Coordinating Radiometals of Copper, Gallium, Indium, Yttrium, and Zirconium for PET and SPECT Imaging of Disease

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1. Introduction

Molecular imaging is the visualization, characterization, and measurement of biological processes at the molecular and cellular levels in humans and other living systems. Molecular imaging agents are probes used to visualize, characterize, and measure biological processes in living systems. These two definitions were put forth by the Society of Nuclear Medicine (SNM) in 2007 as a way to capture the interdisciplinary nature of this relatively new field. The emergence of molecular imaging as a scientific discipline is a result of advances in chemistry, biology, physics, and engineering, and the application of imaging probes and technologies has reshaped the philosophy of drug discovery in the pharmaceutical sciences by providing more cost-effective ways to evaluate the efficacy of a drug candidate and allow pharmaceutical companies to reduce the time it takes to introduce new therapeutics to the marketplace. Finally, the impact of molecular imaging on clinical medicine has been extensive since it allows a physician to diagnose a patient's illness, prescribe treatment, and monitor the efficacy of that treatment noninvasively.

Single-photon emission computed tomography (SPECT) and positron emission tomography (PET) were the first molecular imaging modalities used clinically. SPECT requires the use of a contrast agent labeled with a γ-emitting radionuclide, which should have an ideal γ energy of 100−250 keV. These γ rays are recorded by the detectors of a dedicated γ camera or SPECT instrument and after signal processing can be converted into an image identifying the localization of the radiotracer. PET requires the injected radiopharmaceutical to be labeled with a positron-emitting radionuclide. As the radionuclide decays, it ejects a positron from its nucleus, which travels a short distance before being annihilated with an electron to release two 511 keV γ rays 180° apart that are detected by the PET scanner (Figure 1). After sufficient acquisition time, the data are reconstructed using computer-based algorithms to yield images of the radiotracer's location within the organism. Compared with SPECT, PET has greater advantages with respect to sensitivity and resolution and has been gaining in clinical popularity, with the number of PET-based studies expected to reach 3.2 million by 2010.[1] While SPECT and PET technologies have

Thaddeus J. Wadas was born in Nanticoke, PA, in 1974. He received his B.S. degree in Biology from King's College, Wilkes-Barre, PA, in 1996 and, while working in the environmental science industry, completed his second major in Chemistry in 1998. After completing his second major, he pursued graduate studies at the University of Rochester, Rochester, NY, where he received his M.S. and Ph.D. degrees in Chemistry under the supervision of Richard Eisenberg. His Ph.D. work focused on the synthesis and characterization of luminescent Pt(II) acetylide complexes for photoinduced charge transfer and light-to-chemical energy conversion. On completion of his Ph.D., he moved to the Washington University School of Medicine in St. Louis, MO, to pursue postdoctoral studies with Carolyn Anderson and develop targeted radiopharmaceuticals for diagnostic imaging and radiotherapy. In 2005, he was the recipient of a National Institutes of Health National Research Service Award (NRSA) Fellowship to study bone metastasis imaging with copper-64-labeled peptides, and in 2009 he was promoted to the position of Instructor at the School of Medicine. His current research interests include the application of combinatorial display methods to radiopharmaceutical development and understanding the respective roles gadolinium-based contrast agents and renal insufficiency play in the development of nephrogenic systemic fibrosis.

Edward H. Wong was born in Wuhan, China, in 1946 but was raised in Hong Kong where he discovered the joy of mixing chemicals at St. Louis High School. He then majored in chemistry at the University of California at Berkeley and obtained his Ph.D. doing boron hydride synthesis with William N. Lipscomb at Harvard University in 1974. After a postdoctoral stint with M. Frederick Hawthorne at the University of California, Los Angeles, he began his academic career at Fordham University in 1976 before moving to the University of New Hampshire in 1978. He has performed research in main group boron and phosphorus chemistry as well as metal−phosphine coordination chemistry. In recent years, with his colleague Gary Weisman he has focused on the coordination chemistry of cross-bridged tetraamine macrocycles. Together they have also explored applications of these chelators in copper-based radiopharmaceuticals with Carolyn Anderson and her research group.

