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Measurement of cystic fibrosis transmembrane conductance regulator activity using fluorescence spectrophotometry

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ABSTRACT

Cystic fibrosis (CF) is a frequent autosomal recessive disease caused by mutations that impair the CF transmembrane conductance regulator (CFTR) protein function. CFTR is a chloride channel activated by cyclic AMP (cAMP) via protein kinase A (PKA) and ATP hydrolysis. We describe here a method to measure CFTR activity in a monolayer of cultured cells using a fluorescence spectrophotometer and the chloride-sensitive probe 6-methoxy-*N*-(3-sulfopropyl)quinolinium (SPQ). Modifying a slice holder, the spectrophotometer quartz cuvette was converted in a perfusion chamber, allowing measurement of CFTR activity in real time, in a monolayer of T84 colon carcinoma cells. The SPQ Stern–Volmer constant (K_{Cl^-}) for chloride in water solution was 115.0 ± 2.8 M^{-1}, whereas the intracellular K_{Cl^-} was 17.8 ± 0.8 M^{-1}, for T84 cells. A functional analysis was performed by measuring CFTR activity in T84 cells. The CFTR transport inhibitors CFTR(inh)-172 (5 μM) and glibenclamide (100 μM) showed a significant reduction ($P < 0.05$) in CFTR activity. This simple method allows measuring CFTR activity in a very simple, reproducible, and sensitive way.

Cystic fibrosis (CF)[1] is a frequent autosomal recessive disease. It results from mutations in the *CFTR* gene that encodes for the CF transmembrane conductance regulator (CFTR) protein [1]. CFTR was characterized as a chloride (Cl^-) channel activated by cyclic AMP (cAMP), with this response being the most important characteristic that distinguishes this channel from other Cl^- channels [2]. Several CF model systems have been described, from CF cell lines to CFTR knockout and transgenic mice [3–8]. These model systems need to be validated in a simple way, showing a CFTR-regulated Cl^- transport decrease.

Several methods have been used to measure CFTR activity, including patch clamp [9], microelectrode [10] and ^{36}Cl [11] or ^{125}I tracers [12]. However, these methods are cumbersome and time-consuming, and they often lack sufficient selectivity and/or sensitivity [10,13]. In addition, different halide-sensitive fluorescent probes have been used to measure Cl^- currents and determine Cl^- concentration [14–16]. 6-Methoxy-1-(3-sulfopropyl)quinolinium (SPQ) is the halide-sensitive probe used more extensively for measuring CFTR activity in different models and assays for CFTR and CF studies [17–22]. The probe is quenched by Cl^- through a collisional mechanism; its fluorescence responds very fast to changes in Cl^- concentration (<1 ms), and in aqueous solutions it is not sensitive to HCO_3^-, SO_4^{-2}, NO_3^-, and cations [14].

Different methodologies have been developed to measure CFTR activity using the SPQ probe [14,15,23,24], each with different technical limitations. Mainly, SPQ has low fluorescence and requires ultraviolet excitation; thus, measurement can be associated with high background from the autofluorescence originating in the cells or the noise intrinsic to the instrument [25]. In addition, the methods used most frequently, such as patch clamp and fluorescence microscopy, require specialized skills and equipment, and many individual measurements are needed to obtain valuable data, making these procedures very time-consuming and cumbersome.

The goal of this work was to develop a simple and sensitive method to measure CFTR activity by using a fluorescence spectrophotometer, an instrument commonly found in a laboratory. The method allowed us to perform real-time SPQ measurements with high sensitivity, measuring CFTR activity of the colon carcinoma epithelial cell line T84, under basal and stimulated conditions and in the presence of the CFTR transport inhibitors glibenclamide and CFTR(inh)-172.

Materials and methods

Reagents

Glibenclamide, dimethyl sulfoxide (DMSO, culture grade), dibutyryl cAMP sodium salt, 3-isobutyl-1-methylxanthine (IBMX),

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[1] *Abbreviations used:* CF, cystic fibrosis; CFTR, CF transmembrane conductance regulator; Cl^-, chloride; cAMP, cyclic AMP; SPQ, 6-methoxy-1-(3-sulfopropyl)quinolinium; DMSO, dimethyl sulfoxide; IBMX, 3-isobutyl-1-methylxanthine; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; BSA, bovine serum albumin; PBS, phosphate-buffered saline; I^-, iodide; Ex, excitation; Em, emission.

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(−)-isoproterenol hydrochloride, and valinomycin were purchased from Sigma–Aldrich (St. Louis, MO, USA). CFTR(inh)-172 was obtained from Calbiochem (San Diego, CA, USA), trypsin from Life Technologies (Gibco, Rockville, MD, USA), and SPQ from Invitrogen (Carlsbad, CA, USA). All other reagents were analytical grade.

