated various

TABLE 1

Copper Elution and Zn Leakage from Nonradioactive Zn/Cu Adsorbed Resin (CG-120) with Various Elution Ligands

Ligand (Conc.*)	% Cu elution[†] (1 s.d.)	% Zn leakage[†] (1 s.d.)
Gly (200 mM)	75.0 (3.0)	2.2 (1.3)
Ile (100 mM)	72.6 (5.8)	2.0 (2.0)
Val (100 mM)	53.8 (3.4)	N.D.[‡] (– –)
Met (100 mM)	50.0 (5.3)	3.5 (0.5)
Phe (100 mM)	27.3 (1.8)	1.3 (0.4)
Ala (100 mM)	3.1 (0.3)	0.7 (0.4)
Cys (100 mM)	82.9 (8.1)	2.2 (1.0)
Glu (100 mM)	64.5 (2.1)	0.4 (0.1)
Asp (100 mM)	36.7 (3.5)	3.9 (1.1)
Lys (500 mM)	7.0 (0.3)	3.6 (0.4)
His (100 mM)	3.3 (2.3)	0.1 (0.3)
GG (100 mM)	65.4 (4.1)	N.D.[‡] (– –)
GGH (5 mM)	104.4 (15.6)	3.0 (2.3)
GGG (100 mM)	26.2 (1.1)	N.D.[‡] (– –)
GHL (10 mM)	N.D.[‡] (– –)	N.D.[‡] (– –)
DTPA (0.2 mM)	87.5 (12.1)	3.8 (0.7)
IDA (50 mM)	65.5 (8.0)	1.3 (0.4)
EDDA (30 mM)	73.7 (12.2)	0.5 (0.9)
EDTA (0.1 mM)	42.9 (5.4)	1.6 (0.4)

* Data obtained under optimum ligand concentration conditions for each ligand.
[†] Average (1 s.d.) of three to five experiments.
[‡] Not detectable.

ligands to determine the optimum concentration that allowed high Cu elution with little Zn leakage. Table 1 shows the Cu elution and Zn leakage under the optimum ligand concentration conditions for each elution ligand. Table 2 shows the effect of the glycine concentration on Cu elution and Zn leakage. Under optimum ligand concentration conditions, selective Cu elution was obtained with all ligands, except for alanine, lysine, histidine, and glycyl-histidyl-lysine. Among the peptides, glycyl-glycyl-histidine (synthesized as a model of the selective Cu-binding site of albumin) gave greater Cu elution than other peptides. Among the synthesized amino acid derivatives, DTPA was the most effective.

$^{62}Zn/^{62}Cu$ Column Elution Studies

Four candidates (glycine, cysteine, glycyl-glycyl-histidine and DTPA) were selected from the results of batch studies and used in the $^{62}Zn/^{62}Cu$ column elution studies (Table 3). A comparative study was performed using the generator system reported by Robinson. Under our experimental conditions, Robinson's generator system showed a slightly lower elution efficiency and radionuclidic purity of ^{62}Cu than in the reported data, probably because of differences of column size and/or elution volume. With the glycine system, both the radionuclidic purity and the efficiency of elution of ^{62}Cu were higher than with Robinson's system. The cysteine system also yielded a high ^{62}Cu radionuclidic purity, but there was a slight problem with elution efficiency. In the case of the glycyl-glycyl-histidine system, the elution efficiency of ^{62}Cu was higher than with the cysteine system, but ^{62}Zn leakage was a high 3.3%. With the DTPA system, both radionuclidic purity and elution efficiency were poor. This discrepancy between the results of nonradioactive Zn/Cu and $^{62}Zn/^{62}Cu$ studies may have been due to variations in metal concentration. For the clinical application of a $^{62}Zn/^{62}Cu$ generator system, a high radionuclidic purity of ^{62}Cu in the eluate is important to reduce the radiation dose to patients. In addition, considering the cost of ^{62}Zn pro-

