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ORIGINAL PAPER

A Simple Spectrofluorometric Assay to Measure Total Intracellular Magnesium by a Hydroxyquinoline Derivative

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Abstract The intracellular behaviour of diaza-18-crown-6 appended with two H-substituted hydroxyquinoline groups (DCHQ1) was investigated to explore its application as a new sensor for the evaluation of cell magnesium content and distribution. We used five cells lines characterised by different contents of magnesium and different intracellular membrane-defined compartments. The main result is the definition of the appropriate experimental conditions to quantitatively assess the total cell magnesium by fluorescence spectroscopy. We showed that disrupting cells by sonication, DCHQ1 was capable to assess total cell magnesium in all cell types examined, obtaining overlapping results with atomic absorption spectroscopy (AAS). This new analytical approach requires very small cell samples and a simple fluorimetric technique, and can be a valid alternative to AAS. The fluorescent properties of DCHQ1 in living cells are: (a) it consistently stains live cells, (b) it discriminates small variations of cell Mg contents, (c) cell staining is stable for at least 30 min. We also investigated the role of lipophilic environment on DCHQ1 fluorescence by mimicking cell membranes and described how the composition and structure of lipid vesicles affect Mg-DCHQ1 fluorescence. Thus, DCHQ1 may offer important information also on magnesium distribution in living cells, providing a novel strategy to map the intracellular compartmentalization of this cation.

Keywords Magnesium · Fluorescent probes · Hydroxyquinoline derivatives · Fluorescence spectroscopy · Flow cytometry

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Introduction

Magnesium[1] is an abundant intracellular cation essential for many processes such as ion channel regulation, DNA and protein syntheses, membrane stabilization and cytoskeletal activity [1, 2].

[1] "Magnesium" or "Mg" refer to the total pool of magnesium, i.e. free plus bound forms; "Mg^{2+}" refers specifically to the ionized or free form.

Hundreds of enzymes require $MgATP^{2-}$ as a cofactor or Mg^{2+} as an allosteric regulator [1, 3, 4]. As a consequence, cell functions such as proliferation and death are dependent on Mg^{2+} availability [5, 6]. Despite the concentration of Mg is in the millimolar range in both extracellular and intracellular milieu, many relevant pathological conditions, such as cardiovascular diseases, hypertension, diabetes and dismetabolic syndrome are associated with reduced Mg availability and/or increased excretion either at a systemic level or in specific tissues [7–9]. Furthermore, rare human genetic diseases have been recently described to be associated with mutations of specific genes coding for magnesium channels [10, 11].

Intracellular magnesium content is regulated by specific influx and efflux mechanisms (ion channels and antiporters), some of which have been characterized in depth [12–14]. Nevertheless, magnesium distribution between free and bound forms and its intracellular compartmentalization have not yet been thoroughly elucidated, mainly because of the inadequacy of available techniques to map intracellular magnesium distribution. Some authors showed that free and total magnesium undergo different and independent regulatory mechanisms, suggesting that Mg ions move among cellular sub-compartments following mechanisms not yet fully characterized [15–17].

To date, cell total magnesium is generally measured on cell or tissue acidic extracts by atomic absorption spectroscopy (AAS), a technique that requires large samples (at least tens of millions of cells or several milligrams of tissue). On the other hand, free Mg^{2+} can be measured from the chemical shift of Mg–adenosine triphosphate (ATP) peak by ^{31}P nuclear magnetic resonance spectroscopy [18–20], or by means of intracellular fluorescent probes derived from those developed for calcium (mag–fura, mag–fluo, mag–indo) [21–23]. Lately, research has focussed on the development of new classes of fluorochromes more sensitive and specific to magnesium than the commercially available ones [24–27].

Despite the versatility of fluorescent probes to measure intracellular cations in living cells, the analysis of these data may be biased by interactions between the probes and intracellular moieties, such as proteins or lipophilic structures. The tetra- and try-carboxylate family calcium and magnesium probes, for example, interact with soluble proteins, such as those of mitochondrial matrix, and this interaction influences the physicochemical properties of the probes affecting the direct and accurate ionic quantification [28–30].

We have previously described the photophysical characteristics of some substitutes of diaza-18-crown-6 appended with two hydroxyquinoline groups (DCHQs) [31–33] and proposed their potential applications to measure cell magnesium content and distribution [34].