Cell culture

The human colon carcinoma epithelial cell line T84 (CCL-248, American Type Culture Collection [ATCC], Manassas, VA, USA) was used for validation of the fluorescence spectrophotometer method; these cells express wild-type CFTR. Cells were cultured in Dulbecco's modified Eagle's medium (DMEM)/F12 (Life Technologies, Gibco), supplemented with 10% fetal bovine serum (FBS, Bioser, Buenos Aires, Argentina), 100 U/ml penicillin, 100 μg/ml streptomycin, and 0.25 μg/ml amphotericin B (Life Technologies, Gibco), in a humidified atmosphere containing 5% CO_2. For the experiments, 1×10^6 cells were seeded over p60 plates (2140 mm^2) containing up to four rectangular coverslips (22×8 mm, equivalent to 176 mm^2, Hitachi, Tokyo, Japan). The coverslips were pretreated with a coating solution (10 μg/ml fibronectin, 4.4 μg/ml collagen, and 1.5 μg/ml bovine serum albumin [BSA] in DMEM/F12) for 1 h and washed two times with phosphate-buffered saline (PBS). (The fibronectin coating added to the coverslip prevents the detachment of cells during perfusion and stirring.) The cells were grown to confluence.

Perfusion chamber

The Teflon coverslip holder of the fluorescence spectrophotometer (Hitachi F-2000) was modified by adding one hole and two perfusion tubes (cannulas) to transform the holder and quartz cuvette in a perfusion chamber (Fig. 1). The original injection hole was made wider from the top to the bottom of the holder, using a small drill tip (1 mm), to allow the insertion of a cannula up to the bottom of the holder (fixed with epoxy glue). In addition, a second hole, parallel and near the first hole, was made with a drill tip of 2 mm, just up to the end of the holder cap, in the region that is shorter than the rest of the holder (~1 cm from the top). Then a third hole (2 mm wide) was made to connect the second hole to the external area, for the exit of the second cannula, over the top of the coverslip. The first cannula (1 mm o.d.) was added for the input of buffers and treatments, and the second cannula (2 mm o.d.), the shorter and wider one, was added for the output. The buffer-out cannula was slightly wider than the buffer-in cannula to ensure a constant volume within the cell and to prevent liquid overflow. Fig. 1A shows a photograph of the coverslip holder and cuvette transformed in a perfusion chamber. Both tubes were connected to a peristaltic pump, which was adjusted to ensure faster pumping from the buffer-out tube to prevent overflow. Fig. 1B and C shows a photograph and a schematic representation of the cuvette holder, respectively. In the scheme (Fig. 1C), it is illustrated how some of the excitation light can be 90° reflected by the coverslip and detected by the phototube when measurements are made near the excitation wavelength. However, this reflection signal rapidly disappears if the detected light wavelength is set a few nanometers away from the excitation wavelength (see peaks due to reflection of incident light as dotted lines in Fig. 3). For measurements, confluent monolayers of cells, grown over coverslips, were maintained under perfusion with constant stirring and temperature (37 °C). The perfusion rate was 3 ml/min, and the cuvette volume was 2.4 ml. The temperature was controlled by using an external water circulation system, and the homogeneity of the solution in the chamber was maintained by using the magnetic stirrer of this instrument and a small magnetic bar (Fig. 1) (we recently replaced the stirrer with an electronic magnetic stirrer, Variomag Mini, Thermo Fisher Scientific, Rockford, IL, USA).