TABLE 3
Radionuclidic Purity and Recovery of ^{62}Cu from Generator System

Elution ligand	Radionuclidic purity*	Recovery[†]
Gly	99.8	74.3
Cys	99.9	43.8
GGH	96.7	52.3
DTPA	34.3	—
HCl[‡]	99.5	—
NaCl[‡]	98.9	59.6

* $^{62}Cu/(^{62}Cu + ^{62}Zn)$ in the eluate (cpm) × 100.
[†] Eluted $^{62}Cu/produced$ ^{62}Cu (MBq) × 100.
[‡] Robinson's method (9).

duction and the necessity for radiation protection of the generator, a high degree of ^{62}Cu elution from a column containing little ^{62}Zn (i.e., high elution efficiency for ^{62}Cu) is preferable. Therefore, the glycine system was considered to be the most useful of the tested systems.

A repeat elution study was performed with the glycine generator system to evaluate the reproducibility of ^{62}Cu elution and the effect of radiolysis. Based on the half-lives of ^{62}Zn and ^{62}Cu, the optimal elution interval for reaching the highest ^{62}Cu elution was calculated to be 58 min. However, since 90% of the maximum is reached by 30 min, this was considered to be sufficient for practical purpose. Therefore, elution was performed nine times at 30–40 min intervals. Under these conditions, the radionuclidic purity of ^{62}Cu was 99.93 ± 0.05% (average ± 1 s.d.) and the elution efficiency of ^{62}Cu was also adequate and stable (69.2 ± 6.07%). Although radiolysis of glycine might have occurred in the generator column, no detectable change in ^{62}Cu elution was observed, probably because of the rather high glycine concentration. To confirm the lack of any biological effect on radiolysis product(s), however, further studies would be required.

The carrier-Cu concentration in the ^{62}Cu eluate from the glycine generator system was also studied in the repeat elution studies. When ~4 μg of carrier Cu derived from the target was loaded into the column, most of the carrier Cu was recovered within the early washings and no carrier Cu was detectable in the later eluates (Fig. 1).

Ligand Exchange Studies

To achieve good labeling efficiency by ligand exchange reaction, the labeling ligand must have a higher complex forming ability and higher complex formation rate constant for Cu than the elution ligand used. In this study, we selected kethoxal-bis(N-methylthiosemi-
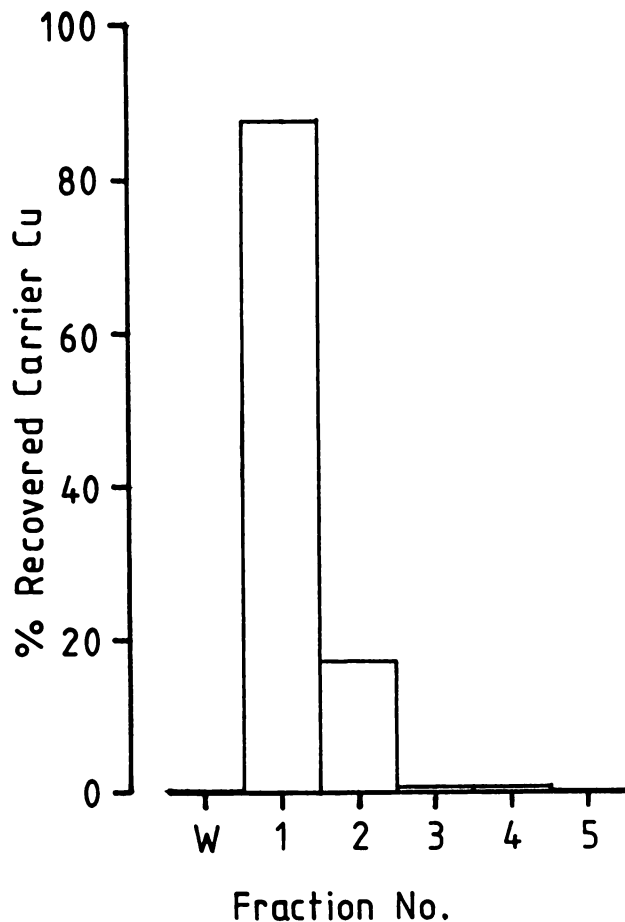
TABLE 2
Effect of Glycine Concentration on Cu Elution and Zn Leakage from Nonradioactive Zn/Cu Adsorbed Resin (CG-120)

