Radiogallium as a probe for magnesium-binding sites*)

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Zusammenfassung

Der isomorphe Ersatz von Magnesium-Ionen durch Radiogallium kann als Sonde für Magnesium-Bindungs-Stellen verwendet werden. Die in-vivo-Verteilung von 67-Gallium zeigt eine Korrelation zwischen chemischer Zusammensetzung der Gewebe und ihrer Fähigkeit, Ionen zu ersetzen. Die Bedeutung der Eigenschaften von Gallium (Radius, elektrische Ladung) beim Zustandekommen dieses Phänomens wird diskutiert.

Summary

The isomorphous ionic replacement of magnesium by radio-gallium can be used as a probe for magnesium-binding sites. The *in vivo* distribution of 67-Gallium indicates a correlation between the chemical composition of the tissues and their ionic replacement capability.

The importance of the characteristics of the cation (radius and electrical charge) in this phenomenon will be discussed.

Résumé

Le remplacement ionique isomorphe par le radiogallium peut être utilisé comme moyen d'identification des sites de fixation du magnésium. La distribution *in vivo* du 67 Gallium indique une corrélation entre la composition chimique du tissu et la capacité de remplacement ionique.

L'importance des caractéristiques ioniques (taille et charge électrique) dans ce phénomène est discutée.

The concept of ionic exchange is of fundamental importance for the interpretation of the behavior of many metal ions in biology. According to the ionic model for chemical bonding [1, 2], in an exchange reaction leading to a complex formation there are stability sequences that depend on the nature of the ligand as well as on the free energy order related to the radius-ratio effect operating on the associated systems in both solid and solution. Consequently, cations with similar atomic or ionic radii would be expected to bind to the same sites, but the binding would be expected to be stronger when the ion possesses a higher charge or valence. A typical example of this ionic exchange is the replacement of calcium by the lanthanides.

In isomorphous replacement in minerals the radius is more important than the charge, but in biology in spite the fact that the radius is an important factor, a higher valence can play a more determinant role in this reaction. This seems to be the reason why the lanthanides, having a radius size close to that of calcium but a higher valence, show excellent characteristics of competitors (probes) for calcium sites [3, 4, 5]. Furthermore, in this type of reaction it should be pointed out that the competitor cation can act in two different ways (depending on the bonding strength): 1) as a substitute for the given ion it affects the functional sense of the biological system (like in Ni^{2+}- and Mn^{2+}-activation of some Mg^{2+}-sensitive enzymes [6, 7]), or 2) in a more drastic manner provoking a complete inhibition of the biological characteristics of that system. The first type of reaction is characteristic for ions with the same valence and the effects are concentration-dependent. The second one, very often irreversible, is presented by competitor ions of a higher valence and a consequent stronger binding which can modify the functional lability of the cation-biological system bonding.

During the study of radiogallium uptake by tumor-bearing animals we have observed a relationship between magnesium content of the tissue and its accumulation of radio gallium (table 1). In addition to this observation there is experimental evidence that complexes of several biological molecules such as nucleic acids, glycosaminoglycans, phospholipids and proteins with magnesium and calcium are able to exchange its cation by radiogallium [8]. Considering the similarity of atomic radii for magnesium and gallium (table 2), this phenomenon can easily be explained on terms of isomorphous replacement. On the other hand, calcium substitution, calcium radius is much larger, must be interpreted as the result of a previous process involving steric adjustment of the ligand to accomodate a cation of a different size.

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Tab. 1: Comparative values of calcium, magnesium and ^{67}Ga specific activity in experimental tumors[8].

