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Determination of cellular thiols and glutathione-related enzyme activities: versatility of high-performance liquid chromatography–spectrofluorimetric detection

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Abstract

A high-performance liquid chromatography (HPLC) method to determine the most important cellular thiols [reduced glutathione (GSH), cysteine, γ-glutamylcysteine and cysteinylglycine] is described. Separation relies upon isocratic ion-pairing reversed-phase chromatography and detection is operated by spectrofluorimetry coupled with post-column derivatization reactions using either *N*-(1-pyrenyl)maleimide (NPM) or *ortho*-phthalaldehyde (OPA). When OPA is used without co-reagent, only GSH and γ-glutamylcysteine are detected (heterobifunctional reaction). However, either the OPA reaction in the presence of glycine in the mobile phase (thiol-selective reaction) or NPM allows the detection of all the cited thiols. The HPLC system has been validated as concerning linearity, accuracy and precision. The low detection limits reached (in the pmol range for each thiol injected) allow the screening and the quantification of thiols (as NPM derivatives) in V79cl and V79HGGT cells as well as the measurement of two cytosolic enzymes related to the glutathione synthesis, using the heterobifunctional OPA reaction.

Keywords: Derivatization, LC; Thiols; Enzymes; Glutathione

1. Introduction

Thiols are an important class of products in biochemical and pharmacological fields. Among endogenous low-molecular-mass non-protein thiols, the tripeptide glutathione (γ-glutamyl-cysteinylglycine) is predominant in cells, mainly in its reduced form (GSH), and plays an important role in cellular protection against injuries by oxidants or free radicals and in detoxification processes by conjugating with electrophilic xenobiotics. Moreover, there is a real interest in the simultaneous monitoring of cysteine and γ-glutamylcysteine (GSH precursors) and cysteinylglycine (GSH breakdown product) when the intracellular metabolism of GSH is studied [1–2].

An up-to-date review of high-performance liquid chromatography (HPLC) methods for thiols has been published [3]. It pointed out the main problems which were met with these analytes: unstability during sample treatment and absence of chromophore for sensitive detection. Concerning this last point, two approaches are possible (i) direct de-

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tection using electrochemistry, either amperometry on gold/mercury amalgamed electrodes [4] or coulometry on porous graphite electrodes [5], (ii) derivatization coupled with UV or spectrofluorimetric detection. The pre-column reactions involve different thiol selective reagents: monobromobimane [6–8], 4-(aminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole and 7-fluoro-2,1,3-benzoxadiazole-4-sulfonate [9], *N*-(1-pyrenyl)maleimide (NPM) [10] and *ortho*-phthalaldehyde (OPA) [11]. The reagents reported for the post-column derivatization of thiols are less numerous than for the pre-column mode since only few of them met the following requirements: a fast kinetic of adduct formation and spectroscopic or spectrofluorimetric properties different from the adduct itself. The main labelling reagents described for this purpose are 5,5′-dithiobis(2-nitrobenzoic acid) [12], NPM [13–15] and OPA [8,15–18]. More recently, interest in capillary electrophoresis for the analysis of thiols is increasing: detection is operated either by UV spectrophotometry at low wavelengths [19] or by laser induced fluorescence after labelling [20].

This paper deals with a HPLC system devoted to the separation of the main biological thiols and the comparison of post-column derivatization reactions using either NPM or OPA (Fig. 1). The respective interest of each reagent was demonstrated through: (i) the assay of the intracellular thiols in two cell lines, i.e., V79cl and V79HGGT fibroblasts, which differ by no or high γ-glutamyl-transferase (GGT, EC 2.3.2.2) expression, respectively and (ii) the activity measurement of γ-glutamylcysteine synthetase (EC 6.3.2.2) and GSH synthetase (EC 6.3.2.3) in the same cell lines.

2. Experimental

2.1. Chemicals, reagents and standards

All chemicals and solvents were of analytical or HPLC reagent grade and used without further purification. GSH, cysteine, *N*-acetylcysteine, γ-glutamylcysteine (as trifluoroacetate salt), cysteinylglycine and 2-aminoethanesulfonic acid were supplied by Sigma (St. Louis, MO, USA). *n*-Decylsulfate (sodium salt) was obtained from Acros (Geel, Belgium). OPA, NPM, 1,4-dithio-D,L-threitol (DTT) were purchased from Fluka (Buchs, Switzerland).

Stock solutions of each standard thiol and calibration curves were prepared daily in 0.1 *M* HCl–2 m*M* EDTA and kept in the dark at +4°C for a maximum of 6 h.