Glycine conc.	% Cu elution* (1 s.d.)	% Zn leakage* (1 s.d.)
1 mM	N.D.[†] (– –)	N.D.[†] (– –)
10 mM	2.2 (0.4)	N.D.[†] (– –)
50 mM	15.7 (1.3)	N.D.[†] (– –)
100 mM	53.0 (6.1)	1.2 (1.4)
200 mM	75.0 (3.0)	2.2 (1.3)
1000 mM	96.1 (0.5)	1.6 (0.4)

* Average (1 s.d.) of three to five experiments.
[†] Not detectable.

FIGURE 1
Carrier Cu elution profile from ^{62}Zn-adsorbed glycine generator system. Volumes of the washing water and glycine solution (200 m*M*) were 2 ml (W) and 2 × 5 ml (Fractions No. 1 to 5), respectively.
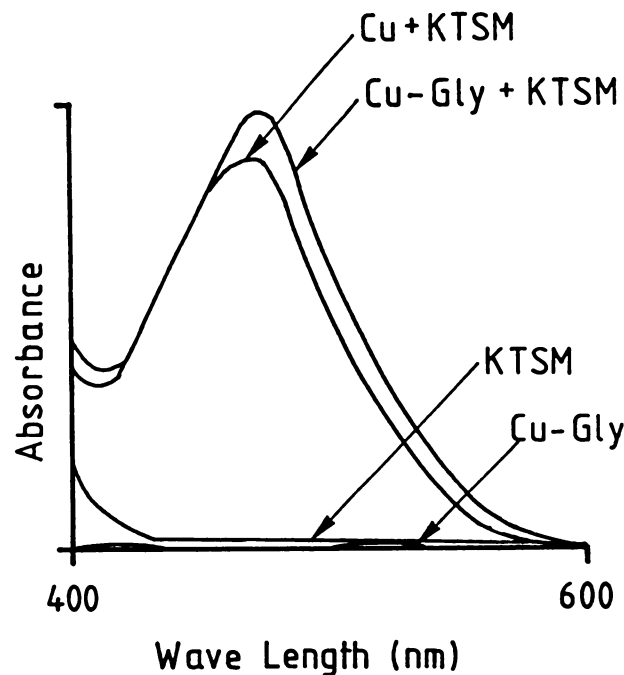

FIGURE 2
Effect of glycine on [Cu]KTSM complex formation (nonradioactive Cu studies).

this generator system, noncarrier added ^{62}Cu could be easily separated from Zn. This ^{62}Cu eluate, having a physiological form of Cu, may be useful not only for Cu-related biologic studies in vivo, but also for radiopharmaceutical labeling by simple ligand exchange reactions.

carbazone) (KTSM), which is a chelating agent used for ^{64}Cu, ^{67}Cu, or ^{99m}Tc labeling (*2,5,13,14*). Even when glycine was present as an elution ligand, [Cu]KTSM complex formation occurred (absorption at 480 nm, in Fig. 2). Also, in the noncarrier added ^{62}Cu-labeling studies, ^{62}Cu readily formed complexes with KTSM; most of the ^{62}Cu radioactivity was detected in the [Cu]KTSM fraction, but had no uv absorbance (Fig. 3). This was a good indication of the degree of [^{62}Cu] KTSM complex formation under noncarrier Cu added conditions. Thus, it was shown that Cu obtained from the glycine generator system should be useful as a ^{62}Cu labeling source for various radiopharmaceuticals having strong Cu-binding sites.