	Adenocarcinoma BW 10232			Fibrosarcoma SaD2		
	A	B	C	A	B	C
Tumor	3.7 ± 0.3*	11.6 ± 1.6*	1.9	4.9 ± 1.7*	12.7 ± 1.1*	4.0
Liver	1.6 ± 0.4	28.3 ± 1.5	3.0	1.5 ± 0.3	24.2 ± 3.0	6.1
Spleen	2.0 ± 0.1	19.3 ± 1.3	2.9	2.8 ± 1.2	14.8 ± 4.2	5.6
Kidney	3.1 ± 0.3	13.2 ± 0.8	2.2	4.3 ± 0.6	14.5 ± 1.7	4.3
Lung	5.2 ± 0.8	7.6 ± 1.1	1.8	7.0 ± 1.6	7.3 ± 1.6	3.0
Stomach	6.5 ± 1.2	12.6 ± 1.3	2.0	16.0 ± 1.7	13.8 ± 1.0	3.2
Blood	2.9 ± 0.4	2.1 ± 0.7	1.0	3.1 ± 0.9	1.3 ± 0.9	1.0
Number of animals : for radiochemical	100			60		
for chemical analyses	20			20		

A = Calcium concentration (μEq/g wet tissue)
B = Magnesium concentration (μEq/g wet tissue)
C = ^{67}Ga specific activity when blood ^{67}Ga specific activity equal to 1.0
* = mean value ± standard deviation

Tab. 2: Atomic radii of cations participating in ionic replacement reactions.

	ATOMIC WEIGHT	ATOMIC NUMBER		ATOMIC RADIUS Å
MAGNÉSIUM	24,32	12	Mg^{2+}	0,65
GALLIUM	69,72	31	Ga^{3+}	0,62
CALCIUM	40,08	20	Ca^{2+}	0,99
SCANDIUM	44,96	21	Sc^{3+}	0,81
YTTRIUM	88,91	39	Y^{3+}	0,93
LANTHANUM	138,91	57	La^{3+}	1,15
CERIUM	140,12	58	Ce^{3+}	1,11
PRASEODYMIUM	140,91	59	Pr^{3+}	1,09

The steric fitting by modification of a flexible 'hole' may explain the binding of gallium to calcium sites, and in certain cases that of calcium to magnesium sites (figure 1). This last reaction is involved in the precipitation of calcium carbonate, oxalate, phosphate and even fluoride commonly occurring in biological systems and due to the smaller solubility product of the calcium salts (radius-ratio effect). It seems likely that the replacement by calcium of magnesium bound to fibrous protein structures outside cells can act triggering the crystalization, possibly using sulfate and carboxylate groups of outer membrane. This suggest that the calcification may be a consequence of the radius-ratio effect as much as is the binding of calcium to outer molecules of cells.

On the basis of the apparent relationship between magnesium concentration in the tissue and radiogallium accumulation we have thought to use the widely available ^{67}Ga as a probe for magnesium sites. This choice over the use of ^{28}Mg is a consequence of the commercial unavailability of this radioactive magnesium. On the other hand, the physical characteristics of ^{67}Ga (table 3) indicate its suitability for biological studies.

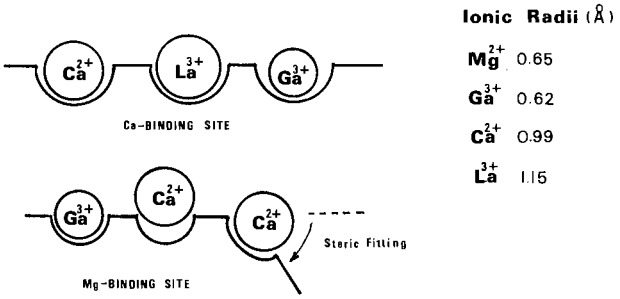

Fig. 1: Ionic replacement.

As a system to assess the probe characteristics of radiogallium for magnesium sites we have chosen the Na^+,K^+-activated and Mg^{2+}-dependent ATPase enzyme. This enzyme represents the biochemical basis for the cation pump responsible for the transport of monovalent cations acrosse cell membranes. The conventional reactions sequence for the ATPase

$$E \xrightarrow{ATP,Mg^{2+},Na^+} E-P \xrightarrow{K^+,\ -Pi} E$$

indicates an initial Na^+- and Mg^{2+}-dependent phosphorylation of the enzyme, followed by a K^+-dependent dephosphorylation.