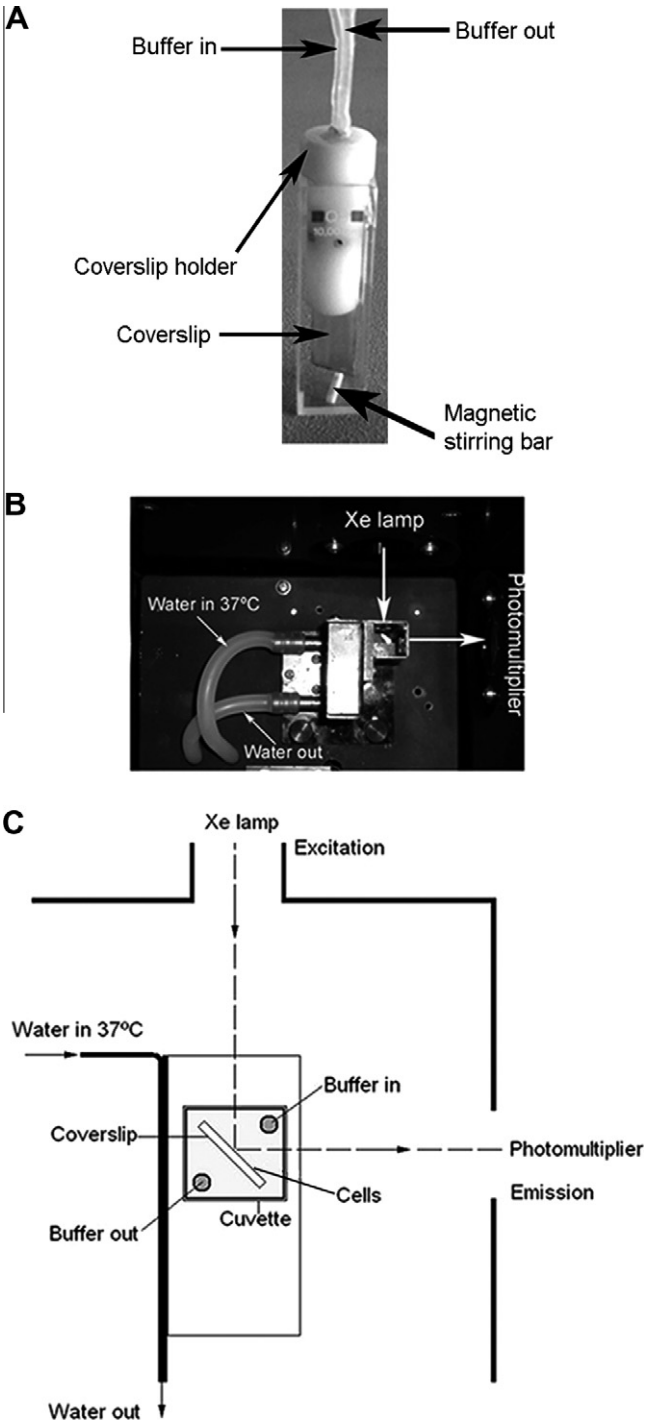

Fig.1. Scheme of the system used to measure SPQ under constant temperature and stirring in a fluorescence spectrophotometer. (A) Photograph of the quartz cuvette and the coverslip holder used, now transformed in a perfusion chamber. One perfusion tube reaches the bottom of the holder before the light path and, therefore, does not interfere with the light path. It is used to add the solutions. The other perfusion tube, which is wider, has its end approximately 1 cm above and is used to extract the solutions and maintain a constant volume. (B) Photograph of the cuvette holder, which maintains a constant temperature by recirculation of external water. It also contains a magnetic stirrer capable of different stirring speeds. The direction of the light is also shown. (C) Schematic representation of the cuvette holder.

SPQ emission and excitation spectra

SPQ is a water-soluble fluorescent dye; its fluorescence is quenched by Cl^- and other halides such as iodide (I^-) and bromide [14]. The emission and excitation spectra of the fluorescent probe were measured with a fluorescence spectrophotometer (Hitachi F-2000) using a 10-μM SPQ solution prepared in ultrapure water (PURELAB Ultra Mk 2 Genetic, ELGA LabWater, Buckinghamshire, UK). The excitation (Ex) spectra were recorded detecting the fluorescence at 443 nm, with Ex from 260 to 425 nm. The emission (Em) spectra were obtained with Ex at 344 nm, detecting the Em from 320 to 550 nm. The equipment settings were as follows: photomultiplier voltage, 700 V; bandpass, 10 nm (Ex and Em); response, 2 s each. For measurements of SPQ Ex and Em spectra inside the cells, T84 cells grown on coverslips were loaded with 5 mM SPQ in serum-free DMEM/F12 for 16 to 20 h. Then the cells were washed with prewarmed PBS (pH 7.4) and inserted into a holder, specially designed by Hitachi for this instrument (F-2000), inside a quartz cuvette of the fluorescence spectrophotometer. The measurements were made under perfusion with nitrate buffer (135 mM $NaNO_3$, 10 mM glucose, 1 mM $CaSO_4$, 1 mM $MgSO_4$, 10 mM Hepes, 2.4 mM K_2HPO_4, and 0.6 mM KH_2PO_4 at pH 7.4).