Gary R. Weisman was born in Mason, Ohio, in 1949, receiving his primary and secondary education in the public school system there. He was interested in chemistry from a young age, working with his cousin Thomas J. Richardson in their substantial home laboratories. He earned his B.S. in Chemistry with Distinction at the University of Kentucky in 1971, carrying out research with Robert D. Guthrie. At the University of Wisconsin—Madison, he worked on conformational analysis of hydrazines and their radical cations under the mentorship of Stephen F. Nelsen, earning the Ph.D. in Organic Chemistry in 1976. After a postdoctoral stint with Donald J. Cram at the University of California, Los Angeles, in 1976−1977, he started his independent academic career at the University of New Hampshire, where he has enjoyed both teaching and research. He was Gloria G. and Robert E. Lyle Professor at UNH from 2005 to 2009 and has been the recipient of Outstanding Teaching awards in 1995 and 2009. He has been a visiting professor at the University of Wisconsin (1986), University of Bristol (1987, 1998), University of Melbourne (2005), and Australian National University (2005). He was a Wilsmore Fellow at the University of Melbourne in 2002. His research interests are in both physical organic and synthetic organic chemistry, with special emphasis on stereochemical aspects. His recent research has centered on the chemistry of polyaza molecules, ligand design and synthesis, and biomedical applications of coordination complexes. He has enjoyed a productive collaboration with Edward H. Wong for almost two decades and more recently with Carolyn J. Anderson.

been around for decades, their use remained limited because

the self-contained radionuclide generator, and the dedicated small animal or clinical SPECT and PET scanners to hospitals and research facilities has increased the demand for SPECT and PET isotopes.

Traditional PET isotopes such as ^{18}F, ^{15}O, ^{13}N, and ^{11}C have been developed for incorporation into small molecules, but due to their often lengthy radiosyntheses, short half-lives, and rapid clearance, only early time points were available for imaging, leaving the investigation of biological processes, which occur over the duration of hours or days, difficult to explore. With the continuing development of biological targeting agents such as proteins, peptides, antibodies and nanoparticles, which demonstrate a range of biological half-lives, a need arose to produce new radionuclides with half-lives complementary to their biological properties. As a result, the production and radiochemistry of radiometals such as Zr, Y, In, Ga, and Cu have been investigated as radionuclide labels for biomolecules since they have the potential to combine their favorable decay characteristics with the biological characteristics of the targeting molecule to become a useful radiopharmaceutical (Tables 1 and 2).[2]

The number of papers published describing the production or use of these radiometals continues to expand rapidly, and in recognition of this fact, the authors have attempted to present a comprehensive review of this literature as it relates

Carolyn Anderson was born in Superior, WI, in 1962, and remained there throughout her school years. In 1985, she graduated Summa Cum Laude with a B.S. in Chemistry from the University of Wisconsin—Superior. In 1984, she received a fellowship to attend the Summer School in Nuclear Chemistry at San Jose State University, and this is where her interest in nuclear and radiochemistry began. She pursued her Ph.D. in Inorganic Chemistry with Prof. Gregory R. Choppin at Florida State University, studying the electrochemistry and spectroscopy of uranium complexes in room-temperature molten salts. On completion of her Ph.D. in 1990, she moved to Washington University School of Medicine (WUSM) in St. Louis, MO, to carry out postdoctoral research with Prof. Michael J. Welch in the development of radiopharmaceuticals for PET imaging. In 1993, she was promoted to Assistant Professor of Radiology, and she is currently Professor in the departments of Radiology, Biochemistry & Molecular Biophysics, and Chemistry. Her research interests include the development of radiometal-labeled tumor receptor-based radiopharmaceuticals for PET imaging and targeted radiotherapy of cancer and cancer metastasis. She greatly enjoys the productive collaboration with Edward Wong and Gary Weisman on the development of novel chelation systems for attaching metal radionuclides to biomolecules for nuclear medicine imaging applications.

certain aspects of the production, coordination chemistry, or application of these radiometals,[2−18] very few exhaustive reviews have been published.[10,12] Additionally, this review has been written to be used as an individual resource or as a companion resource to the review written by Anderson and Welch in 1999.[12] Together, they provide a literature survey spanning 50 years of scientific discovery. To accomplish this goal, this review has been organized into three sections: the first section discusses the coordination chemistry of the metal ions Zr, Y, In, Ga, and Cu and their chelators in the context of radiopharmaceutical development; the second section describes the methods used to produce Zr, Y, In, Ga, and Cu radioisotopes; and the final section describes the application of these radiometals in diagnostic imaging and radiotherapy.

