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Quercetin Exhibits a Specific Fluorescence in Cellular Milieu: A Valuable Tool for the Study of Its Intracellular Distribution

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The elaboration of novel techniques for flavonoid intracellular tracing would elucidate the compounds' absorption and bioavailability and assist molecular and pharmacological approaches, as they are promising candidates for drug development. This study exploited the properties of quercetin (3,3′,4′,5,7-pentahydroxyflavone), found in high concentrations in the majority of edible plants. Through the use of UV−vis spectroscopy, confocal microscopy, and HPLC-ESI-MS, native quercetin, at physiologically relevant concentrations, was found to exhibit a specific fluorescence (488 nm_{ex}/500−540 nm_{em}) upon internalization. This fluorescence shift is due to a non-covalent binding to intracellular targets (probably proteins) and compatible with the settings applied in confocal microscopy. This property provides a valuable, selective alternative technique for quercetin tracing in cellular systems, permitting the quantitative evaluation of its transit at pharmacologically relevant concentrations and the validation of a number of already described molecular functions.

KEYWORDS: Quercetin; flavonoids; fluorescence; hepatocytes

INTRODUCTION

Flavonoids constitute a large family of plant-derived phenolic compounds present in vegetables, fruits, brews, and spices and normally taken in through the diet (*1*). Their hydrogen-donating abilities and their propensity for nitration make them powerful scavengers of reactive oxygen and nitrogen species. In addition, recent evidence suggests their interference with redox-sensitive cell signaling pathways (*2*). Epidemiological and intervention studies implicated polyphenol-rich diets in reduced chronic disease incidence and morbidity, including cardiovascular diseases and cancer (*3*, *4*). Moreover, flavonoids and flavonoid derivatives, alone or in conjunction with established treatment, were found to be of therapeutic value in a number of clinical trials (*5*, *6*).

Quercetin (3,3′,4′,5,7-pentahydroxyflavone) is a major flavonol, found in most edible plants, in its native form or as glucoside metabolites. Quercetin aglycone and glucosides are absorbed from the upper gastrointestinal tract, probably the duodenum, whereas quercetin-3-rutinoside is absorbed from the distal part, probably the colon (*7*). The major amount of quercetin detected in biological fluids is in the form of quercetin metabolites (*8*). Marked interindividual variations in quercetin-3-rutinoside bioavailability have been observed: native quercetin was more bioavailable in women, especially during oral contraceptive treatment, compared with men. Finally, radioactive quercetin administration revealed that flavonol absolute bioavailability ranged from 36 to 53%, whereas a substantial portion of quercetin was excreted by the lungs as CO_2 (*9*). Absorption or elimination of flavonoids is also affected by diet components, as fat content increased quercetin bioavailability (*10*). Nevertheless, after the ingestion of quercetin, the native compound could be detected in plasma, at concentrations of ~5 μM (*7*, *11*), suggesting that some of the actions of the compound might be attributed to the native molecule itself.

Quercetin was reported to interact with a number of signaling molecules (see ref *12* for a recent review). The majority of these studies were made in solution with purified molecules, using flavonol concentrations much higher than those found in biological fluids. However, in order to attribute a possible biological role to the compound, at concentrations compatible with those found in the cellular milieu (<5 μM), native quercetin should be internalized. Hitherto, the absorption of native quercetin and its metabolites by cells was assayed either with

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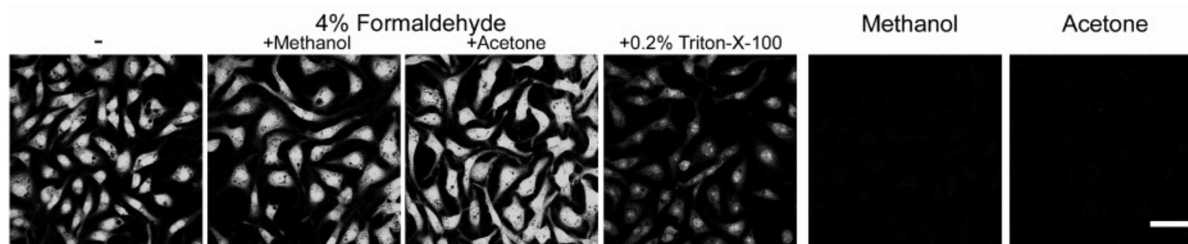

Figure 1. Quercetin fluorescence is a tool for its tracing within cells. HepG2 cells were treated for 30 min with 3 μM quercetin in PBS, and fluorescent staining was observed in a confocal microscope at 488 nm_{ex}/500–540 nm_{em}. Fluorescence signal was preserved with formaldehyde. Subsequent postfixation treatment with polar solvents (methanol or acetone) induced diffusion of the staining. Postincubation with Triton-X-100, a classic nonionic detergent used for cell permeabilization in immunofluorescence protocols, resulted in signal edulcoration. Interestingly, methanol or acetone alone eliminated intracellularly trapped quercetin (scale bar = 50 μm).