8-Hydroxyquinoline-substituted (DCHQ1) or 5-chloro-8-hydroxyquinoline-substituted (DCHQ2) derivatives bind Mg^{2+} with much higher affinity than any other available probe (K_d=44 and 73 μM, respectively) and show a strong fluorescence increase upon complexation. Remarkably, their fluorescence is not significantly affected either by other divalent cations, most importantly Ca^{2+}, or by pH changes within the physiological range. We also found that DCHQ1 readily permeates cells and seems to detect Mg distribution and movements in live cells by confocal imaging [34]. The evaluations of cell total magnesium by DCHQ1 fluorescence in leukemic (HL60) and mammary epithelial (HC11) cells resulted in a three to fourfold overestimation compared to Mg measured by AAS. The hydrophobic nature of DCHQ1 and the lifetime measurements performed by single-photon counting spectrofluorometry [34] suggested that the enhancement obtained in intact cells could be due to the entrapment of the probe in membrane compartments. Indeed, it has been described that hydrophobic derivatives of hydroxyquinoline preferentially distribute into lipophilic moieties [35]. Furthermore we showed that upon disruption of HL60 cells, DCHQ1 fluorescence substantially decreased [34], approaching corresponding AAS values.

This observation led us to a deeper evaluation whether DCHQ1 could be exploited to measure cell total magnesium contents in alternative to AAS. To this aim we investigated the fluorescent properties of DCHQ1 in several cells lines characterised by different contents of magnesium and of intracellular membranes.

Materials and methods

Materials All chemicals were purchased from Sigma Aldrich, unless otherwise specified. All reagents were of Ultrapure grade. Dulbecco phosphate buffered saline (DPBS) without Ca^{2+} and Mg^{2+} (NaCl 8 g/l, KCl 0.20 g/l, Na_2HPO_4 0.20 g/l, KH_2PO_4 0.20 g/l, pH 7.2) was prepared in bidistilled water. The fluorescent probe DCHQ1 was synthesised as reported in [36]. A 4 mM stock solution was prepared in DMSO, and aliquots were stored at 4 °C in the dark.

Cell lines Rat1 (rat fibroblast), Cos7 (monkey fibroblast), HC11 (mouse mammary epithelial) and HL60 (human promyelocytic leukemia) cells were routinely cultured in Roswell Park Memorial Institute 1640 medium (Gibco, UK) supplemented with 10% heat-inactivated fetal calf serum (Euroclone, UK) and L-glutamine 2 mM, at 37 °C in 5% CO_2 atmosphere.

For fluorescence and flow cytometry measurements, cells were prepared as previously reported [34]. Briefly, cells were harvested, washed three times in DPBS without Ca^{2+}

and Mg^{2+} and re-suspended at a concentration of 5×10^5 cells per milliliter in the same buffer. DCHQ1 was added at a concentration of 25 μM, unless otherwise specified, and cells were incubated in the dark for 5 min. Blood from Wistar rats was collected in DPBS and heparin, centrifuged to remove plasma and washed in DPBS by centrifugation at 1,500×*g* for 10 min at 20 °C. Samples of erythrocytes at 4% hematocrit were used for fluorimetric analyses [37].

Cell disruption was obtained by sonication of cell samples kept in slurry ice for 10 s (Misonix USA).

Determination of cell volume To evaluate the mean cellular volume, HL60 cells were counted on a double-threshold Z1 Coulter Counter (Beckman Coulter, USA) and the thresholds were set to cover the interval from 65 to 3,600 fl, each step corresponding to an increase of 2 μm in cell diameter. The mean cellular volume was estimated from the Gaussian distribution of the data.

Vesicle preparation Vesicles were prepared by dissolving 10 mg of phospholipids in chloroform and, after solvent evaporation, the lipid film was resuspended in 1 ml DPBS. Unilamellar vesicles were prepared either by sonication or by extrusion through a membrane with pores of 0.1 μm. Vesicles consist of a mixture of phospholipids (MPL) mimicking natural membranes, namely 30% phosphatidylcholine, 30% phosphatidylethanolamine, 15% phosphatidylserine and 25% sphingomyelin [38].

Fluorescence spectroscopy DCHQ1 fluorescence spectra were recorded on a PTI Quanta Master C60/2000 spectrofluorimeter (Photon Technology International, Inc., NJ, USA) upon excitation at 363 nm.