Stern–Volmer constant for SPQ quenching by Cl^-

First, the Stern–Volmer constant for SPQ quenching by Cl^- (K_{Cl^-}) was determined in ultrapure water using a Cl^- concentration curve (0–100 mM) with 2.5 μM SPQ. Next, the intracellular K_{Cl^-} for SPQ in T84 cells was measured by using the double ionophore method with some modifications [26]. Briefly, T84 cells were grown on coverslips and loaded overnight with 5 mM SPQ. After that, the cells were perfused at 37 °C with a concentration gradient of Cl^- made using two high-K buffers, one with KNO_3 and the other with KCl, to form the gradient (high-KCl buffer: 1.3 mM Ca-gluconate, 100 mM KCl, 40 mM K-gluconate, 3.7 mM NaH_2PO_4, 0.4 mM KH_2PO_4, 4.2 mM $NaHCO_3$, 0.7 mM $MgSO_4$, and 5.5 mM D-glucose; high-KNO_3 buffer: 1.3 mM Ca-gluconate, 100 mM KNO_3, 40 mM K-gluconate, 3.7 mM NaH_2PO_4, 0.4 mM KH_2PO_4, 4.2 mM $NaHCO_3$, 0.7 mM $MgSO_4$, and 5.5 mM D-glucose) containing the ionophores nigericin (5 μM) and tributyltin (10 μM). A gradient former was used for establishing the Cl^- gradient, and 10 nm fluorescein was added to the high-KCl buffer as a standard control to determine the Cl^- concentration at each measurement point and to make sure that the solution was equilibrated and homogeneous. A calibration curve for fluorescein, fluorescence versus fluorescein/Cl^- concentration, was made by serial dilutions of the high-KCl buffer containing 10 nm fluorescein in high-KNO_3 buffer. Fluorescence intensity was measured with the fluorescence spectrophotometer (Hitachi F-2000) as described above, now using dual wavelength for Ex and Em. For SPQ we used Ex = 344 nm and Em = 443 nm, and for fluorescein we used Ex = 495 nm and Em = 519 nm. At each measurement point, the flow rate was stopped to allow reaching homogeneity in the perfusion chamber and to reach equilibrium between the intracellular and the external Cl^- concentrations. After equilibration, values were recorded during 60 s and the average value for each calculated concentration was used to construct the Stern–Volmer plot.

Functional CFTR assay

A 50-mM SPQ stock solution was prepared in serum-free DMEM/F12 medium and stored at −20 °C until used to load the cells. The method described by Verkman [16] and Tondelier and coworkers [27] was used with some modifications. The SPQ fluorescence was measured in cells grown over coverslips using a fluorescence spectrophotometer (Hitachi F-2000). T84 cells were grown in p60 plates containing rectangular coverslips (22 × 8 mm, Hitachi). The coverslips were pretreated with a coating solution (10 μg/ml bovine fibronectin, 4.4 μg/ml bovine collagen, and 1.5 μg/ml BSA, made in DMEM/F12 medium) for 1 h at 37 °C and were washed with PBS. After plating, the cells were grown to near confluence. Then the cells were cultured for 24 h in serum-free DMEM/F12 medium, and some samples were treated with inhibitors of CFTR Cl^- transport activity. In the initial experiments, the sulfonylurea glibenclamide (100 μM) was used. Then we tested the inhibition by using the thiazolidinone CFTR inhibitor CFTR(inh)-172 (5 μM), which was reported to be more potent and specific than glibenclamide [5,28]. The stock solutions for both inhibitors were made 1000× in DMSO. Treatments were performed while the cells were loaded with 5 mM SPQ in serum-free DMEM/F12 for 16 to 20 h. Then the cells were washed twice with iodide buffer (135 mM NaI, 10 mM glucose, 1 mM $CaSO_4$, 1 mM $MgSO_4$, 10 mM Hepes, 2.4 mM K_2HPO_4, and 0.6 mM KH_2PO_4 at pH 7.4) and maintained at 37 °C for 30 min in the incubator (under CO_2) to ensure the quenching of intracellular SPQ. Finally, each coverslip was placed in a separated culture dish and covered from the light. For measurements, confluent monolayer cells on the coverslips were inserted in a holder that was placed inside a quartz cuvette containing iodide buffer. By using the build chamber shown in Fig. 1A, the cells were maintained under perfusion and stirring at 37 °C. The wavelengths used to measure the SPQ fluorescence were Ex = 344 nm and Em = 443 nm. Initially, a perfusion with iodide buffer was performed to measure the baseline fluorescence (F_b) for 100 s. Next, the cells were perfused with nitrate buffer (described above) for 200 s, followed by perfusion with the same buffer containing a cocktail of CFTR activators (200 μM dibutyryl cAMP, 200 μM IBMX, and 20 μM isoproterenol in nitrate buffer) for another 200 s. Finally, the cells were perfused with quenching buffer (iodide buffer and 5 μM valinomycin) for 100 s. The stock solutions for valinomycin, IBMX, and dibutyryl cAMP were made 1000× in DMSO culture grade and stored at −20 °C. The isoproterenol was prepared in water at 1000× concentration. The controls were treated with the same DMSO concentration. The collected data were plotted as $F/F_i - 1$ versus time, where F_i represents initial fluorescence and F is the fluorescence for each point of time. For analysis, the areas under the curve were calculated by numerical integration and the average values and standard deviations were calculated for each set. All figures are representative of two independent experiments, each made by triplicate. The results were analyzed by analysis of variance (ANOVA) and Tukey tests.