HPLC-NMR methods (*13*−*15*) or with the use of radiolabeled compounds (*16*−*20*). In the present paper, we propose an easy alternative tool to investigate native quercetin distribution in living cells, namely, the specific fluorescence of the flavonol in a cellular milieu. We show that the agent is non-covalently associated with intracellular targets, exhibiting a specific fluorescence exclusively in the cellular environment, in settings compatible with those applied in confocal microscopy. This property could provide a valuable, selective technique for quercetin tracing in cellular systems, permitting the quantitative evaluation of its transit and the validation of a number of already described molecular functions.

MATERIALS AND METHODS

Chemicals. All biochemicals were obtained from Sigma-Hellas (Athens, Greece) and Tocris (Bristol, U.K.). Culture media were from Gibco BRL (Life Technologies, Paisley, U.K.). Quercetin (3,3′,4′,5,7-pentahydroxyflavone) was prepared from total red wine polyphenol extract, by semipreparative HPLC. Its purity (>99%) was confirmed by analytical HPLC and proton nuclear magnetic resonance, to obtain the highest possible purity of the compound. Quercetin powder was conserved in a dark bottle, at −20 °C, under nitrogen.

Spectroscopic Analysis. Quercetin was initially dissolved in absolute ethanol (10^{-2} M) and subsequently in the appropriate vehicle. CsCl ultrapurified supercoiled pGL2 plasmid DNA (Promega, Madison, WI), human genomic DNA, purified with midi-prep columns (Qiagen N.V., Venlo, The Netherlands), or BSA fraction V was used for intercalation experiments. Reagents have been mixed either in nanopure H_2O or in PBS, and fluorescence spectra were recorded on an Aminco Bowman series 2 fluorometer (Spectronics Instrument, Rochester, NY), with the appropriate vehicle as blank. Quercetin absorbance spectra were recorded on a Perkin-Elmer Lambda 6 UV−vis spectrophotometer and analyzed with Spectrum v 2.00 software (Perkin-Elmer Co., Wellesley, MA). To study possible interactions of quercetin with cellular proteins we used a total HepG2 lysate: Cells (DSMZ; Braunschweig, Germany) were harvested and homogenized by sonication in 50 mM Tris buffer, pH 7.4, containing freshly added protease inhibitors (10 μg/mL PMSF and 1 μg/mL aprotinin). Unbroken cells were removed by centrifugation at 2500*g* for 15 min, and supernatants were used immediately.

DNA Electrophoresis. Quercetin and linear DNA (100 bp DNA ladder, Invitrogen, Carlsbad, CA) were mixed at equimolar concentrations and incubated at room temperature for 30 min. A sample of the mixture (containing 1 μg of DNA) was separated in 2% agarose gel in 0.5× TBE running buffer (44 mM Tris-base, 44 mM boric acid, and 1 mM EDTA, pH 8). Gels were photographed under UV light and thereafter stained in a bath of EtBr (2.5 mg/L).

Protein Electrophoresis. HepG2 protein extract was prepared as described above. Lysates were mixed with quercetin, incubated on ice for 30 min, and separated in 8% polyacrylamide gels, under denaturing or native conditions. After electrophoresis, gels were photographed under UV light, and thereafter proteins were stained with Coomassie Brillant Blue (CBB, 0.5%). Alternatively, gel strips with total HepG2 proteins, separated in either native or denaturing conditions, were incubated in quercetin solutions (10^{-2}−10^{-5} M), observed under UV light, and subsequently stained with CBB.

Confocal Microscopy. HepG2 cells were cultured in RPMI 1640 (10% FBS), at 37 °C and 5% CO_2 and subcultured weekly. Cells were plated in poly-l-lysine-coated coverslips in six-well plates (10^5 cells/well), co-incubated with quercetin (3 μM), quickly washed with PBS, and fixed with 4% formaldehyde for 5 min. Subsequently, coverslips were put upside-down on glass slides, with a drop of Mowiol antifading reagent. Specimens were observed with a Leica TCS SP confocal scanner system (Heidelberg, Germany), using a 40x oil immersion objective and zoom software options.

HPLC Analysis of Quercetin Metabolites. Cells were incubated for 30 min with 3 μM quercetin in PBS, quickly washed, extracted with acid methanol (0.2% acetic acid), scrapped, sonicated, and centrifuged. The supernatant was dried in vacuo, rediluted in 25 μL of acetonitrile, and analyzed with HPLC-ESI-MS. We used a Waters 1525 binary HPLC pump system with a Waters oven (Waters, Milford, MA) and online DAD-FLD-ESI/MS detection (Thermo Electron, Waltham, MA). UV signal was recorded from a Waters 2996 photodiode array detector, and data obtained by a Waters 2475 multi λ fluorescence detector were further monitored with Empower software. The outlet flow was fully directed to a Thermo Finnigan LCQ Advantage ion-trap mass spectrometer with an electron spray ionization source (ESI). For HPLC analysis an Atlantis RP18 column (5 μm, 250 × 4.6 mm i.d.) with a guard column (2 cm) was used. Elution was performed at 30 °C with a 500 μL/min flow rate, and an isocratic solvent system of water/acetonitrile/acetic acid (42:58:2, v/v/v) was applied. UV spectra were recorded from 200 to 600 nm (2 nm resolution) and fluorescence spectra from 400 to 600 nm (345 nm_{ex}). The mass spectrometer was operated in either negative or positive mode at a 4.5 kV voltage source voltage source, −38.0 V (+9.0 V) capillary voltage, −40 V (−10 V) tube lens offset, and 200 °C capillary temperature.