Using dog kidney Na^+, K^+-activated, Mg^{2+}-dependent, ouabain-sensitive ATPase (SIGMA) the effect of gallium ion (Ga^{3+}) on the enzyme activity was assayed by measuring the hydrolisis of ATP to ADP and Pi. After a preincubation of the enzyme with increasing concentrations of gallium at 37°C for 10 minutes, ATP was added and incubated for 1 hour. Then the enzymatic hydrolisis was stopped adding perchloric acid in an ice-bath (4°C), and released Pi measured in an aliquot by photocolorimetry. The buffer solution

Tab. 3: Physical characteristics of radioisotopes of magnesium and gallium.

Nuclides of (Mass No)	Half-Life	Production Reaction	Production Cross Section, barns	Specific Activity (Theory), Ci/g	Yield,Ci/g Half-Lives 1	2	1 year(or saturation, if shorter)	Gamma Energies (Mev (%)	Beta Energies, Mev(%)	Interfering Isotopes Isotope	Energy and Type	Decay Product
MAGNESIUM												
(28)	21.3 h	$^{26}Mg(T,p)^{28}Mg$		5.3×10^6	Cyclotron,yield not available			1.35(70) 3 others to 0.95	0,46(100)	^{28}Al	1.78 γ 2.87 β	^{28}Al ^{28}Si
GALLIUM												
(66)	9.5 h	$^{63}Cu(\ ,n)^{66}Ga$		4.5×10^6	Cyclotron,yield not available			0.5(114) 1.1(37) 2.7(25)	4.23-(57)			^{66}Zn
(67)	9.5 h	$^{67}Zn(p,n)^{67}Ga$		5.0×10^5	Cyclotron,120 mc,hr			0,09(40) 0.18(24) 0.30(22) 0.39(7)	0.09e-(15)			^{62}Zn
(72)	14.1 h	$^{71}Ga(n,\gamma)^{72}Ga$	5.0	3.0×10^6	0.23	0.25	0.48	0.6-1.9(142 2.2-2.5(59)	0.6-1.5(83) 2.5-3.2(17)			^{72}Ge