Cell magnesium concentrations were calculated from the changes of DCHQ1 fluorescence emission monitored at 505 nm, by Eq. 1:

$$[\mathrm{Mg}]_{\mathrm{tot}} = K_{\mathrm{d}}(F - F_{\mathrm{min}})/(F_{\mathrm{max}} - F) + [\mathrm{probe}](F - F_{\mathrm{min}})/(F_{\mathrm{max}} - F_{\mathrm{min}}) \quad (1)$$

where F is the fluorescence intensity of the sample incubated with the probe, F_{max} is the fluorescence intensity of the sample upon addition of a saturating amount of $MgSO_4$ (8 mM), F_{min} is the fluorescence intensity of the sample upon addition of ethylenediaminetetraacetic acid (EDTA) (17 mM) and [probe] is the loading concentration of DCHQ1 (25 μM, unless otherwise specified). It is noteworthy that F_{max} can be determined in the presence of whole cells, because cell membranes are permeable to DCHQ1.

DCHQ1 fluorescence in the presence of lipid moieties To model cell environment we used 40 μg/ml MPL vesicles, in the presence of 20 μM Mg^{2+} to mimic physiological Mg content and upon loading with DCHQ1, fluorescence was recorded before and after sonication.

Flow cytometry Flow cytometric measurements were performed on a Bryte HS cytometer (BioRad, UK), equipped with a Hg lamp and a filter set with an excitation band centered on 360 nm and two emission bands centered respectively on 500 nm (DCHQ1 fluorescence) and 600 nm (propidium iodide fluorescence). Cells loaded with 25 μM DCHQ1 were counterstained with 5 μg/ml propidium iodide (PI) to identify dead cells. Fluorescence was recorded using a logarithmic scale. Cell cycle was estimated according to [39].

Cell total magnesium measurements Total magnesium content was assessed by AAS on acidic cellular extracts. Harvested cells were washed twice in cold PBS by centrifugation and then pelleted at 1,000×*g*. Ion extractions were obtained by overnight treatments of the cell pellets with 1.0 N HNO_3. After agitation and centrifugation of samples, magnesium was assayed on cell supernatants by AAS (Instrumentation Laboratory mod. S11, USA or Perkin Elmer AA200) equipped with an air/acetylene flame.

Statistical analysis Data are expressed as mean±SD. Student's *t* test was performed on data reported in Table 1. $P<0.05$ was considered to be significant.

Table 1 Magnesium contents in different cell lines

	Cell magnesium (nmol/10^6 cells)		
	Intact cells (DCHQ1)	Cell extracts (AAS)[a]	Sonicated cells (DCHQ1)[a]
Cos7	62.2±5.3	17.0±3.8	23.0±2.0
Rat1	23.0±7.7	9.2±3.6	10.6±2.9
HC11	86.7±8.4	30.9±3.8	35.9±4.5
HL60	41.2±11.9	11.5±4.9	11.9±1.5
HL60-DMSO treated cells	20.4±8.4	6.6±3.5	7.2±1.8

