New perspective in the assessment of total intracellular magnesium

Azzurra Sargenti,[1] Lucia Merolle,[1] Giulia Andreani,[2] Concettina Cappadone,[1] Giovanna Farruggia,[1,3] Stefano Iotti,[2,3] Chiara Marraccini[4]

[1]Department of Pharmacy and Biotechnologies, University of Bologna; [2]Department of Veterinary Medical Sciences, University of Bologna; [3]National Institute of Biostructures and Biosystems, Roma; [4]Department of Life Sciences, University of Modena and Reggio Emilia, Italy

Abstract

Magnesium (Mg) is essential for biological processes, but its cellular homeostasis has not been thoroughly elucidated, mainly because of the inadequacy of the available techniques to map intracellular Mg distribution. Recently, particular interest has been raised by a new family of fluorescent probes, diaza-18-crown-hydroxyquinoline (DCHQ), that shows remarkably high affinity and specificity for Mg, thus permitting the detection of the total intracellular Mg. The data obtained by fluorimetric and cytofluorimetric assays performed with DCHQ5 are in good agreement with atomic absorption spectroscopy, confirming that DCHQ5 probe allows both qualitative and quantitative determination of total intracellular Mg.

Introduction

Magnesium (Mg) is essential for numerous biological processes, but its cellular homeostasis has not been thoroughly elucidated; yet, some peculiarities in its regulation are emerging, indicating that free and total Mg undergo two independent regulatory mechanism and therefore discriminating between the two pools is crucial. Atomic absorption spectroscopy (AAS) is the reference technique to measure total Mg content but requires volatilization of the sample and a high number of cells (several millions). Recently, particular interest has been raised by a new family of fluorescent probes, diaza-18-crown-hydroxyquinoline (DCHQ), that shows remarkably high affinity and specificity for Mg. Specifically, DCHQ1 (Figure 1) is capable to image intracellular Mg in living cells and to assess total cellular in small sample, providing overlapping results with AAS.[1] However, DCHQ1 showed some limitation, such us poor intracellular retention and a certain degree of fluorescence instability, invalidating its use, for instance, in time based measures of Mg cellular fluxes. In addition, DCHQ1 is excitable only in the UV range (360 nm). A new phenyl-derivate, DCHQ5 (Figure 1) is highly retained within cells and maintaining stable fluorescence intensity up to 30 min of light exposure.[2] Furthermore, DCHQ5 displayed higher fluorescence enhancement upon Mg binding than DCHQ1, and is excitable also in the visible range. Aim of this study is to perform a quantitative assessment of total intracellular Mg by this new hydroxyiquinoline derivative chemosensor.

Correspondence: Azzurra Sargenti, Department of Pharmacy and Biotechnologies, University of Bologna, via Belmeloro 6, 40126 Bologna, Italy.
Tel./Fax: +39.051.2099700.
E-mail: azzurra.sargenti@unibo.it

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Materials and Methods

HL60 cells were maintained in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 2 mM L-Glutamine, 10% foetal bovine serum (FBS) at 37°C and 5% CO2. Cells were washed twice in PBS without Ca^{2+} and Mg^{2+}, counted and resuspended at $5x10^5$ cells/mL. For flow cytometric assays, cells resuspended at $5x10^5$ cells/mL were incubated with DCHQ5 5 µM in phosphate buffered saline (PBS) without Ca^{2+} and Mg^{2+} for 15 min in the dark, counterstained with Propidium Iodide 5 µg/mL and analyzed by flow cytometry exciting DCHQ5 fluorescence at 360 or 488 nm and collecting on a logarithmic scale the fluorescence emission at 525 nm. Samples were lysed by sonifier, and 200 µL of the sample were added to 1.8 mL of DCHQ5 15 µM in methanol and oil production system (MOPS) 20 mM/Methanol 50%. Fluorescence spectra were collected with $_{ex}$ 360 nm Mg concentration was assessed comparing fluorescence intensity at $_{em}$ 510 nm of the samples with a calibration curve prepared with $MgSO_4$. For AAS, the sonified samples were digested in HNO3 1 N, centrifuged and the supernatant analyzed by a spectrometer equipped with an air/acetylene flame.

Results and Discussion

In this work we tested the analytical capability of DCHQ5. The probe can be applied for the cytofluorimetric assay of intracellular total Mg, assessing intracellular variations of Mg content in different proliferative states. It is known that dimethyl sulfoxide (DMSO)-induced differentiation in HL60 promielocitic leukemic cells halves cellular Mg content, from an average of 25 to 12 nmol/10^6 cell.[1] DCHQ5 allowed to detect the Mg decrease in G0/G1 blocked cells, as reported in Figure 2.

Flow cytometric assays, performed both by UV and visible excitation, showed a decrease of about 50% of the mean fluorescence intensity (evaluated as mean channel of the fluorescence distribution) of differentiated *vs* control cells: in the experiment showed in Figure 2, for example, the geometric mean channel of control cells and DMSO differentiated cells were respectively 94 and 45 with Visible excitation and 83 and 43 with UV excitation.

Therefore, we planned a protocol to quantitatively assess total intracellular Mg in sonicated cellular samples by using a simple spectrofluorimetric assay. We compared the data obtained by DCHQ5 with AAS, the reference technique for the quantification of total intracellular Mg (Table 1).

Table 1. Quantification of total intracellular magnesium in different samples by DCHQ5 and atomic absorption spectroscopy.

Sample at different passage	DCHQ5 Mg (nmol/10^6 cells)	AAS Mg (nmol/10^6 cells)
HL60 121p	19.75±0.76	22.78±0.38
HL60 124p	22.93±1.30	20.70±0.61
HL60 128p	23.77±1.14	24.70±0.89
HT29 67p	24.31±1.13	23.94±0.89
HT29 69p	23.96±0.37	21.17±0.71

AAS, atomic absorption spectroscopy.

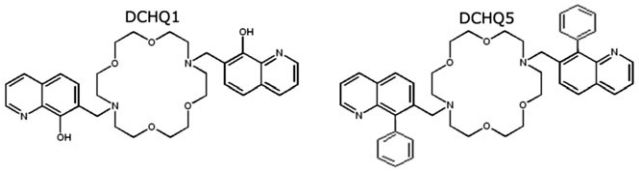

Figure 1. Structure of the DCHQ1 e DCHQ5 probes.

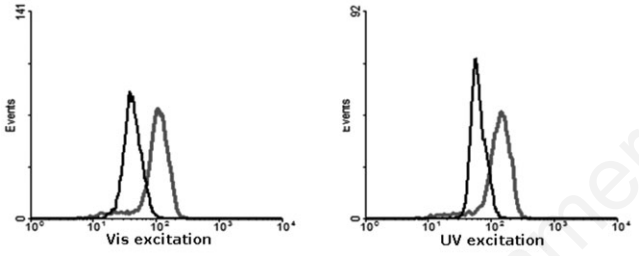

Figure 2. DCHQ5 fluorescence distribution of control (grey line), and dimethyl sulfoxide-differentiated (black line) HL60 cells.

The data obtained by DCHQ5 are in good agreement with atomic absorption spectroscopy, confirming that DCHQ5 probe allows both qualitative and quantitative determination of total intracellular Mg.

Conclusions

In conclusion, we demonstrate that DCHQ5 can be considered the future reference molecular probe for the study of intracellular Mg homeostasis, broadening the field of application of DCHQ chemosensors.

References

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