RESULTS

Native Quercetin Exhibits a Specific Fluorescent Profile in Living Cells. Incubation of HepG2 cells with quercetin (3 μM in PBS or culture medium) resulted in a yellow-green fluorescent staining under UV light. This signal could be also detected in a confocal microscope, upon excitation with an Ar−Kr laser at 488 nm and in the emission window of 500−540 nm (**Figure 1**). Fluorescent signal was preserved with formaldehyde, but swilled with repeated washes. We assumed that the flavonol was trapped, upon binding and chemical cross-linking, within unknown protein molecules. Indeed, alternative fixation conditions using polar solvents resulted in quercetin elution. This could be due to either the molecular nature of quercetin or the inevitable disposal of soluble proteins and lipids that could serve as quercetin intracellular targets. When methanol or acetone was applied after formaldehyde fixation, the fluorescent signal was still intense but diffused within cells, indicating that polar agents are not able to abolish formaldehyde-stabilized quercetin molecules. Moreover, postfixation treatment

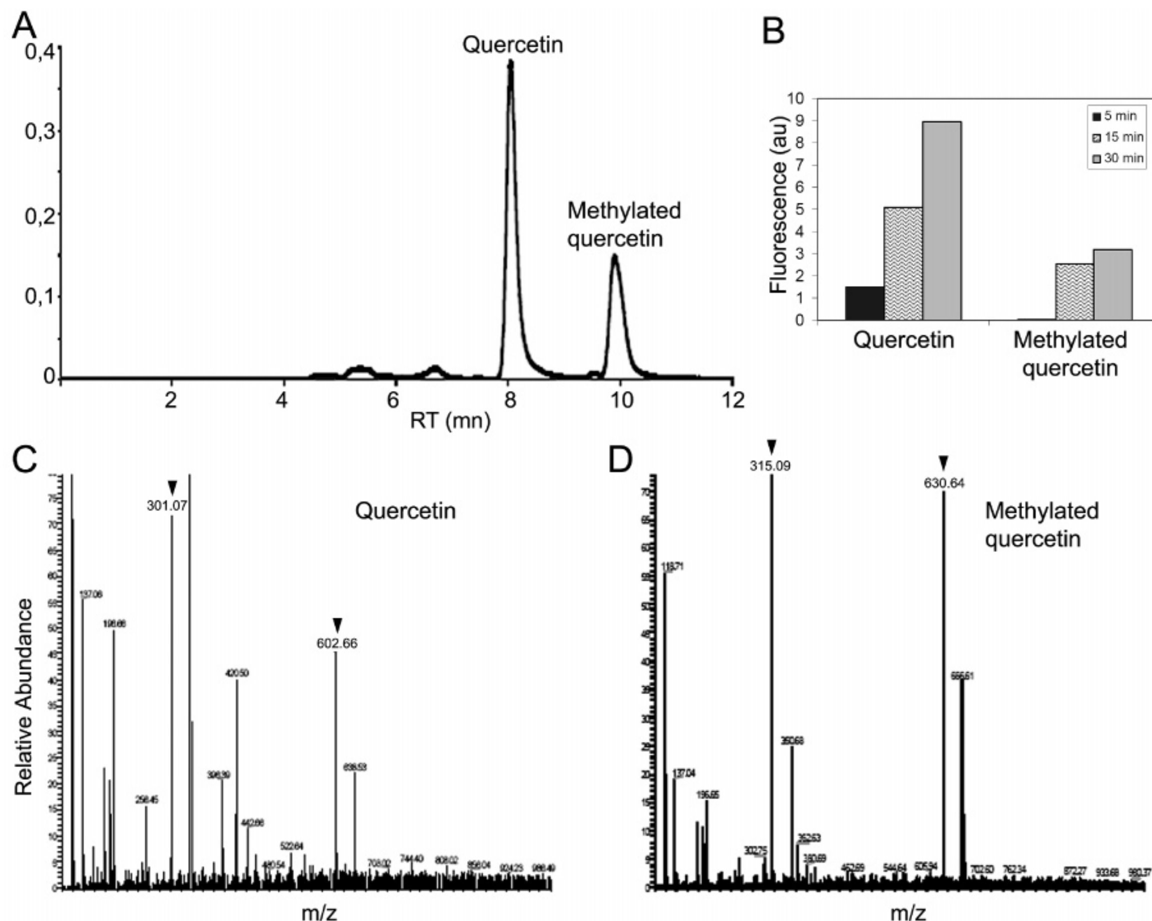

Figure 2. HPLC analysis of internalized quercetin. HepG2 cells were treated with quercetin (3 μM in PBS) for 5–30 min and extracted with methanol/0.2% acetic acid, and samples were subjected to HPLC-ESI-MS analysis: (**A**) HPLC chromatogram obtained at 369 nm DAD absorption signal; (**B**) quercetin and *O*-methylquercetin intracellular levels, based on HPLC signals from corresponding time samples; (**C**) mass spectrum analysis, obtained from TIC between 7.94 and 8.22 nm, corresponding to quercetin (molecular ion $[M - H]^-$ at *m/z* 301.07); (**D**) mass spectrum analysis, obtained from TIC between 9.83 and 10.09 nm, corresponding to methylated quercetin (molecular ion $[M - H]^-$ at *m/z* 315.09). Dimer ions appeared at *m/z* 602.66 and 630.64, corresponding, respectively, to quercetin and its methyl derivative (arrowheads).

with Triton X-100, a nonionic surfactant, resulted in fluorescence quenching. No differentiation of the fluorescence settings was observed upon fixation. Considering the nature of fixatives and their implication in flavonol intracellular detection, we concluded that quercetin binding is non-covalent and requires protein targets.