[a] The reported values were not statistically different

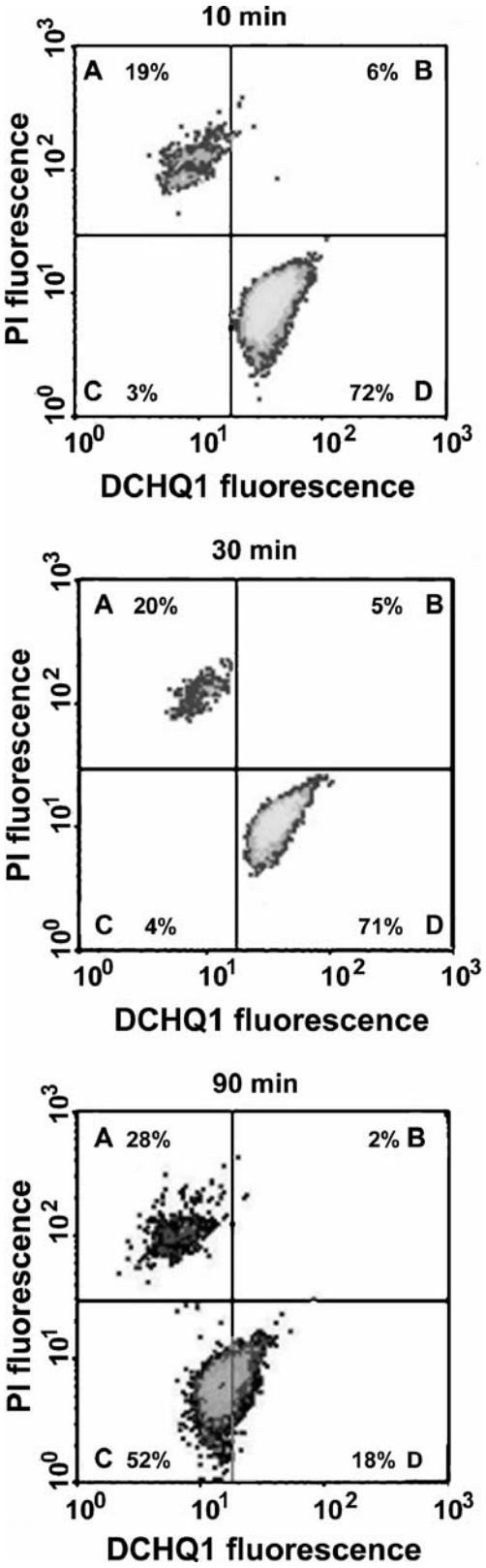

Fig. 1 Cytograms of Cos7 cells loaded with DCHQ1 and PI analyzed after 10, 30 and 90 min of incubation at room temperature. *Quadrant A*: PI positive/damaged cells with low DCHQ1 fluorescence; *quadrant C*: PI negative/viable cells with low DCHQ1 fluorescence; *quadrant D*: PI negative/viable cells with high DCHQ1 fluorescence. The percentage of cell population is indicated in each *quadrant*. Cos7 cells 5×10^5/ml; DCHQ1 25 μM; PI 10 μM. Results from a typical experiment repeated three times with similar results

Results and discussion

DCHQ1 distribution between cells and medium

To analyse the characteristics of DCHQ1 fluorescence in biological samples, we took advantage of fluorimetric methods to detect intracellular and extracellular distribution of the probe. Cytofluorimetric analyses of HL60, Rat1 and Cos7 cells loaded with DCHQ1 and counterstained with PI allowed to discriminate live from damaged cells and selectively analyse intracellular DCHQ1 fluorescence. Figure 1 reports the cytograms of Cos7 cells: PI positive cells (quadrant A) are distinct from DCHQ1 fluorescent cells (quadrant D), suggesting that DCHQ1 selectively stains live cells. This feature is consistent with the high intracellular Mg content of live cells.

Fluorescence of viable Cos7 cells loaded with DCHQ1 remained substantially unaltered for 30 min, whereas at a later time the signal decreased indicating a leaking of Mg, likely due to the metabolic suffering of the cells incubated in pure saline buffer (data not shown). Similar results were obtained for all the cell lines investigated. To assess the sensitivity of DCHQ1 in detecting changes in cell Mg contents we took advantage of HL60 cells which, upon differentiation (1.3% DMSO for 5 days), undergo a sizable decrease of cell Mg [16]. Differentiation process was verified by the flow cytometric assay of cellular DNA content which revealed that upon DMSO treatment 94±4% of HL60 cells were in the G0/G1 phase compared to 48± 7% of control cells. Figure 2 reports DCHQ1 fluorescence distributions in control and differentiated HL60 cells: DMSO-induced growth arrest led to a clear cut decrease of DCHQ1 fluorescence which is consistent with the expected decrease of cell Mg content as found by assessing Mg from DCHQ1 fluorescence spectroscopy or AAS (see Table 1). These data suggest that evaluating cell Mg from DCHQ1 fluorescence can provide sensitive qualitative and possibly, quantitative results.

Next, we used fluorescence spectroscopy to further investigate DCHQ1 cell loading and the distribution of the probe between cells and medium. The amount of probe inside the cells was derived from the difference between the fluorescence intensity of the total DCHQ1 in cell suspensions (F_{max}) and that remained in the supernatants upon centrifugation of the cells (F_{max}-supernatant), as shown in Fig. 3a. The DCHQ1 distribution between cells and medium was studied at different loading concentrations of DCHQ1, ranging between 25 and 100 μM (Fig. 3b). We found that at all loading concentrations, about 40–45% of