2. The Coordination Chemistry of Cu, Ga, Y, In, and Zr

2.1. General Considerations

The development of metal-based radiopharmaceuticals represents a dynamic and rapidly growing research area that requires an intimate knowledge of metal coordination chemistry and ligand design. This section of the review covers general considerations regarding the parameters that are important in developing stable, kinetically inert radiometal complexes that can be incorporated into radiopharmaceuticals. Additionally, the aqueous coordination chemistry of these metals and their coordination complexes that are most relevant to radiopharmaceutical development are discussed below.

Relevant properties in aqueous solution of the five metal cations covered in this review are presented in Table 3. The acidic cations Ga(III), In(III), and especially Zr(IV) present precipitation problems at neutral pH in the absence of suitable complex formation. In terms of plausible aqueous redox processes relevant to radiopharmaceutical applications, only Cu(II) and its complexes are susceptible to reduction chemistry, although the possibility of an ascorbic acid reduction of a ^{89}Zr(IV) complex has been postulated.[19] Based on Pearson's hard−soft acid−base theory, the tetravalent Zr(IV) is an extremely hard acidic cation, followed by Y(III), Ga(III), and In(III). The Cu(II) cation is considered a borderline acid.

Table 1. γ- and β-Emitting Radiometals

Isotope	$t_{1/2}$ (h)	production methods	decay mode	E_γ (keV)	E_β (keV)	ref
^{67}Cu	62.01	accelerator ^{67}Zn(n,p)	β^- (100%)	91, 93, 185	577, 484, 395	578
^{67}Ga	78.26	cyclotron	EC (100%)	91, 93, 185, 296, 388		578
^{90}Y	64.06	^{90}Sr/^{90}Y generator	β^- (72%)		2288	578
^{111}In	67.9	cyclotron, ^{111}Cd(p,n)^{111}n	EC (100%)	245, 172		578

Table 2. Positron-Emitting Radiometals

isotope	$t_{1/2}$ (h)	methods of production	decay mode	E_{β^+} (keV)	ref
^{60}Cu	0.4	cyclotron, ^{60}Ni(p,n)^{60}Cu	β^+ (93%) EC (7%)	3920, 3000 2000	578
^{61}Cu	3.3	cyclotron, ^{61}Ni(p,n)^{61}Cu	β^+ (62%) EC (38%)	1220, 1150 940, 560	578
^{62}Cu	0.16	^{62}Zn/^{62}Cu generator	β^+ (98%) EC (2%)	2910	578
^{64}Cu	12.7	cyclotron, ^{64}Ni(p,n)^{64}Cu	β^+ 19(%) EC (41%) β^- (40%)	656	578
^{66}Ga	9.5	cyclotron, ^{63}Cu(α,nγ)^{66}Ga	β^+ (56%) EC (44%)	4150, 935	578
^{68}Ga	1.1	^{68}Ge/^{68}Ga generator	β^+ (90%) EC (10%)	 1880, 770	578
^{86}Y	14.7	cyclotron, ^{86}Sr(p,n)^{86}Y	β^+ (33%) EC (66%)	2335, 2019 1603, 1248 1043	578