Quercetin Metabolism. Hepatocytes are metabolically active cells, accumulating flavonoid conjugates. To verify whether the aforementioned fluorescence is due to the native (unconjugated) quercetin or its metabolites, we have treated HepG2 cells with 3 μM quercetin and extracted them with acidified methanol after 5, 15, and 30 min. HPLC analysis followed by mass spectroscopy (**Figure 2**) revealed that native quercetin represents the major fraction of the detected intracellular flavonols. In addition, moderate levels of an O-methylated metabolite were detected after 15 min of incubation, whereas no oxidative derivatives were found. We concluded that cell fluorescence is due to the native molecule, which can accumulate into the cells, when applied at physiologically relevant concentrations, after a short incubation time.

Preliminary Analysis of Quercetin Fluorescent Profile in a Cellular Milieu. Analysis of quercetin absorbance spectra (3 μM in PBS) comprises two peaks at 310 and 335 nm. Further monochromatic excitation versus emission analysis showed quercetin optimum settings at 430 nm_{ex}/500 nm_{em} (**Figure 3A**,**B**), whereas an additional weak peak at 335 nm_{ex}/390 nm_{em} was observed, only at low flavonoid concentrations. Nevertheless, as described above, quercetin-treated cells exhibited a specific fluorescent signal at 488 nm_{ex}/500−540 nm_{em}. These findings do not correlate with the in vitro quercetin fluorescent profile. We assumed that the fluorescence shift of quercetin in a cellular milieu might be attributed either to energy transfer phenomena (as the flavonol is concentrating into the cells) or to quercetin binding to macromolecules, as previously reported (*21*, *22*). In this respect, we co-incubated quercetin with albumin, plasmid, or genomic DNA. Our results showed a quenching of the fluorescent signal upon quercetin co-incubation at 430 nm_{ex}/500 nm_{em} (**Figure 4A**). No signal was obtained at 488 nm_{ex}/500−540 nm_{em}. We further hypothesized that the differential profile of quercetin excitation in vivo could be possibly due to the intracellular milieu, including pH, salt concentration, presence of metal ions, and presumptive interaction with other macromolecules (*23*). Indeed, quercetin co-incubation with HepG2 lysate, at neutral pH, revealed an enhancement of the emitted fluorescence in the 500−600 nm window, after excitation at 488 nm (**Figure 3C**,**D**), whereas fluorescence shift was dose-dependent, indicating a specific binding among the flavonol and nonidentified proteins.

In Vitro Interactions of Quercetin with Nucleic Acids and Proteins. Although our spectroscopy data impugned quercetin/DNA interaction, we used an alternative approach to further study the above phenomenon: if such a binding occurs,

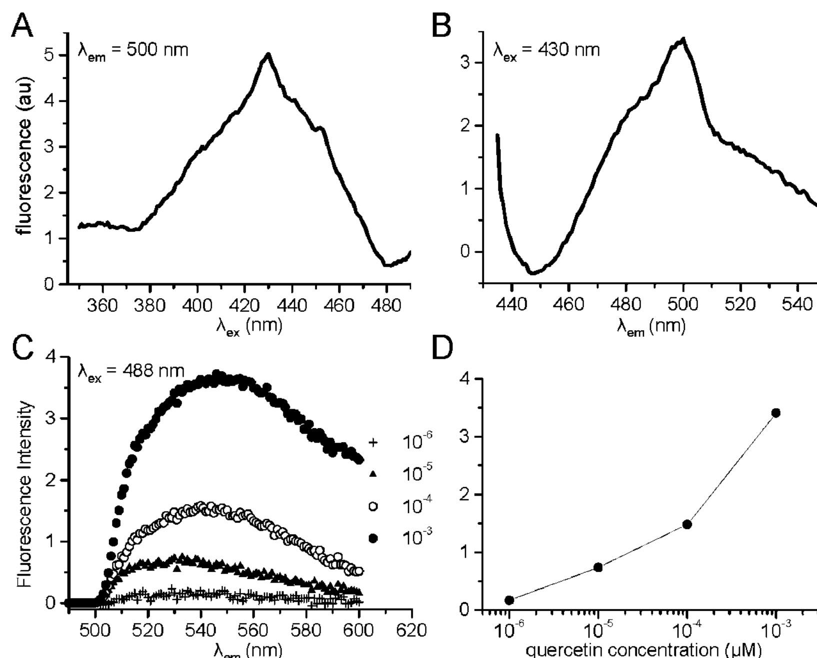

Figure 3. Quercetin exhibits a specific fluorescent profile in a cellular milieu: (**A**, **B**) quercetin (3 μM in PBS) optimum settings in solution [(**A**) excitation spectrum at 500 nm_{em}; (**B**) emission spectrum after excitation at 430 nm]; (**C**, **D**) fluorescence shift upon quercetin incubation with a HepG2 cell lysate [(**C**) emission spectra (λ_{ex} = 488 nm) subtracted from the corresponding values of cell lysate; (**D**) fluorescence intensity shift at 488 nm_{ex}/530 nm_{em} of quercetin–lysate mixtures is proportional to quercetin concentrations].