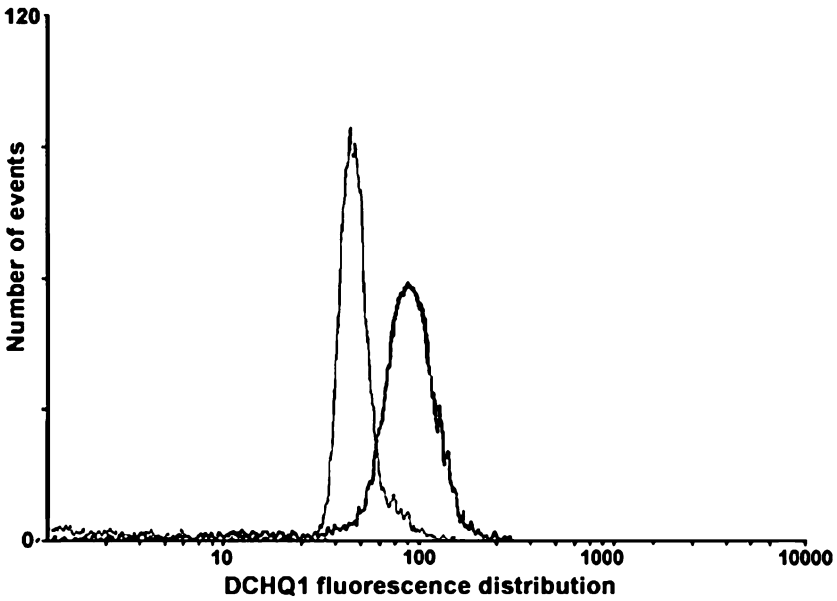

Fig. 2 Effect of HL60 cell differentiation on DCHQ1 fluorescence. DCHQ1 fluorescence distribution by flow cytometry in control (*black*) and DMSO-treated (*grey*) HL60 cells. For further details see "Materials and methods." Results from a typical experiment

the probe was present within the cells, showing that DCHQ1 distributed almost equally between extracellular and intracellular compartments.

Considering the total number of HL60 cells, with a mean cellular volume of 1,760 fl, we estimated that intracellular DCHQ1 concentrations were 13±3, 23±4 and 46±11 mM at loading concentrations of 25, 50 and 100 μM DCHQ1, respectively. These results are in agreement with data reported for other dyes, which are accumulated in the intracellular compartments more than 1,000 times compared to the loading concentration [26]. The millimolar concentrations of intracellular DCHQ1, results in the same order of magnitude as cell total Mg, which combined with the low K_d of DCHQ1, indicates that the intracellular available probe is potentially detecting all cell Mg.

Calculating cell Mg contents in HL60 cells from Eq. 1, the values obtained for DCHQ1 loading concentrations of 25 and 50 μM were quite close (about 20 mM) and consistent with the values calculated from data in Table 1 (see next paragraph), whereas at loading concentration of 100 μM the value was much higher (about 30 mM). These results can be explained by data reported in Fig. 3b, where

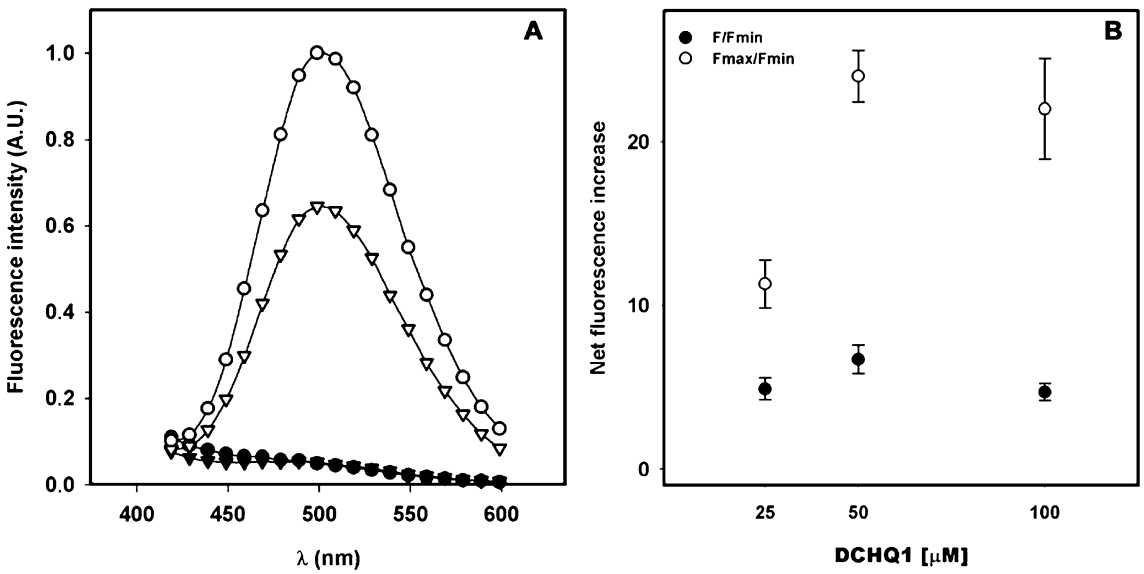

Fig. 3 DCHQ1 fluorescence in HL60 cells. **a** Distribution of the probe between cells and medium: fluorescence spectra of DCHQ1 in HL60 cell suspension (5×10^5 cells per milliliter) and cell-free supernatants at a loading concentration of 50 μM DCHQ1: fluorescence of cell suspension (*filled circles*); F_{max} of cell suspension (*empty circles*); fluorescence of cell-free supernatant (*inverted filled triangles*); F_{max} of cell-free supernatant (*inverted empty triangles*), typical spectra. **b** Calibration of DCHQ1 cell loading: F/F_{min} and F_{max}/F_{min} at increasing concentrations of the probe. Data are mean±SD of at least three independent experiments