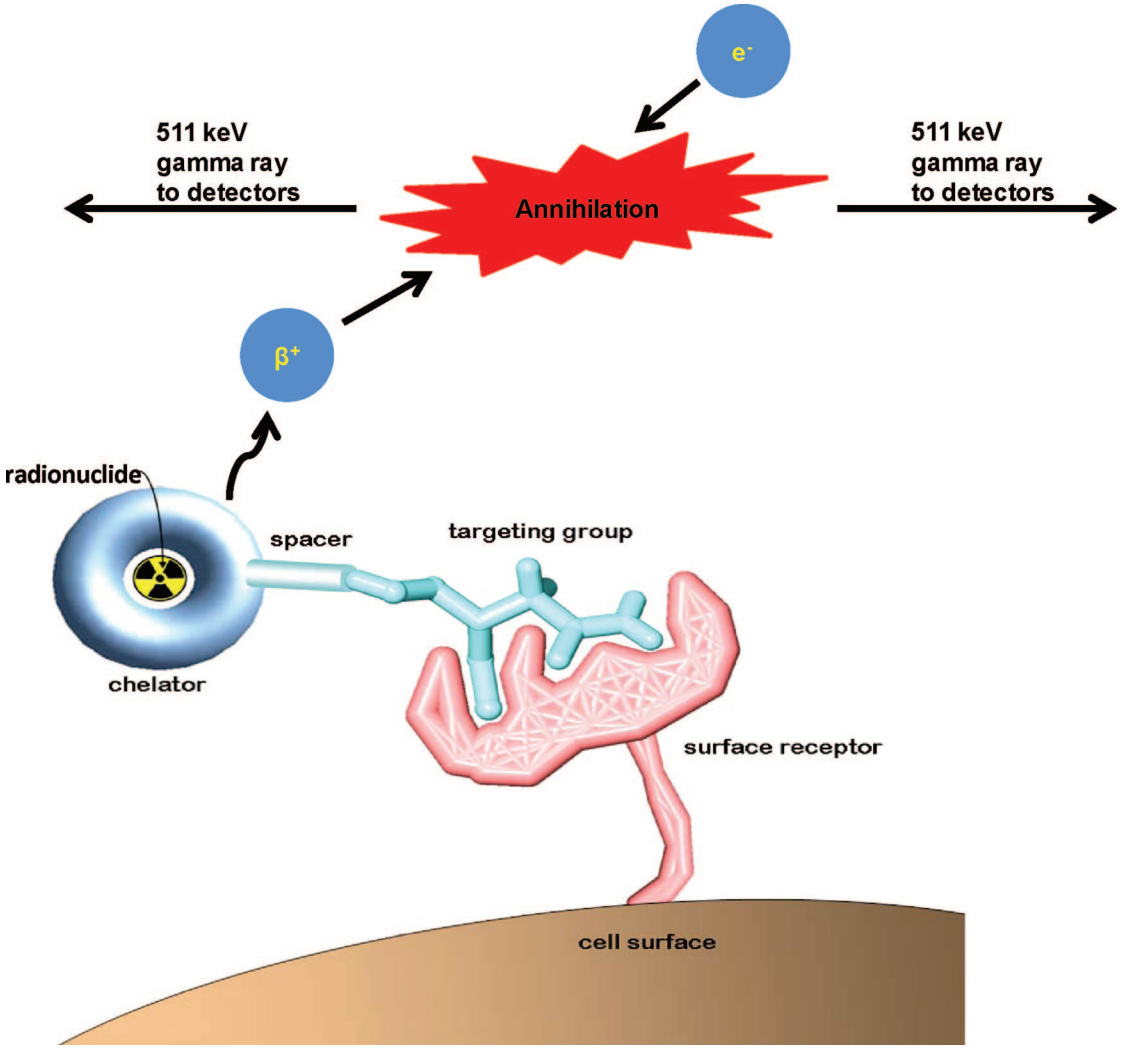

Figure 1. Cartoon depicting the fundamental principle of positron emission tomography (PET). As the targeting group interacts with the cell surface receptor, the positron-emitting radiometal decays by ejecting β^+ particles from its nucleus. After traveling a short distance in the electron-rich tissue, the positron recombines with an electron in a process called annihilation. During annihilation, the mass of the positron and electron are converted into two high-energy photons (511 keV γ rays), which are released approximately 180° apart to ensure that energy and momentum are conserved. Although attenuation is possible, these two γ rays are usually energetic enough to escape the organism and be collected by the detectors of a PET scanner.