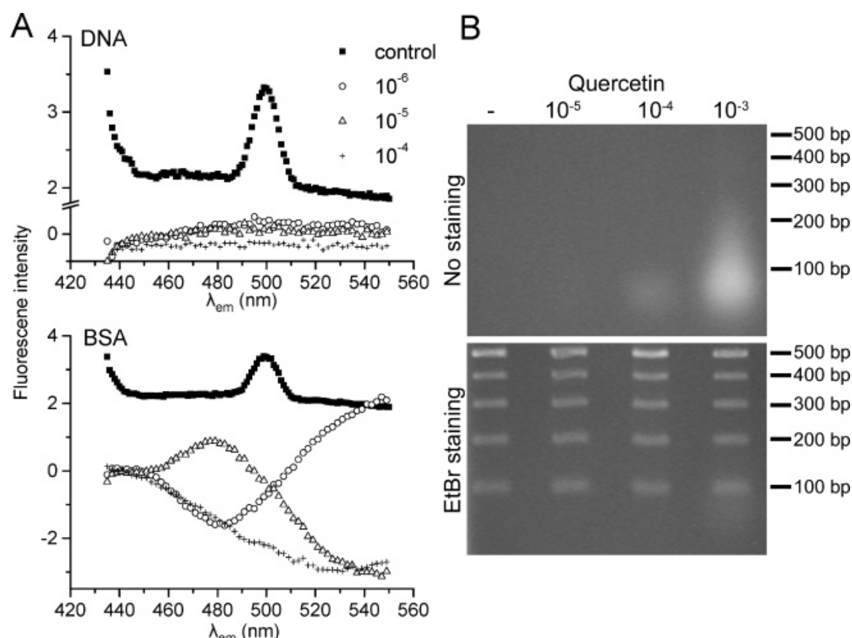

Figure 4. Quercetin in vitro interactions with nucleic acids or proteins. (**A**) Quercetin was co-incubated with either plasmid DNA (10 μM, calculated in base pairs) or BSA (10 μM) in PBS. Fluorescence emission spectra were recorded with ascending quercetin concentrations (10^{-6}–10^{-4}M) at 430 nm_{ex}/435–550 nm_{em} and normalized with the respective emission spectra of the flavonol alone. At these settings all reagents were found to produce a signal; however, co-incubation resulted in fluorescence quenching of the putative complexes. (Control = nucleic acids or BSA spectra in the absence of quercetin). (**B**) Quercetin does not intercalate to DNA, as EtBr stains equally treated or untreated DNA ladder.

quercetin should be able to eliminate other DNA interacalators' access (*24*). Therefore, we incubated quercetin with DNA and separated them by electrophoresis in agarose gel. Gel observation under UV light showed no DNA–quercetin colocalization. Quercetin was found to migrate along the electric field from negative toward positive potential, in front of DNA ladder fragments, <100 bp (**Figure 4B**), as postelectrophoresis staining with EtBr allowed band visualization. It is interesting to note that the known intercalator EtBr stained equally quercetin-treated and untreated samples. Similarly, quercetin co-incubation with HepG2 cellular extracts showed no fluorescent signal, whether proteins were separated under native or denaturing conditions. Moreover, no signal was obtained when gel lanes were soaked in quercetin solutions after PAGE (data not shown). Considering spectroscopy data, we concluded that the quercetin fluorescent shift is due to an interaction with a specific protein or a macromolecular complex; however, due to its nature, it could not be visualized using standard electrophoresis protocols.

DISCUSSION

Flavonoids, including quercetin, were characterized as a class of plant-derived xenobiotics that confer immediate antioxidant protection. Interestingly, they were also shown to precondition cell oxidative status and defense mechanisms and further modulate several molecular pathways, denoting rapid cellular uptake and direct interaction with macromolecules (see ref *12* for a recent review). Hitherto, flavonoid intracellular accumulation was studied using radiolabeled analogues, in combination with a number of separation/analytical techniques (see ref *1* for a discussion). However, it is not established whether these micronutrients may enter the cell and colocalize with their presumptive molecular targets. The elaboration of novel techniques for intracellular tracing would assist molecular and pharmacological approaches. Here, we show that native quercetin exhibits a specific fluorescence profile (488 nm_{ex}/500−540 nm_{em}) upon cellular internalization. This was further verified using UV−vis spectroscopy, confocal microscopy, and HPLC coupled to mass spectroscopy. This specific fluorescence could be further exploited to study in vivo flavonol transit and possible interactions, particularly at the infracellular level.