the cellular (F) and maximum (F_{max}) DCHQ1 fluorescence are reported as net increase with respect to autofluorescence of the dye (F_{min}) for the different DCHQ1 loading concentration. As expected, the ratio F/F_{min} and F_{max}/F_{min} increased between 25 and 50 μM DCHQ1, while at 100 μM DCHQ1 both values decreased remarkably.

These data clearly show that the optimal loading concentrations are in the range between 25–50 μM where the fluorescence response to Mg binding is proportional to the dye concentration, while at higher probe concentrations an inner filter effect probably takes place [40, 41].

Fluorimetric evaluation of cell magnesium content by DCHQ1

To investigate the sensitivity of DCHQ1 in detecting cell Mg contents, we used several cell lines, characterized by very different total Mg contents (see Table 1).

Figure 4 reports the fluorescence spectra of DCHQ1 in the presence of intact or sonicated Cos7 cells. The fluorescent spectra of DCHQ1 loaded cells show a marked decrease in fluorescence signals in sonicated compared to intact cells; similar results were obtained in all the cell lines herein investigated. We ascertained that this decrease in fluorescence was not due to a modification in the probe–cation binding induced by sonication itself, since DCHQ1 F_{max} values were the same before and after this procedure (see also the following figures).

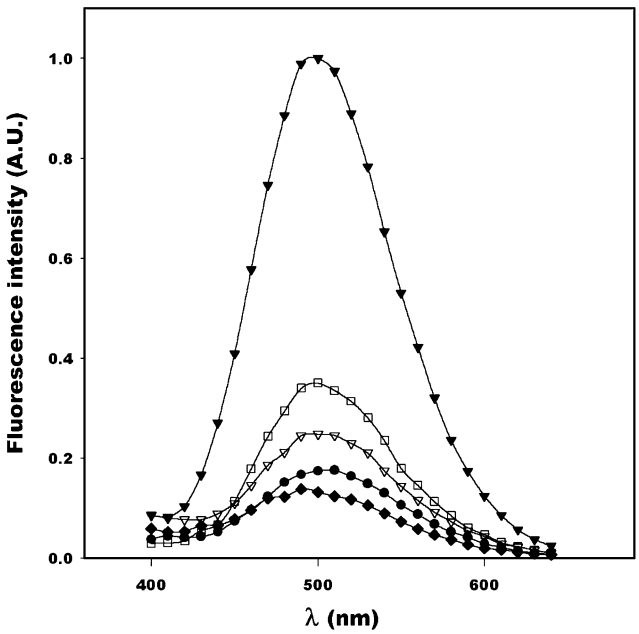

Fig. 4 Fluorescence spectra of Cos7 cells loaded with DCHQ1. DCHQ1 in DPBS (*filled circles*), after addition of intact (*empty squares*) or sonicated (*empty inverted triangles*) cells (5×10^5 cells per milliliter). Fluorescence calibration in both intact and sonicated cells by addition of 8 mM Mg^{2+} (*filled inverted triangles*; F_{max}) and 17 mM EDTA (*filled diamonds*; F_{min}). Results from a typical experiment

To investigate whether DCHQ1 could be used to measure total intracellular magnesium we compared values calculated from DCHQ1 fluorescence by Eq. 1 in whole and sonicated cells with those obtained by AAS measurements on parallel cell acidic extracts (Table 1). Interestingly, Mg contents measured by DCHQ1 fluorescence always correlated to the total amount of Mg in each cell line. Cos7 cells contain twice as much Mg as Rat1 fibroblasts because of their higher proliferation rate, as cell Mg content increases proportionally to the amount of cells engaged in the cell cycle [5]. These results were confirmed also by comparing control and differentiated HL60 cells, as discussed in the previous paragraph. Notably, cell magnesium contents derived from DCHQ1 fluorescence of sonicated samples are much smaller than those measured in whole cells, and they overlap the values given by AAS measurements, as confirmed by statistical analysis. The possibility that this decrease was due to a simple dilution of the probe bound to Mg was ruled out, first because the probe can freely permeate cell membranes and equally distributed inside and outside the cells, second because of the results obtained by using liposomes mimicking cell suspensions and physiological magnesium, as detailed in the next paragraph. Furthermore, the potential competition between cell ATP and the probe to bind Mg due to their closely related micromolar K_d values, was overcome by the rapid ATP hydrolysis following cell sonication [42] These results indicate therefore that DCHQ1 fluorescence of sonicated samples is a sensitive and accurate approach alternative to AAS to measure intracellular Mg on few millions cells.