2.2. Instrumentation

The HPLC system consisted of a two-solvent delivery pump (Model P 2000, Thermo Separation Products, Les Ulis, France), an injection valve equipped with a 20-μl sample loop (Model 7725i, Rheodyne, Cotati, CA, USA), a column oven (Model Croco-Cil, Thermo Separation Products) set at 40°C, a three-way post-column reagent delivery pump (Model Hitachi 655 A-13, Merck-Clevenot, Nogent/Marne, France) and a fluorescence detector (Model Shimadzu RF-551, Touzart-et-Matignon, Vitry/Seine, France or Model Jasco FP-920, Prolabo, Fontenay-sous-Bois, France). Derivatization took place in a PTFE knitted open tubular reactor (3 m×0.5 mm I.D.) (Model 5-9206, Supelco, St. Quentin Falavier, France). All data capture and calculations were performed using an integrator (Model Chromjet) connected to a data station with Winner on Windows (Thermo Separation Products) as software.

2.3. Chromatography, derivatization and detection conditions

The guard columns (4×4 mm I.D.) and analytical columns (125×4 mm I.D.) used were pre-packed with different octadecyl silica stationary phases of 5-μm particle size: LiChrospher 100 RP 18 end-capped (Merck, Darmstadt, Germany), Spherisorb ODS-2 (Phase Separations, Deeside, UK) or Nucleosil C_{18} (Macherey Nagel, Düren, Germany).

The optimized mobile phase consisted of a mixture of acetonitrile (5%, v/v) and 10 m*M* phosphate buffer (pH 2.5) containing 0.1 m*M* disodium EDTA and 0.5 m*M* sodium *n*-decylsulfate. In the case of the thiol-selective OPA reaction, the mobile phase buffer contains additional 0.5 m*M* glycine. The NPM reaction was carried out using two co-solutions: 2% (v/v) triethylamine–1% (v/v) Brij–35 in CH_3CN–