Results

SPQ emission and excitation spectra

To determine the best Ex and Em wavelengths in our assay conditions, we first measured the Em and Ex spectra of 10 μM SPQ in ultrapure water. As shown in Fig. 2, two Ex peaks were detected for SPQ in water, one at 320 ± 10 nm and the other at 344 ± 10 nm, whereas one Em peak was observed at 443 ± 10 nm. Then the Ex and Em spectra of the SPQ fluorescent probe were measured within T84 cells loaded with SPQ maintained under continuous perfusion with nitrate buffer (Fig. 3). Similar values of maximal SPQ excitation (Ex = 344) and emission (Em = 443) were obtained in ultrapure water or in nitrate buffer with SPQ-loaded cells (Figs. 2 and 3). In addition to the Em and Ex peaks observed for SPQ inside the cells, a small signal was detected in the Em spectrum at 344 nm due to coverslip reflection of the Em light (Fig. 3A, dotted lines). This reflection was at a wavelength far from the recording value of Em = 443 nm and, therefore, did not interfere with the

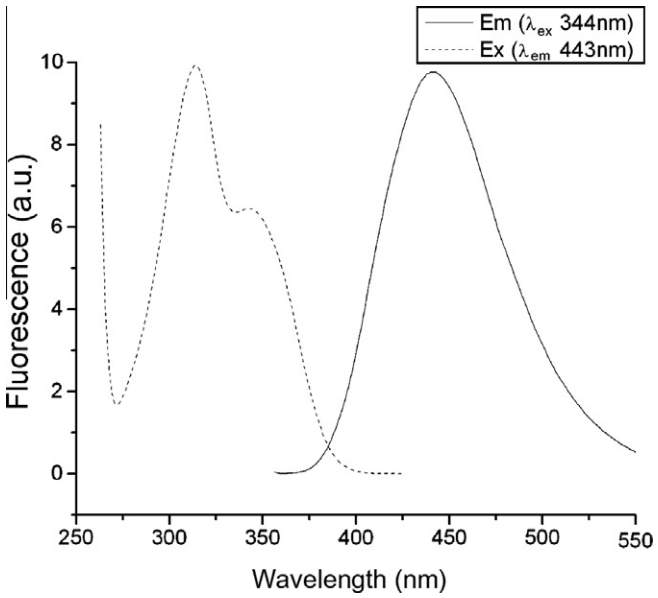

Fig.2. Emission and excitation spectra of SPQ in water. A solution of 10 μM SPQ in ultrapure water was used for measurements. The dotted line shows the excitation spectra obtained at 443 nm (Em = 443 nm), and the solid line represents the emission spectra obtained with excitation at 344 nm (Ex = 344 nm). a.u. indicates arbitrary units.

measurements. This coverslip reflection was also observed in the Ex spectrum at 443 nm (Fig. 3B).

Stern–Volmer constant

The SPQ fluorescence was measured as a function of Cl^- concentration to determine the Stern–Volmer constant (K_{Cl^-}) in our system. The results (Fig. 4A) show the typical decay of the SPQ fluorescence signal (2.5 μM SPQ solubilized in ultrapure water) when the Cl^- concentration was increased from 0 to 100 mM. The equation $F_o/F - 1 = K_{Cl^-}$ [Cl^-] was applied to obtain the Stern–Volmer plot and to calculate K_{Cl^-} (Fig. 4B), where F_o is the SPQ fluorescence without Cl^- and F is the fluorescence at each point. As shown in Fig. 4B, the SPQ fluorescence was quenched by Cl^-, showing a linear relationship with the Cl^- concentration. The K_{Cl^-} obtained in ultrapure water (PURELAB Ultra MK 2 Genetic system, ELGA LabWater, Woodridge, IL, USA) was 115.0 ± 2.8 M^{-1}, consistent with previously published data [14,24]. Similar results (not shown) were observed using two different concentrations of SPQ in water (7.5 and 10 μM). Other authors have used 5 and 10 μM for this purpose [14,15,23]. Then the intracellular SPQ quenching was determined in T84 cells by the double ionophore technique using nigericin and tributyltin to equilibrate intracellular and extracellular Cl^- concentration in high-potassium buffers [26]. Fig. 5A shows the intracellular SPQ fluorescence signal decay with increasing Cl^- concentrations and the corresponding fluorescence values of fluorescein as internal standard. The Cl^- concentrations indicated in the graph were calculated by using a fluorescein calibration curve (fluorescence vs. Cl^- concentration). The flux was stopped at each measurement point to allow the stabilization of the signal, and the mixing of the Cl^- solution by the stirrer, to obtain a homogeneous Cl^- concentration. Applying the equation $F_o/F - 1 = K_{Cl^-}$ [Cl^-], the Stern–Volmer constant obtained for the intracellular quenching of SPQ in T84 cells was K_{Cl^-} = 17.8 ± 0.8 M^{-1} (mean ± standard error, n = 3) (Fig. 5B). This intracellular K_{Cl^-} value, lower than the K_{Cl^-} obtained for SPQ in water solution, is similar to the values reported previously, albeit using different cell lines [24,25].