The effects of lipophilic moieties on DCHQ1 fluorescence

The higher DCHQ1 fluorescence of whole vs sonicated cells, which resulted in a two to four times overestimation of cell Mg contents, could be ascribed to the effect of intracellular phospholipids on DCHQ1 fluorescence, as suggested by the increase of the excited-state lifetimes previously described [34]. In this case, the increase in DCHQ1 fluorescence in whole cells should be proportional to the amount of intracellular membranes and/or lipophilic environments. In order to address this possibility we compared DCHQ1 fluorimetric analyses of rat erythrocytes, which can be assumed as nearly one-compartment cells, with those of nucleated cells. Results are reported in Fig. 5. It is worth noting that (1) the fluorescence increase detected in the presence of erythrocytes is modest, due to the small Mg content and the extremely small volume of these cells, and (2) fluorescence spectra of intact vs sonicated erythrocytes are very similar, especially around 500 nm, in contrast to the clear-cut decrease observed in nucleated cells, as shown in Fig. 4. These results suggest that the increased DCHQ1 fluorescence in whole cells

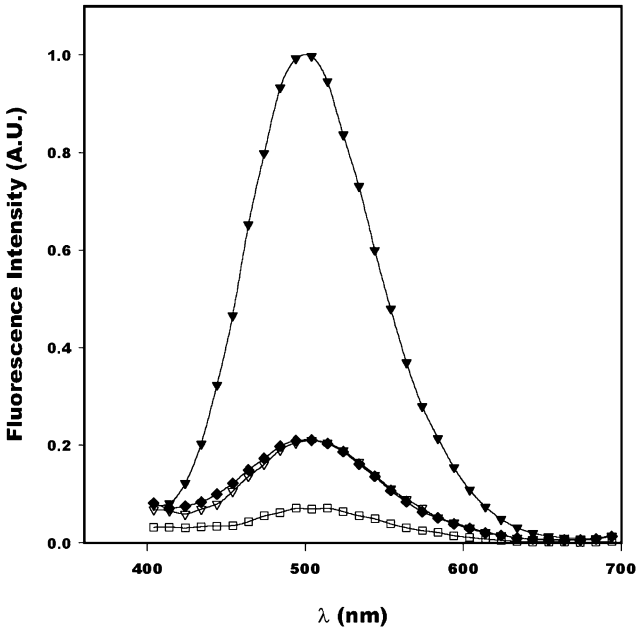

Fig. 5 Fluorescence spectra of rat erythrocytes (Rbc) loaded with DCHQ1. Intact (1×10^6/ml) Rbc (*filled diamonds*); after cell sonication (*empty inverted triangles*). Fluorescence calibration by addition of 8 mM Mg^{2+} (*filled inverted triangles*; F_{max}) and 17 mM EDTA (*empty squares*; F_{min})

correlates with the amount of membrane-defined intracellular compartments.

To further support this hypothesis, we prepared vesicles of MPL, at a concentration comparable to that present in our HL60 cell suspension, namely 40 μg/ml, and we added an amount of Mg to mimic that present in the cell suspension, namely 20 μM. Upon loading of these liposomes with the usual DCHQ1 concentration, we acquired fluorescence spectra before and after sonication (Fig. 6).

Figure 6b shows that by sonicating the vesicles, the fluorescence intensity of DCHQ1-Mg complex decreased. This is due exclusively to the disruption of the lipid bilayer structure since: (1) the spectra of intact or sonicated vesicles are comparable in the presence of a saturating amount of Mg^{2+} (cf. Fig. 6a,b); and (2) dilution of DCHQ1–Mg probe does not affect fluorescence since in this case Mg is already distributed in the medium and not released into the extracellular milieu by sonication, as in the case of whole cells. These observations confirm that (1) the presence of lipophilic moieties does not affect the maximum binding of the probe with Mg^{2+}, (2) the sonication procedure does not affect the binding between the probe and Mg^{2+}. Altogether these data clearly demonstrate that the structural organization of MPL was responsible for the enhanced DCHQ1 fluorescence, similarly to what observed in whole cells. We can therefore describe the increase of DCHQ1 fluorescence observed in whole cells as a "membrane enhancement effect" due to an interaction between DCHQ1 and lipophilic moieties, provided they are in a vesicular conformation, similar to intracellular membrane-defined compartments.