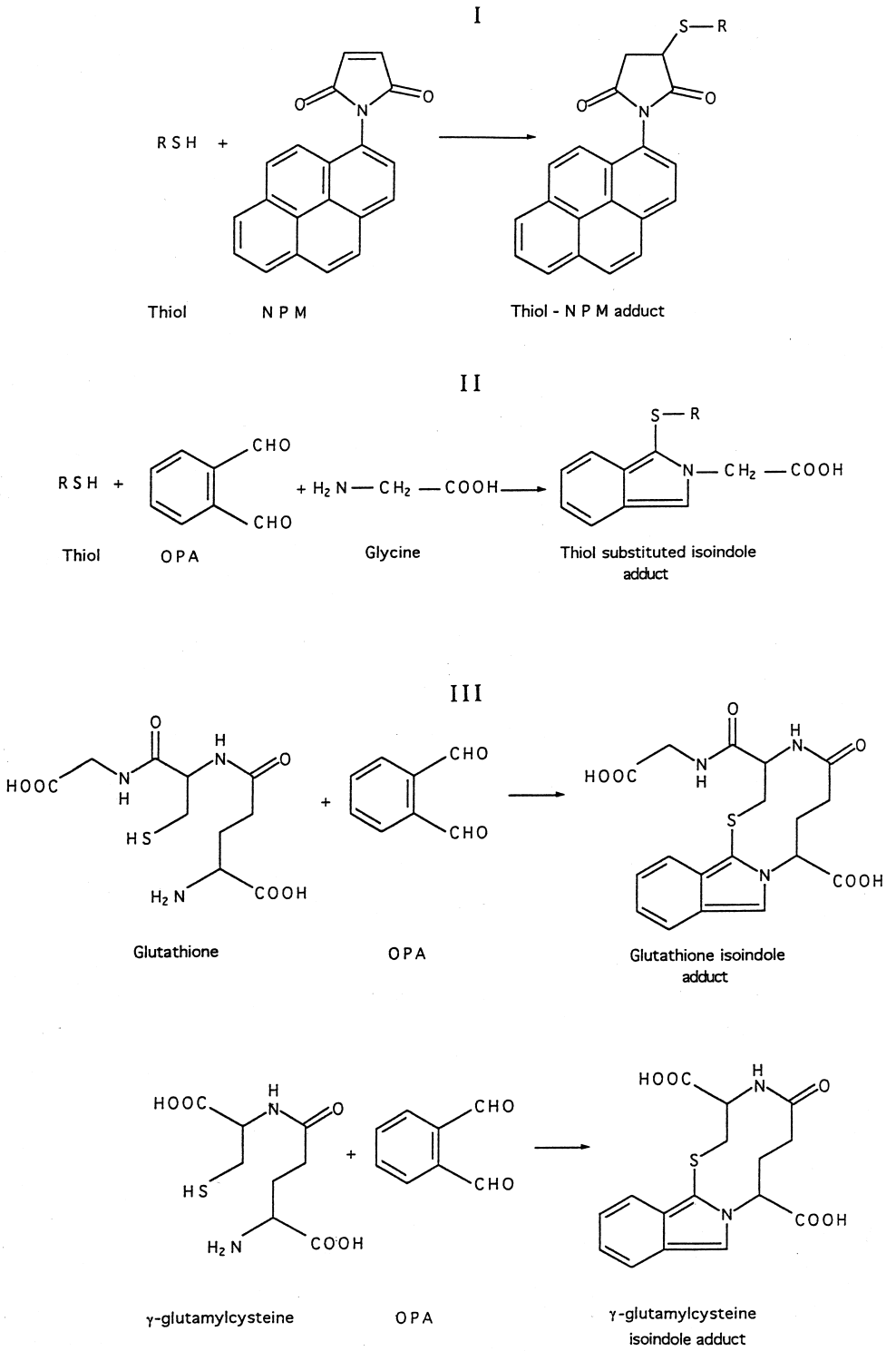

Fig. 1. Schemes of the different post-column derivatization reactions of thiols: NPM (I), thiol-selective OPA (II) and heterobifunctional OPA (III).

water (30:70, v/v) and 0.015 g l^{-1} NPM in acetonitrile successively added to the mobile phase.

OPA reactions were performed using a 0.8 g l^{-1} OPA solution prepared in 0.35 *M* borate buffer, pH 10.5 containing 0.4% (v/v) Brij-35.

The flow rate of the mobile phase was 1.2 ml min^{-1}. All the post-column reagent solutions, i.e., OPA and the two co-reagent solutions used for NPM reaction, were introduced into the mobile phase at a similar flow rate of 0.3 ml min^{-1}. Fluorescence detection was operated at respective excitation and emission wavelengths of either 342 and 389 nm for NPM derivatives, or 340 and 440 nm for OPA derivatives.

2.4. Cell culture conditions

V79cl (Chinese hamster lung fibroblasts V79) as negative-GGT control and V79HGGT [21] expressing a high level of GGT activity were used. Both cell lines were grown at 37°C in humidified air with 5% CO_2 as monolayer cells in RPMI medium (Gibco BRL, Cergy Pontoise, France) supplemented with L-glutamine (2 m*M*), 10% (v/v) heat-inactivated fetal calf serum (Boehringer Mannheim, Meylan, France) and 1% (v/v) antibiotic–antimycotic solution 100X (Gibco BRL) (medium A). For the different experiments performed in triplicate, 100-mm diameter dishes containing 10 ml medium A were inoculated with $0.8 \cdot 10^6$ trypsinized cells and incubated at 37°C for 48 h.

2.5. Measurement of intracellular thiols

After 48 h of culture, cells in all dishes were washed twice, then incubated at 37°C for 5 min with 3 ml medium B (medium A supplemented with 2.5 m*M* GSH and 25 m*M* glycylglycine). The control dishes were treated in a similar way but using 3 ml medium A. Then, cells in all dishes were washed, scraped and harvested according to previously optimized conditions [22]. After centrifugation at 10 000 *g* at 4°C for 5 min, the supernatants were kept at −80°C whereas the pellets were solubilized in 1.0 *M* NaOH for protein assay according to Lowry et al. [23] using bovine serum albumin as standard.

Before the analysis of thiols, the supernatants were thawed out at 37°C for 30 s, vortexed and immediately diluted (2- to 10-fold) in 0.1 *M* HCl–2 m*M* EDTA. The thiols were assayed by HPLC coupled with the post-column NPM reaction. The concentrations of thiols were calculated on the basis of peak areas according to standard curves and expressed in nmol mg $protein^{-1}$.