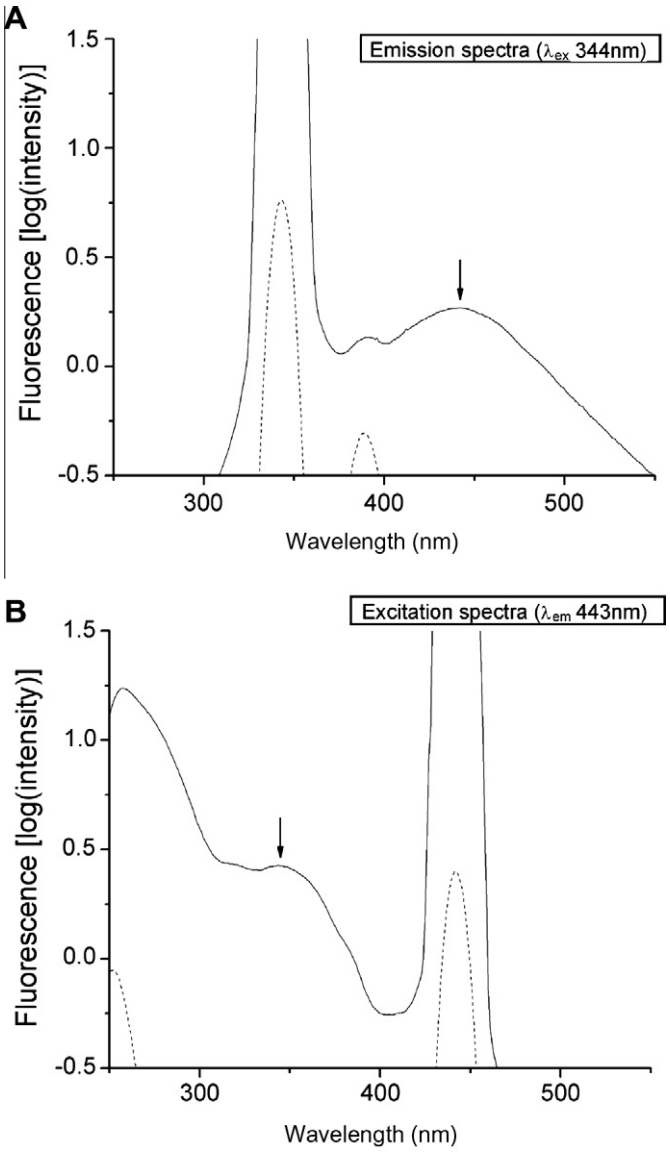

Fig.3. Emission and excitation spectra of SPQ in cells. The T84 cell monolayers were loaded overnight with 5 mM SPQ. Measurements were performed under continuous perfusion with nitrate buffer. (A) Em spectra measured at Ex = 344 nm. (B) Ex spectra measured at Em = 443 nm. The arrows show the wavelengths, without autofluorescence or reflections, used for all of the experiments (Ex = 344 nm and Em = 443 nm). The dotted lines represent the light reflected from the coverslip in the absence of SPQ.

Functional assay

The T84 cells grown over rectangular coverslips were incubated in DMEM/F12 medium (plus 10% FBS) until confluence. Then the coverslips were placed into the holder and perfusion chamber and were perfused with iodide buffer until a stable fluorescence baseline was obtained (~100 s). At this point, the intracellular SPQ was quenched and the initial fluorescence (F_i) value was calculated as the minimal value read. Then nitrate buffer was pumped into the perfusion chamber to permit the intracellular exchange between the intracellular I^- and the extracellular NO_3^- (200 s). At this point, the system showed basal CFTR activity. This perfusion was followed by a perfusion with the same nitrate buffer now containing the CFTR stimulation cocktail (cAMP–IBMX–isoproterenol). After stimulation (200 s), the chamber was perfused with a quenching buffer. As shown in Fig. 6A, an increase in the basal SPQ fluorescence was ob-