Quercetin has been reported to exhibit fluorescence at specific settings, especially upon binding to macromolecules (proteins or nucleic acids). It was shown to interact in solution with albumin (*22*), insulin (*25*), and actin (*26*). However, data about quercetin−albumin complexes' fluorescent properties are contradictory: quercetin−BSA interactions could be detected at 425 nm, although increased protein concentration obstructed complex formation (*27*); contrariwise, saturation kinetics analysis showed that these complexes could be detected at 485 nm_{ex}/530−550 nm_{em} (*28*). Nevertheless, no signal was detected with quercetin and BSA mixtures at 488 nm_{ex}/500−540 nm_{em}, indicating that this interaction does not intervene in the fluorescence emission, upon quercetin internalization. In addition, quercetin induced a fluorescence quenching at 430 nm_{ex}/500 nm_{em} upon incubation with BSA, in accordance with previous results (*29*). Preliminary results indicate that albumin−quercetin conjugates, non-covalent in nature (*30*), could determine the bioavailability of the flavonol in cellular systems or in vivo. On the other hand, preliminary data do not confirm a quercetin−actin association in a cellular system.

Quercetin was further shown to interact with purified nucleic acids and form covalent complexes that could be detected as a fluorescence shift at 395 nm (*18*, *24*). The flavonol initially stabilized the DNA secondary structure, whereas prolonged treatment led to an extensive disruption of the double helix (*31*), although Kang et al. suggested a non-covalent binding via electrostatic interactions (*32*). Quercetin redox properties are essential during molecular assembly, inducing DNA strand scission or albumin oxidative degradation (*33*−*36*). Only oxidized quercetin metabolites were capable of covalent binding, and the effect was dramatically decreased in the presence of reduced glutathione (*17*, *18*). Strand scission is also facilitated in the presence of Cu^{2+} (*37*) and rare earth metals, such as lanthanoids (*38*), as the respective complexes could modify DNA stability. However, the above studies have been performed in solution and at quercetin concentrations higher than those found normally in the cellular milieu. The experimental discrepancies could be further due to the applied method. Indeed, linear dichroism spectra and infrared analysis revealed that electrostatic forces are favored in high DNA concentrations (*39*). Within this work, quercetin co-incubation with equimolar DNA concentrations in solution resulted in fluorescence quenching (430 nm_{ex}, **Figure 2**). Contrariwise, using cellular extract or cell monolayers, we observed a substantial enhancement of fluorescence emission at 500−540 nm (488 nm_{ex}), which paralleled quercetin concentration (**Figure 1A**,**B**). Upon further attempts to visualize quercetin−DNA complexes by standard electrophoresis, the flavonol was found to migrate independently, in front of DNA fragments, whereas it did not affect DNA staining with EtBr. Moreover, the absence of quercetin oxidized metabolites during cell treatment argued for a non-covalent interaction with cellular components. We conclude that quercetin−DNA interactions could occur mainly in vitro and at high (supraphysiological) concentrations, possibly due to the chemical nature of the compounds. In a cellular environment, the presence of an excess of specific macromolecules, serving as quercetin targets, undermines such nonspecific interactions. Indeed, although quercetin intercalative properties were implicated in its antiproliferative effects, experimental studies in animals showed that the compound is protective rather than clastogenic (*40*) and thus safe for prospective applications in humans. The observed DNA fragmentation in tumor cells should be considered as a final step of the flavonoid-induced apoptosis (*41*). Quercetin dietary intake by healthy individuals increased resistance against DNA oxidative damage in subjects' leucocytes (*42*, *43*) and inhibited cisplatin-induced DNA adducts in hepatocytes (*44*).

In conclusion, within this work, we show that quercetin internalization is coupled to a transient binding of the native molecule to specific protein targets. This interaction could be visualized at 488 nm_{ex}/500−540 nm_{em}. Considering the compelling need for advanced techniques in flavonoid research, this property provides an expedient tool to define its target tissues and determine the intracellular domains, where it accumulates. Moreover, as the quercetin fluorescent signal is maintained with formaldehyde fixation, it would be feasible to combine quercetin uptake with standard immunofluorescence or FRET protocols. The sensitivity of the method is very high, as it can visualize internalized flavonol molecules applied at the low micromolar range. In addition, preliminary results show that quercetin is gradually imported but not retained by hepatocytes, indicating an energy-dependent transport. The identification of the implicated transporters and possible molecular partners could provide additional information about the bioavailability and mechanism of action of the agent.

LITERATURE CITED

(1) Spencer, J. P.; Abd-el-Mohsen, M. M.; Rice-Evans, C. Cellular uptake and metabolism of flavonoids and their metabolites: implications for their bioactivity. *Arch. Biochem. Biophys.* **2004**, *423*, 148−161.

(2) Williams, R. J.; Spencer, J. P.; Rice-Evans, C. Flavonoids: antioxidants or signalling molecules? *Free Radical Biol. Med.* **2004**, *36*, 838−849.

(3) Graf, B. A.; Milbury, P. E.; Blumberg, J. B. Flavonols, flavones, flavanones, and human health: epidemiological evidence. *J. Med. Food* **2005**, *8*, 281−290.