2.6. Activity measurement of enzymes

After culture for 48 h in medium A, cells were washed with 5 ml of ice-cold phosphate-buffered saline (PBS), then scraped and harvested in 1.5 ml of ice cold PBS, sonicated for 5 s (Model Vibra Cell, Sonics and Materials, Danbury, CT, USA ; power: 40 W) and centrifuged at 10 000 *g* at 4°C for 5 min. The resulting supernatants (crude cytosolic fractions) were used for the enzyme activity measurement (medium composition according to Nardi and Cipollaro [24]) and for protein assay. γ-Glutamylcysteinyl synthetase activity was measured in the presence of 6 m*M* DTT, 3 m*M* L-cysteine and 15 m*M* L-glutamic acid. The reaction was started by adding supernatant (80 μl) to incubation medium (220 μl) and continued for 20 min at 37°C. Then samples were immediately diluted (5- to 10-fold) in 0.1 *M* HCl–2 m*M* EDTA. GSH synthetase activity was measured in similar conditions but in the presence of 3 m*M* γ-glutamylcysteine and 30 m*M* glycine instead of cysteine and glutamic acid. γ-Glutamylcysteine and glutathione were assayed by HPLC and detected by heterobifunctional OPA reaction. Activity values were expressed in nmol of reaction product min^{-1} mg $protein^{-1}$.

3. Results

3.1. Chromatographic and post-column derivatization conditions

The chromatographic separation of the biological thiols was optimized by successively (i) selecting a stationary phase and (ii) testing different elution parameters.

(i) Three columns which were pre-packed with different silica based C_{18} stationary phases (LiChrospher, Nucleosil and Spherisorb) were tested. The overall elution time of the analytes with optimized

mobile phase ranged from 15 min (Spherisorb) to 25 min (LiChrospher). Spherisorb was selected since it gave the shortest elution times with baseline resolution between analytes.

(ii) The ruggedness of the method was evaluated by testing different parameters related to mobile phase composition: buffer pH (2.2 to 3.0), counterion concentration (0.25 to 1.0 m*M*) and acetonitrile content (3 to 10%, v/v). The capacity factor variations of the different analytes are shown in Fig. 2.

A typical chromatogram obtained under the optimized conditions (pH: 2.5; counterion concentration: 0.5 m*M*; acetonitrile: 5%, v/v) is shown in Fig. 3A and the relative retention times (expressed vs. GSH) are given in Table 1.

The HPLC method which is presently developed offers selectivity with regard to *N*-acetylcysteine (used as a source of cysteine to restore GSH content after oxidative stress) and homocysteine (a substance potentially present in biological tissues). For each analyte, the number of theoretical plates is around 30 000 plates m^{-1} and the asymetrical factor is ca. 1.05.

The operating conditions of post-column derivatization were previously reported by us for the NPM [14–15] and the heterobifunctional OPA [15, 18] reactions. The thiol-selective OPA reaction needs the presence of an amino compound: 2-aminoethanesulfonic acid and glycine were tested through their addition to the mobile phase [17]. Their influence on the capacity factor of the thiols and on the fluorescence response of the derivatives was evaluated. 2-Aminoethanesulfonic acid acting as an anionic ion-pairing agent alters the thiols retention without increasing fluorescence response. When using glycine (0.1 to 1.0 m*M*), no significant variations were observed and this compound was selected at a concentration of 0.5 m*M*. Chromatograms obtained with a solution of standard thiols using the three different derivatization processes are shown in Fig. 3.

The main validation parameters of the HPLC methods have been studied (Table 2): linearity ($r>$ 0.99) and coefficients of variation (C.V.s) ($n=5$), which did not exceed 5.4 %. Recoveries from spiked cultured cells (with medium concentration of the analytes relatively to the linearity range) comparatively to blank controls were 90±4, 102±2, 97±2 and 98±2% ($n=5$) for cysteine, GSH, cysteinylglycine and γ-glutamylcysteine, respectively.

3.2. Fluorescence properties of the derivatives

3.2.1. Glutathione adducts

Close relative fluorescence intensities were observed for GSH reacting with OPA (1.00) and NPM (0.96), but a lower value was obtained in the case of the thiol-selective OPA reaction (0.73). The detection limit of GSH estimated on standard solution is around 0.4 ng (1.30 pmol) injected onto the column (signal-to-noise ratio of 3).

3.3.1. Biological thiols adducts

The excitation and emission spectra of the different adducts obtained using the scanning function of the detector show no significant shifts of the maximum wavelength values. For each derivatization reaction, the fluorescence response obtained with the different thiols is expressed vs. that of the GSH adduct (Table 1). Although relative fluorescence responses of the thiol-NPM adducts are quite similar (0.85 to 1.27), higher variations (0.10 to 2.03) were noted in the case of the isoindole derivatives obtained with the thiol-selective OPA reaction; moreover this system is not convenient for cysteine (response factor of 0.10).