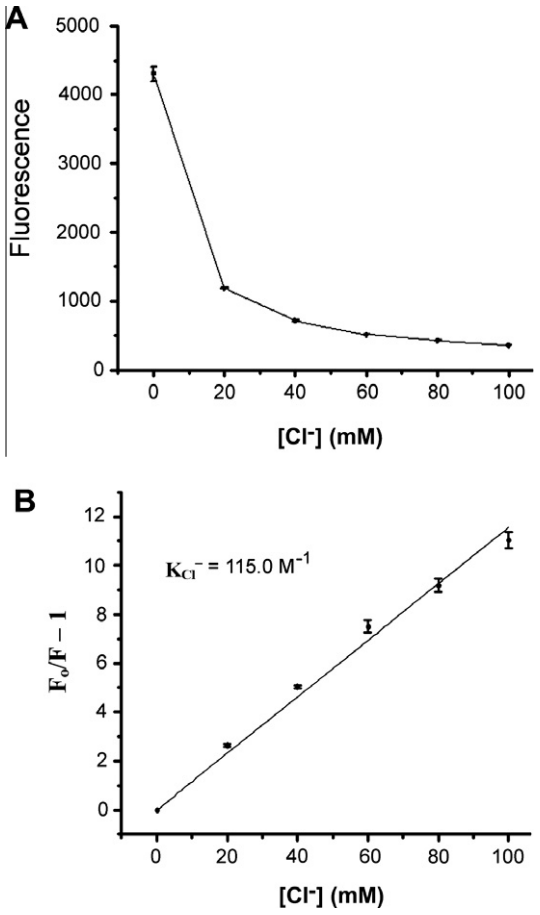

Fig.4. Quenching of SPQ fluorescence in water solution by chloride. (A) SPQ fluorescence under different Cl^- concentrations. The fluorescence decreased hyperbolically with increasing concentrations of Cl^-. (B) Quenching of SPQ by Cl^- shown as a Stern–Volmer plot ($F_o/F - 1$ vs. [Cl^-]). The solid line represents a linear fit of $F_o/F - 1$ to the data. A Stern–Volmer constant (K_{Cl^-}) of 115.0 ± 2.8 M^{-1} (mean ± standard error, $n = 3$) was obtained in ultrapure water. The results are representative of two independent measurements, each made by triplicate.

served when the initial I^- was replaced by NO_3^-. This is due to the efflux of I^- from the cells, because NO_3^- ions do not quench SPQ fluorescence, and represents basal CFTR activity. Then the cells were stimulated with the CFTR stimulation cocktail, and a clear increment in CFTR activity was observed. To demonstrate that the increased fluorescence after the addition of the CFTR-stimulating cocktail was due to CFTR activation, another set of T84 cells was previously treated with pharmacological inhibitors of the CFTR channel (100 μM glibenclamide and 5 μM CFTR(inh)-172) during the SPQ loading and perfusion steps (the concentrations of the inhibitors were maintained during the entire measurements). As expected, cells treated with CFTR inhibitors showed only a weak response. The differences between stimulated cells and the cells treated with the different CFTR inhibitors were significant ($P > 0.05$).

Discussion

The measurement of CFTR activity in CF system models is essential to validate the CF phenotype. Here we have described a very simple method to measure CFTR activity by using a fluorescence spectrophotometer and the fluorescent probe SPQ as an indicator of Cl^- concentrations and efflux. The Ex and Em wavelength spectra of SPQ measured in water were in close agreement with those obtained by Illsley and Verkman [14]. In addition, the Stern–Volmer constants (K_{Cl^-}) in water and inside the cells were also similar to data reported previously [14,24], indicating that the current method is at least similar to other methods regarding sensitivity and linearity.

To validate the method for a CFTR assay, the T84 human colonic adenocarcinoma cell line was used. These cells have a high expression of CFTR wild type and have been used extensively as a model system to study epithelial Cl^- secretion [29–34]. We previously used these cells to study the effects of CFTR activity on the expression of CFTR-dependent genes after treatment with CFTR transport inhibitors [3–5]. On the other hand, the response to cAMP allows distinguishing CFTR transport activity from activity of other Cl^- channels. The results obtained when the cells were stimulated using the cAMP–isoproterenol–IBMX cocktail showed a significant response, similar to the responses obtained with previous methods [15,21,25,35–37]. In addition, as expected, the CFTR inhibitors impaired the response to the CFTR stimulation cocktail. Altogether, these results indicate that the fluorescence spectrophotometric method described here is a suitable approach to measure CFTR activity.