Conclusion

In this paper we further characterized the interaction between DCHQ1 and the cell environment to define the

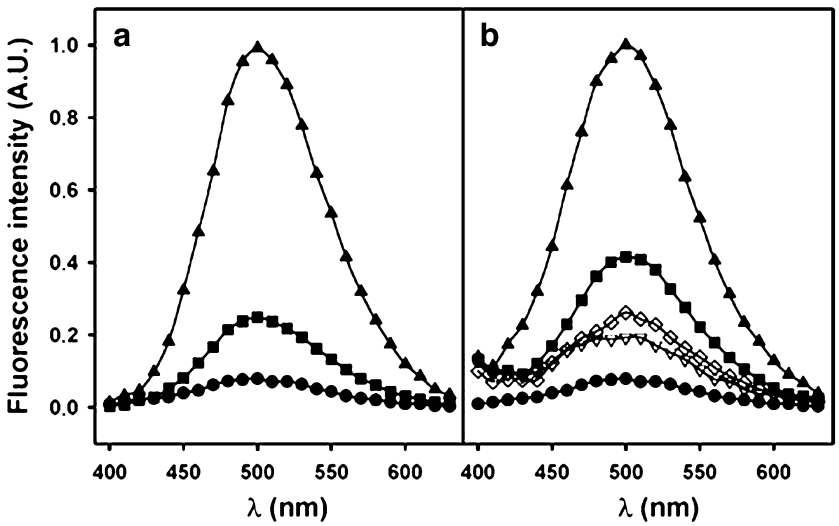

Fig. 6 Modelling cell lipophilic environment and physiological Mg content: effect of MPL vesicles and low Mg on DCHQ1 fluorescence. **a** 25 μM DCHQ1 in DPBS (*filled circles*), addition of 20 μM $MgSO_4$ (*filled square*); addition of 10 mM Mg^{2+} (F_{max}; *filled upright triangles*). **b** 25 μM DCHQ1 in DPBS (*filled circles*), addition of 40 μg/mL MPL vesicles (*empty inverted triangles*), addition of 20 μM Mg^{2+} (*filled squares*), sonication of MPL vesicles in the presence of 20 μM Mg^{2+} (*empty diamonds*), addition of 10 mM Mg^{2+} (F_{max}; *filled upright triangles*)

most suitable conditions to measure Mg in live cells and to assess the capability of this probe to provide an accurate quantification of the total intracellular magnesium. We showed that disrupting cells by sonication, DCHQ1 was capable to assess total cell magnesium in HL60 leukemia, HC11 mammary cells, Cos7 and Rat1 fibroblasts, characterized by different magnesium contents, obtaining overlapping results with AAS. These results provide the basis for a new analytical approach offering several advantages compared to AAS such as: (a) the requirement of very small cell samples, (b) the use of simple fluorimetric techniques more widely diffused than AAS and (c) last but not least, a much faster sample preparation procedure.

Furthermore, the study of DCHQ1 fluorescent properties in living cells, allowed to define that: (a) DCHQ1 stains selectively live cells; (b) its fluorescence signal is directly correlated with cell Mg content and (c) its sensitivity allows to discriminate fine variations of cell Mg contents in samples of small numbers of cells. Notably DCHQ1 cell staining is stable for at least 30 min, indicating that the probe is well tolerated by the cells, thus allowing prolonged observations.

However, the assessment of cell Mg in living cells by DCHQ1 fluorescence is affected by the amount of membrane-defined intracellular compartments. We found that in a mixture of phospholipids that mimics the composition of cell membranes, the increase of DCHQ1 fluorescence upon Mg binding was reduced by vesicle sonication, reproducing the results obtained in disrupted cells. These data suggest that a "membrane enhancement effect" takes places when the lipophilic environment is structured in a similar manner to intracellular membranes. To confirm this interpretation, we show that in red blood cells, possessing few intracellular compartments, DCHQ1 fluorescence in whole and sonicated cells were much more similar compared to those found in the nucleated cell lines examined.

This peculiar characteristic of DCHQ1 fluorescence, if properly evaluated, e.g. by fluorescence lifetime imaging microscopy, may offer important information on magnesium distribution in the intracellular lipophilic moieties and can represent a further potential application of these probes to the study of cell magnesium homeostasis.

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