Since the preponderance of radiometal complexes of note feature at least tetradentate ligands, we have restricted our discussion here to ligands with four or more donor sites coordinating the cation of interest. Rather than exhaustive coverage of all chelators of potential interest, we will discuss only selected representatives of the most-frequently reported ligands, especially those with more complete data of relevance. For the chosen representative chelators of each cation, we have listed available pertinent data on their denticity, coordination geometry, and thermodynamic stability. Where X-ray structural data are available, geometrical data on the coordination mode can provide useful insight into the "goodness of fit" for a specific cation−chelator pairing, the caveat being that actual solution structures or indeed number of species may be distinct from solid-state observations. For the four diamagnetic cations, solution NMR spectroscopic studies can be used to supplement X-ray data. Despite the difficulty of comparing stability constants of complex formation between ligands of different basicity and denticity, the listed log K_{ML}'s provide a convenient gauge of their relative affinities for a specific metal.

Table 3. Properties of Relevant Metal Cations

cation/electron configuration	ionic radius[a] (CN)	pK_a[b]	$k_{exchange}$,[c] s^{-1}	E_{red},[d] V, (acid)	hardness classification (I_A)[e]
Cu(II)/[Ar]3d^9	57 (4) 65 (5) 73 (6)	7.53	2×10^8	+0.34 (Cu^0) +0.16 (Cu^I)	borderline (2.68)
Ga(III)/[Ar]3d^{10}	47 (4) 55 (5) 62 (6)	2.6	7.6×10^2	−0.56 (Ga^0) −0.65 (Ga^{II})	hard (7.07)
In(III)/[Kr]4d^{10}	62 (4) 80 (6) 92 (8)	4.0	4.0×10^4	−0.34 (In^0) −0.49 (In^{II})	hard (6.30)
Y(III)/[Kr]	90 (6) 102 (8) 108 (9)	7.7	1.3×10^7	−2.37 (Y^0)	hard (10.64)
Zr(IV)/[Kr]	59 (4) 72 (6) 84 (8) 89 (9)	0.22		−1.54 (Zr^0)	hard

[a] Picometers. [579] [b] Aqua hydrated cation. [580] [c] In, H$_2$O. [581] [d] Versus NHE.

plexes are less kinetically inert than macrocyclic complexes of comparable stability.[22−26] By the same token, acyclic chelators typically have faster metal-binding kinetics compared with their macrocyclic analogues, which can be a significant advantage for shorter-lived radiometals.[27−30] There have been efforts to enhance the binding rate of macrocycles by incorporation of an acyclic polydentate pendant arm.[31]

A variety of *in vitro* assays of metal−chelator complex integrity can be found in the literature.[32−35] A popular assay of aqueous kinetic inertness is acid decomplexation. This has some relevance in biological environments that are relatively acidic such as in hypoxic tissues and certain cell vesicles. However, the extremely high acidities, for example, 1−5 M HCl, often required to decompose relatively inert complexes clearly have no parallel to any *in vivo* conditions. Nor can such data be relied upon, without considerations of other factors, as the sole predictor of biological behavior.[36] Typically, the decomplexation of Cu(II) complexes is readily monitored through their electronic spectra. Demetalation of the diamagnetic Ga(III), In(III), Y(III), and Zr(IV) complexes can usually be followed by proton and ^{13}C NMR spectroscopy in acidified D_2O solutions. Where feasible, ^{71}Ga, ^{115}In, and ^{89}Y NMR studies can also be undertaken.[37−39] Although detailed mechanistic investigations are sometimes reported, more commonly only pseudo-first-order half-lives are reported, which should only be used to rank inertness qualitatively. Nonetheless, such data remain useful as a preliminary indicator of the *in vivo* viability of specific metal-based radiopharmaceuticals.