3.3.2. DTT as reducing reagent

DTT is frequently used as a reducing agent in the incubation media to determine the activities of thiol-related enzymes. Its excess is generally not eliminated from the sample before injection into the HPLC system and so it is of particular interest to get a low response for its derivative. Considering this fact, the derivatization of thiols by the NPM or the heterobifunctional OPA reaction is more advisable than using the thiol-selective OPA reaction (Table 1).

3.3.2.1. *Measurement of cellular thiols and glutathione-related enzyme activities*. Two applications were realized in the present work: (i) identification and quantification of the intracellular thiols in V79cl and V79HGGT cell lines, which differ by no or high GGT expression respectively and (ii) measurement of two cytosolic glutathione-related enzyme ac-

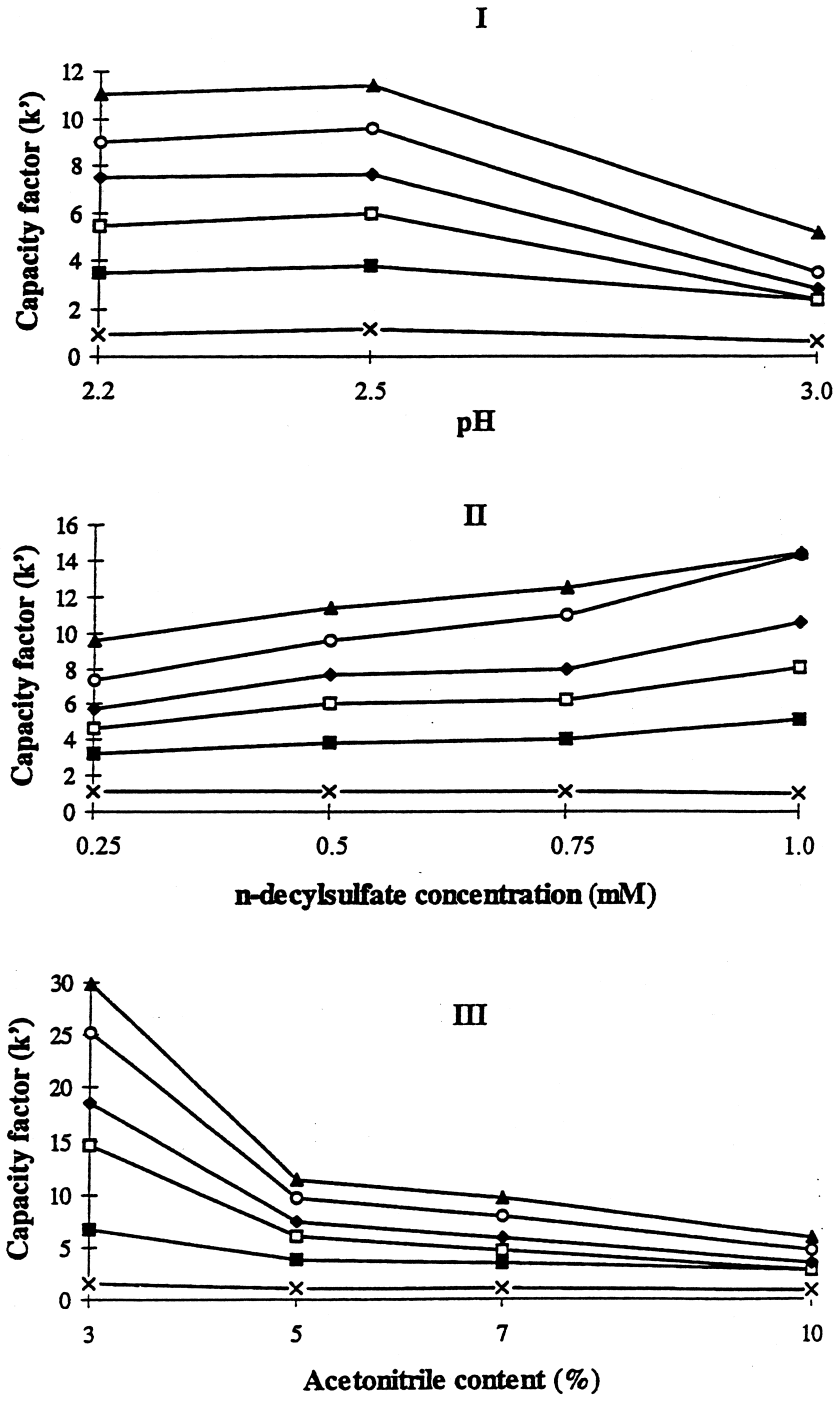

Fig. 2. Capacity factor (k') variations of thiols as a function of different mobile phase parameters. (I) Buffer pH [*n*-decylsulfate: 0.5 m*M*; acetonitrile: 5% (v/v)]; (II) *n*-decylsulfate concentration [buffer pH: 2.5; acetonitrile: 5% (v/v)]; (III) acetonitrile content (buffer pH: 2.5; *n*-decylsulfate: 0.5 m*M*). Column: Spherisorb ODS-2 (5 μm) 125×4 mm I.D.; flow-rate: 1.2 ml min^{-1}; temperature: 40°C. *N*-Acetylcysteine (×), cysteine (■), GSH (□), γ-glutamylcysteine (♦), homocysteine (○) and cysteinylglycine (▲).