(4) Ames, B. N.; Gold, L. S.; Willett, W. C. The causes and prevention of cancer. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 5258−5265.

(5) Senderowicz, A. M. Novel direct and indirect cyclin-dependent kinase modulators for the prevention and treatment of human neoplasms. *Cancer Chemother. Pharmacol.* **2003**, *52* (Suppl. 1), S61−73.

(6) Stickel, F.; Brinkhaus, B.; Krahmer, N.; Seitz, H. K.; Hahn, E. G.; Schuppan, D. Antifibrotic properties of botanicals in chronic liver disease. *Hepatogastroenterology* **2002**, *49*, 1102−1108.

(7) Erlund, I. Review of the flavonoids quercetin, hesperetin, and naringenin. Dietary sources, bioactivities, bioavailability, and epidemiology. *Nutr. Res.* **2004**, *24*, 851−874.
(8) Cermak, R.; Landgraf, S.; Wolffram, S. The bioavailability of quercetin in pigs depends on the glycoside moiety and on dietary factors. *J. Nutr.* **2003**, *133*, 2802−2807.
(9) Walle, T.; Walle, U. K.; Halushka, P. V. Carbon dioxide is the major metabolite of quercetin in humans. *J. Nutr.* **2001**, *131*, 2648−2652.
(10) Lesser, S.; Cermak, R.; Wolffram, S. Bioavailability of quercetin in pigs is influenced by the dietary fat content. *J. Nutr.* **2004**, *134*, 1508−1511.
(11) Radtke, J.; Linseisen, J.; Wolfram, G. Fasting plasma concentrations of selected flavonoids as markers of their ordinary dietary intake. *Eur. J. Nutr.* **2002**, *41*, 203−209.
(12) Moskaug, J. O.; Carlsen, H.; Myhrstad, M.; Blomhoff, R. Molecular imaging of the biological effects of quercetin and quercetin-rich foods. *Mech. Ageing Dev.* **2004**, *125*, 315−324.
(13) Moon, J. H.; Tsushida, T.; Nakahara, K.; Terao, J. Identification of quercetin 3-*O*-β-D-glucuronide as an antioxidative metabolite in rat plasma after oral administration of quercetin. *Free Radical Biol. Med.* **2001**, *30*, 1274−1285.
(14) Spencer, J. P.; Kuhnle, G. G.; Williams, R. J.; Rice-Evans, C. Intracellular metabolism and bioactivity of quercetin and its in vivo metabolites. *Biochem. J.* **2003**, *372*, 173−181.
(15) van der Woude, H.; Boersma, M. G.; Vervoort, J.; Rietjens, I. M. Identification of 14 quercetin phase II mono- and mixed conjugates and their formation by rat and human phase II in vitro model systems. *Chem. Res. Toxicol.* **2004**, *17*, 1520−1530.
(16) Mullen, W.; Graf, B. A.; Caldwell, S. T.; Hartley, R. C.; Duthie, G. G.; Edwards, C. A.; Lean, M. E.; Crozier, A. Determination of flavonol metabolites in plasma and tissues of rats by HPLC-radiocounting and tandem mass spectrometry following oral ingestion of [2-(14)C]quercetin-4′-glucoside. *J. Agric. Food Chem.* **2002**, *50*, 6902−6909.
(17) Kaldas, M. I.; Walle, U. K.; van der Woude, H.; McMillan, J. M.; Walle, T. Covalent binding of the flavonoid quercetin to human serum albumin. *J. Agric. Food Chem.* **2005**, *53*, 4194−4197.
(18) Walle, T.; Vincent, T. S.; Walle, U. K. Evidence of covalent binding of the dietary flavonoid quercetin to DNA and protein in human intestinal and hepatic cells. *Biochem. Pharmacol.* **2003**, *65*, 1603−1610.
(19) Ueno, I.; Nakano, N.; Hirono, I. Metabolic fate of [14C] quercetin in the ACI rat. *Jpn. J. Exp. Med.* **1983**, *53*, 41−50.
(20) Graf, B. A.; Mullen, W.; Caldwell, S. T.; Hartley, R. C.; Duthie, G. G.; Lean, M. E.; Crozier, A.; Edwards, C. A. Disposition and metabolism of [2-14C]quercetin-4′-glucoside in rats. *Drug Metab. Dispos.* **2005**, *33*, 1036−1043.
(21) Solimani, R. Quercetin and DNA in solution: analysis of the dynamics of their interaction with a linear dichroism study. *Int. J. Biol. Macromol.* **1996**, *18*, 287−295.
(22) Sengupta, B.; Sengupta, P. K. The interaction of quercetin with human serum albumin: a fluorescence spectroscopic study. *Biochem. Biophys. Res. Commun.* **2002**, *299*, 400−403.
(23) Zsila, F.; Bikadi, Z.; Simonyi, M. Circular dichroism spectroscopic studies reveal pH dependent binding of curcumin in the minor groove of natural and synthetic nucleic acids. *Org. Biomol. Chem.* **2004**, *2*, 2902−2910.