Competition or challenge assays of complexes of interest with excess biometals and biochelators are relevant since their typical concentrations are orders of magnitude higher than the radiolabeled complex's, requiring high chelator selectivity for the radiometal. For example, copper homeostasis is tightly regulated in biology,[40] and as a result, a variety of copper-binding biomolecules are present in extracellular (serum albumin, ceruloplasmin, transcuprin, etc.) and intracellular (transporters, chaperones, metallothioneins, superoxide dismutase, cytochrome *c* oxidase, etc.) environments.[41−43] A viable Cu(II) chelator should therefore be both thermodynamically stable and kinetically inert to transchelation challenges by these species. Highly charged cations like Y(III) and Zr(IV) may also have high affinity for bone tissues, while the avid Ga(III) binding of transferrin is well-established.[44−46] Serum stability studies using radiometal-labeled chelator complexes or their bioconjugates are routinely used in inertness assays. These are readily monitored by radio-TLC, HPLC, and LC-MS techniques.[47−49] *In vitro* uptake studies using specific cell lines have also been carried out in many assays. While simulating extracellular environments to an extent, these studies cannot always accurately forecast *in vivo* behavior. Ultimately, studies of animal biodistribution and bioclearance using radiometal-labeled complexes or bioconjugates need to be carried out to obtain realistic data on their *in vivo* performance.

The following discussion of pertinent acyclic and macrocyclic ligands and their specific metal coordination chemistry is organized according to their denticity. Most of these ligands have been designed to provide a minimum of four donor atoms, usually also incorporating anionic sites for charge balance (See Figures 2 and 3). While all are given

complexes involving these ligands are also provided where appropriate. They were prepared from published CIF files using CrystalMaker 8.2 for Mac (CrystalMaker Software Ltd., Centre for Innovation & Enterprise, Oxford University Begbroke Science Park, Sandy Lane, Yarnton, Oxfordshire, OX5 1PF, UK; http://www.crystalmaker.com). Each atomic sphere is scaled to 0.4 times the covalent atomic radius, using the recently updated radii of Alvarez and co-workers.[50] In addition to the labeled and uniquely colored metal atoms, common elements are color coded as follows: C = gray, Cl = green, F = light green, N = blue, O = red, P = orange, and S = yellow. Hydrogen atoms have been omitted from the structures for clarity.

2.2. Aqueous Copper Coordination Chemistry

While +1 and +3 oxidation states are both accessible for copper in the presence of suitable donors, $3d^9$ Cu(II) remains the predominant state for radiocopper chemistry in protic media. The aqueous cupric ion was long believed to have a tetragonally distorted hexa-aqua structure until a 2001 report suggested only five-coordination.[51] Its water-exchange rate has been found to be very rapid compared with most common first-row transition metal cations and as a result it has relatively facile substitution chemistry despite having some crystal-field stabilization. This is usually ascribed to the Jahn−Teller distortion that elongates one or more of its coordinated ligands. Classified as a cation of borderline hardness, the high affinity of Cu(II) for borderline nitrogen donors is well-established. With a relatively small ionic radius of between 57 and 73 pm for coordination numbers 4−6, it is particularly suitable for the formation of five-membered chelate rings; indeed the chelate effect is epitomized in its ethylenediamine family of complexes.[52] The popular use of polyazamacrocycles, especially cyclen and cyclam, for strong binding of Cu(II) is a consequence of the added advantage of the macrocyclic effect,[53] as borne out by their extensive coordination literature.[54−57]

The importance of *in vivo* redox activation of metallodrugs incorporating Pt(IV), Ru(III), and Co(III) has received increasing attention.[58−61] The role of bioreduction in copper radiopharmaceutical efficacy has been intensively studied in their thiosemicarbazone complexes, especially Cu-ATSM (**L9**).[62−64] Convincing evidence for the formation and selective retention/decomplexation of Cu(I)-intermediates from Cu(II) precursors in hypoxic tissues has been presented.[65,66] Whether Cu(II)/Cu(I) bioreduction is also a viable pathway for irreversible *in vivo* radiocopper loss from other chelator complexes and their bioconjugates is an intriguing possibility. There is some compelling evidence for the deteriorated *in vivo* performance of related Cu(II) complexes differing only in their reduction propensities. Specifically, the "long arm" dicarboxyethyl pendant-armed Cu(II) complex of cross-bridged cyclam has an E_{red} almost 400 mV higher (or more positive) than that its carboxymethyl-armed analogue, Cu-CB-TE2A (**L57**).[67] The former has been found to exhibit significantly inferior bioclearance behavior despite very similar coordination geometry and acid-inertness. More structure−activity studies, including the consequence of protonation on reduction feasibility, are warranted. Most polyazamacrocyclic complexes of Cu(II), however, have rather negative reduction potentials that are well below the

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