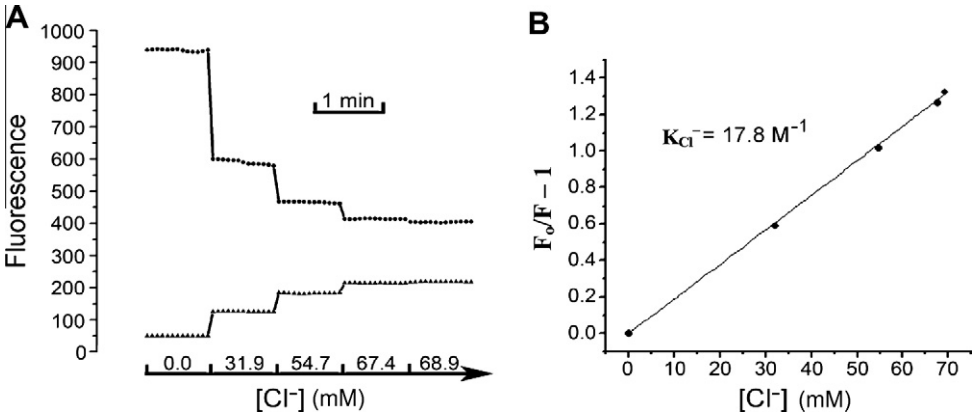

Fig.5. Intracellular quenching of SPQ by chloride in T84 cells. (A) Intracellular SPQ fluorescence under different Cl^- concentrations. A Cl^- concentration gradient was formed by perfusion of 100 mM Cl^- in a high-K buffer containing the ionophores nigericin (5 μM) and tributyltin (10 μM) to equilibrate extracellular and intracellular Cl^- concentrations. Fluorescein was used as internal standard to determine the Cl^- concentration at each measured point. Perfusion was stopped for 1 to 2 min each 5 to 10 min of perfusion to allow the Cl^- equilibration between the perfusion chamber and the intracellular Cl^- concentration and to have a homogeneous Cl^- concentration; the fluorescence values at these points are plotted. The Cl^- concentration in each stopped point was determined by using a standard curve of fluorescein and chloride (serial dilutions of fluorescein [10 nm] and chloride [100 mM]). The graph shows the points in which the perfusion was stopped for 60 s. The last 10 values were averaged to obtain the Stern–Volmer plot. ●, SPQ; ▲, fluorescein. (B) Stern–Volmer plot analysis ($F_o/F - 1 = K_{Cl^-}$ [Cl^-]) for the double ionophore calibration. The K_{Cl^-} obtained was 17.8 ± 0.8 M^{-1} (mean ± standard error, $n = 3$).

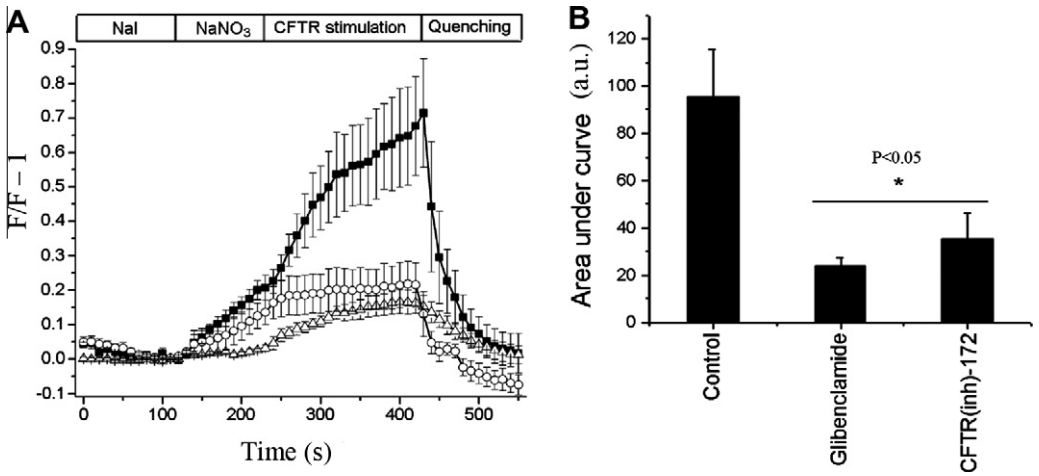

Fig.6. Functional analysis of CFTR activity measurement. (A) CFTR activity corresponding to T84 cells treated with DMSO (control, ■) and the CFTR inhibitors glibenclamide (100 μM, △) and CFTR(inh)-172 (5 μM, ○). F indicates fluorescence intensity, and F_i indicates the minimal fluorescence value when the nitrate buffer was perfused and equilibrated. $F/F_i - 1$ is the ratio used to normalize the fluorescence intensity to the lower value measured. (B) Quantification of the areas shown in panel A obtained by numerical integration of the values obtained from the addition of the CFTR-stimulating cocktail (200 μM dibutyryl cAMP, 200 μM IBMX, and 20 μM isoproterenol) up to the addition of the quenching cocktail. a.u. indicates arbitrary units. $^*P < 0.05$. The figure is representative of two independent experiments, each made by triplicate ($n = 3$).

The method has several advantages. The perfusion chamber, using a quartz cuvette, has a low fluorescence background. In addition, it allows stirring of the liquid in the perfusion chamber, which is very important to obtain a uniform Cl^- concentration for measurements and to rapidly reduce the I^- concentration around cells, a procedure that cannot be done with plate readers. Most important, it allows the measurement of thousands of cells at the same time, increasing the signal/noise ratio, and provides samples that are more representative of the entire population of cells, reducing the amount of replicates needed to obtain accurate and representative results. Finally, there is no need for costly equipment and specialized skills. In conclusion, the results show that by using a simple perfusion chamber and a common fluorescence spectrophotometer, it is possible to measure CFTR activity in a reproducible, accurate, and sensitive way.

Acknowledgments

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