(24) Ahmed, M. S.; Ramesh, V.; Nagaraja, V.; Parish, J. H.; Hadi, S. M. Mode of binding of quercetin to DNA. *Mutagenesis* **1994**, *9*, 193−197.
(25) Gutzeit, H. O.; Henker, Y.; Kind, B.; Franz, A. Specific interactions of quercetin and other flavonoids with target proteins are revealed by elicited fluorescence. *Biochem. Biophys. Res. Commun.* **2004**, *318*, 490−495.
(26) Bohl, M.; Czupalla, C.; Tokalov, S. V.; Hoflack, B.; Gutzeit, H. O. Identification of actin as quercetin-binding protein: an approach to identify target molecules for specific ligands. *Anal. Biochem.* **2005**, *346*, 295−299.
(27) Kitson, T. M. Spectrophotometric and kinetic studies on the binding of the bioflavonoid quercetin to bovine serum albumin. *Biosci., Biotechnol., Biochem.* **2004**, *68*, 2165−2170.
(28) Papadopoulou, A.; Green, R. J.; Frazier, R. A. Interaction of flavonoids with bovine serum albumin: a fluorescence quenching study. *J. Agric. Food Chem.* **2005**, *53*, 158−163.
(29) Rawel, H. M. S. K. F.; Meidtner, K.; Kroll, J.; Schweigert, F. J. Determining the binding affinities of phenolic compounds to proteins by quenching of the intrinsic tryptophan fluorescence. *Mol. Nutr. Food Res.* **2006**.
(30) Rawel, H. M.; Meidtner, K.; Kroll, J. Binding of selected phenolic compounds to proteins. *J. Agric. Food Chem.* **2005**, *53*, 4228−4235.
(31) Alvi, N. K.; Rizvi, R. Y.; Hadi, S. M. Interaction of quercetin with DNA. *Biosci. Rep.* **1986**, *6*, 861−868.
(32) Kang, J.; Zhuo, L.; Lu, X.; Liu, H.; Zhang, M.; Wu, H. Electrochemical investigation on interaction between DNA with quercetin and Eu-Qu3 complex. *J. Inorg. Biochem.* **2004**, *98*, 79−86.
(33) Ahmed, M. S.; Ainley, K.; Parish, J. H.; Hadi, S. M. Free radical-induced fragmentation of proteins by quercetin. *Carcinogenesis* **1994**, *15*, 1627−1630.
(34) Fazal, F.; Rahman, A.; Greensill, J.; Ainley, K.; Hadi, S. M.; Parish, J. H. Strand scission in DNA by quercetin and Cu(II): identification of free radical intermediates and biological consequences of scission. *Carcinogenesis* **1990**, *11*, 2005−2008.
(35) Rahman, A.; Shahabuddin; Hadi, S. M.; Parish, J. H. Complexes involving quercetin, DNA and Cu(II). *Carcinogenesis* **1990**, *11*, 2001−2003.
(36) Rahman, A.; Shahabuddin; Hadi, S. M.; Parish, J. H.; Ainley, K. Strand scission in DNA induced by quercetin and Cu(II): role of Cu(I) and oxygen free radicals. *Carcinogenesis* **1989**, *10*, 1833−1839.
(37) Annaraj, J.; Srinivasan, S.; Ponvel, K. M.; Athappan, P. Mixed ligand copper(II) complexes of phenanthroline/bipyridyl and curcumin diketimines as DNA intercalators and their electrochemical behavior under Nafion and clay modified electrodes. *J. Inorg. Biochem.* **2005**, *99*, 669−676.
(38) Zhou, J.; Wang, L. F.; Wang, J. Y.; Tang, N. Synthesis, characterization, antioxidative and antitumor activities of solid quercetin rare earth(III) complexes. *J. Inorg. Biochem.* **2001**, *83*, 41−48.
(39) Solimani, R. The flavonols quercetin, rutin and morin in DNA solution: UV−vis dichroic (and mid-infrared) analysis explain the possible association between the biopolymer and a nucleophilic vegetable-dye. *Biochim. Biophys. Acta* **1997**, *1336*, 281−294.
(40) Okamoto, T. Safety of quercetin for clinical application (review). *Int. J. Mol. Med.* **2005**, *16*, 275−278.
(41) Nagata, S. DNA degradation in development and programmed cell death. *Annu. Rev. Immunol.* **2005**, *23*, 853−875.
(42) Beatty, E. R.; O'Reilly, J. D.; England, T. G.; McAnlis, G. T.; Young, I. S.; Geissler, C. A.; Sanders, T. A.; Wiseman, H. Effect of dietary quercetin on oxidative DNA damage in healthy human subjects. *Br. J. Nutr.* **2000**, *84*, 919−925.
(43) Boyle, S. P.; Dobson, V. L.; Duthie, S. J.; Kyle, J. A.; Collins, A. R. Absorption and DNA protective effects of flavonoid glycosides from an onion meal. *Eur. J. Nutr.* **2000**, *39*, 213−223.
(44) Cross, H. J.; Tilby, M.; Chipman, J. K.; Ferry, D. R.; Gescher, A. Effect of quercetin on the genotoxic potential of cisplatin. *Int. J. Cancer* **1996**, *66*, 404−408.

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