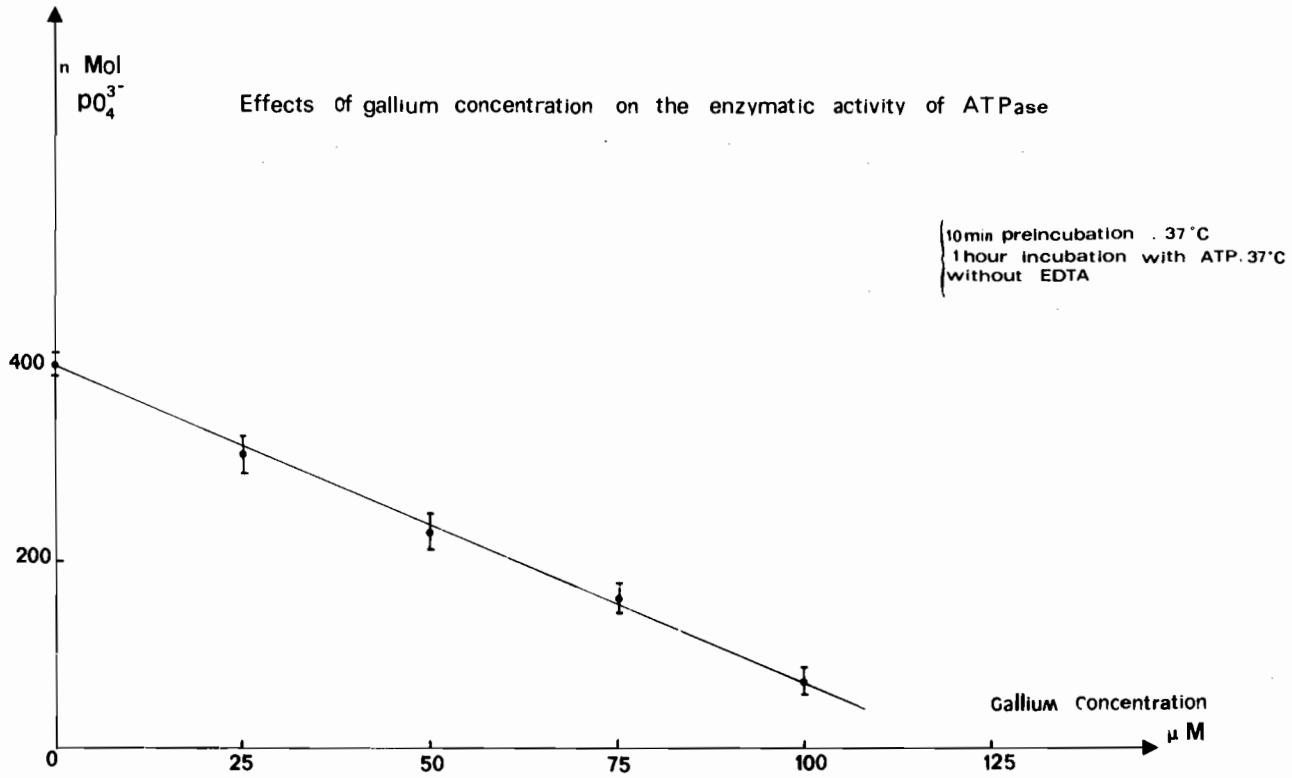

Fig. 2: Effects of gallium ion (Ga^{3+}) on Na^+,K^+-activated, Mg^{2+}-dependent, ouabain-sensitive ATPase enzyme activity.

used for the incubations contained 200 mM NaCl, 10 mM KCl, 100 mM Tris and 10 mM $MgCl_2$.

The experimental results indicate a lineal relationship between gallium concentration and enzyme activity inhibition (figure 2).

The fact that the Mg^{2+}-dependent, Na^+, K^+-activated ATPase enzyme is sensitive to gallium, as it is known to be to calcium [9], seems to indicate that an ion replacement process is responsible for this inhibition. This experimental observation together with the before mentioned *in vivo* and *in vitro* substitution of magnesium by radiogallium suggest its use as a probe for magnesium sites.

References

[1] *Pauling, L.:* In: The Nature of the Chemical Bond. Cornell Univ. Press, Ithaca, N. Y., 1960, 3.

[2] *Phillips, C. S. G.* and *Williams, R. J. P.:* In: Inorganic Chemistry: II Metals, Clarendon Press, Oxford, 1966, 80.

[3] *Cuatrecasas, P., Fuchs, S. and Anfinsen, C. B.:* Catalytic Properties and Specificity of the Extracellular nuclease of Staphylococcus aureus, J. Biol. Chem. **242** (1967) 1541—1547.

[4] *Lehninger, A. L.:* Mitochondria and Calcium Ion Transport. Biochem. J. **119** (1970) 129—138.

[5] *Mela, L.* and *Chance, B.:* Spectrophotometric Measurements of the Kinetics of Ca^{2+} and Mn^{2+} Accumulation in Mitochondria. Biochemistry **7** (1968) 4059—4063.

[6] *Cohn, M.:* Magnetic resonance studies of enzymesubstrate complexes with paramagnetic probes an illustrated bu creatine kinase. Quart. Rev. Biophys. **3** (1970) 61—89.

[7] *Peck, E. J.* and *Ray, W. J.:* Role of Bivalent Cations in the Phosphoglucomutase System — II. Metal ion binding and the structure of binary enzyme — metal complexes. J. Biol. Chem. **244** (1969) 3748—3753.

[8] *Anghileri, L. J.* and *Heidbreder, M.:* On the mechanism of accumulation of ^{67}Ga by tumors. Oncology **34,** (1977) 74—77.

[9] *Glick, N. B.:* In: Metabolic Inhibitors. *Holchster, R. M., Kates, M.* and *Quastel, J. H.,* (eds.), Academic Press, N. Y. (1972), Vol. III, 1—44.

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