CONCLUSION

A new ^{62}Zn/^{62}Cu generator system was developed using a glycine (200 m*M*) solution as the eluant. Using
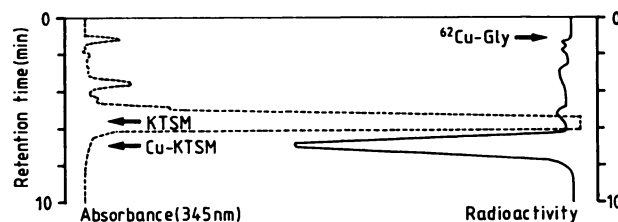
ACKNOWLEDGMENT

FIGURE 3
HPLC elution profile of [^{62}Cu]KTSM. Continuous line: radioactivity, broken line: uv absorption at 345 nm. Arrows indicate the retention time of the reference samples.

REFERENCES

1. In: Lederer CM, Shirley VS eds. *Table of isotopes.* 7th ed. New York: John Wiley, 1978.
2. Green MA, Klippenstein DL, Tennison JR. Copper(II) bis(thiosemicarbazone) complexes as potential tracers for evaluation of cerebral and myocardial blood flow with PET. *J Nucl Med* 1988; 29:1549–1557.
3. In: Smith RM, Martell AE, eds. *Critical stability constants.* Vol. 1–4. New York: Plenum, 1975.
4. Fujibayashi Y, Matsumoto K, Konishi J, Yokoyama A. Generator produced positron emitting ^{62}Cu-labeled human serum albumin for regional plasma volume measurement [Abstract]. *J Nucl Med* 1988; 29:930.
5. Yokoyama A, Hosotani T, Arano Y, et al. Development of neutral and bifunctional radiopharmaceuticals using ^{62}Cu-dithiosemicarbazone (DTS) chelate; basic studies on ^{64}Cu chelates. *Radioisotopes* 1986; 35:249–255.
6. Green MA. A potential copper radiopharmaceutical for imaging the heart and brain: copper-labeled pyruvaldehyde bis(N^4-methylthiosemicarbazone). *Nucl Med Biol* 1987; 14:59–61.
7. O'Dell BL. Biochemistry and physiology of copper in vertebrates. In: Prasad AS, Oberleas D, eds. *Trace elements in human health and disease.* Vol. 1. Zinc and copper. Academic Press, New York: 1976:391–413.
8. Yagi M, Kondo K. A ^{62}Cu generator. *Int J Appl Radiat Isot* 1979; 30:569–570.
9. Robinson GD, Zielinski FW, Lee AW. The zinc-62/copper-62 generator: a convenient source of copper-62 for radiopharmaceuticals. *Int J Appl Radiat Isot* 1980; 31:111–116.
10. Ueda N, Nakamoto S, Tanaka Y, et al. Production of Zn-62 and development of Zn-62/Cu-62 generator system [Abstract]. *J Nucl Med* 1983; 24:P124.
11. Martin RB. Complexes of alpha-amino acids with chelatable side chain donor atoms. In: Sigel H, ed. *Metal ions in biological systems.* Vol. 9. New York: Marcel Dekker, 1979:1–39.
12. Petering HG, Buskirk HH, Underwood GE. The antitumor activity of 2-keto-3-ethoxybutyraldehyde bis(thiosemicarbazone) and related compounds. *Cancer Res* 1964; 24:367–372.
13. Yokoyama A. Recent progress in the development of bifunctional radiopharmaceuticals. *Radiopharm Label Comp* 1985; 251–265.
14. Arano Y, Yokoyama A, Magata Y, et al. Synthesis and evaluation of a new bifunctional chelating agent for ^{99m}Tc labeling proteins: p-carboxyethylphenyl-glyoxal-di(N-methylthiosemicarbazone). *Int J Nucl Med Biol* 1986; 12:425–430.