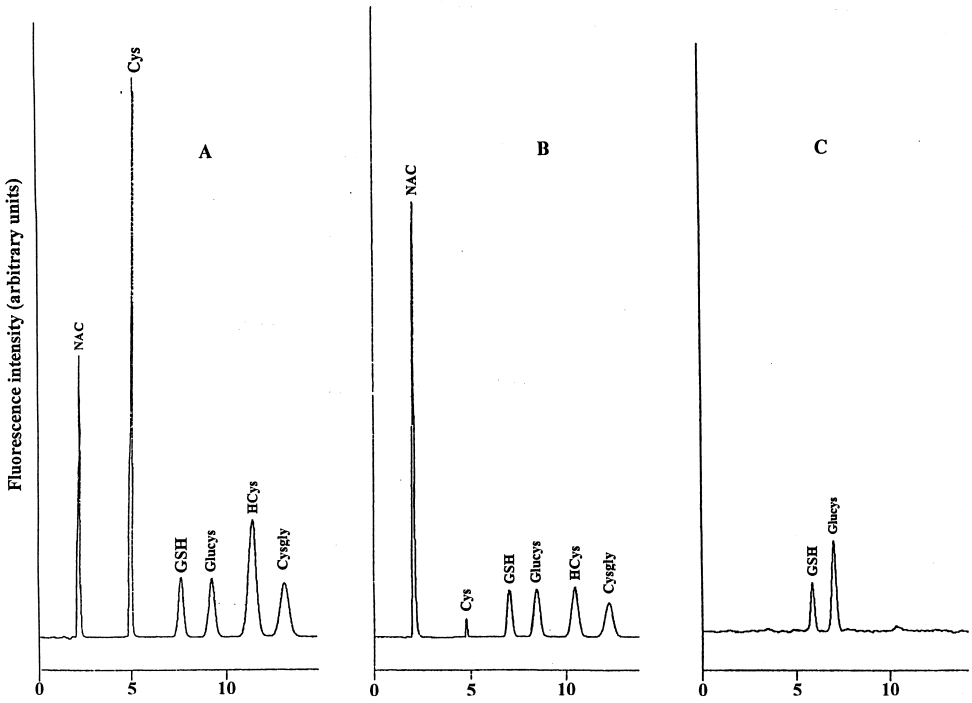

Fig. 3. Chromatograms corresponding to a solution of standard thiols: (A) NPM reaction (λ_{ex}=342 nm, λ_{em}=389 nm), (B) thiol-selective OPA reaction and (C) heterobifunctional OPA reaction (λ_{ex}=340 nm, λ_{em}=440 nm). Optimized HPLC conditions as in section 2.3. Mobile phase contains 0.5 m*M* glycine in the case of thiol-selective OPA reaction. NAC, *N*-acetylcysteine; Cys, cysteine; GSH, glutathione; Glucys, γ-glutamylcysteine; HCys, homocysteine; Cysgly, cysteinylglycine.

tivities, i.e., γ-glutamylcysteine and glutathione synthetases.

For the first application, the NPM post-column reaction was selected since it allows detection of all the thiols with a similar detection limit. Under standard conditions of culture (medium A), no statistical difference in GSH levels was observed between V79cl cells (15.0±1.2 nmol mg protein^{-1})

Table 1
Relative retention time (RRT) of thiols and relative fluorescence intensities of their adducts after post-column derivatization (NPM, thiol-selective OPA and heterobifunctional OPA reactions)[a]

Thiols	RRT	Relative fluorescence intensity[b]		
		NPM reaction	Thiol-selective OPA reaction	Heterobifunctional OPA reaction
N-Acetylcysteine	0.27	1.06	2.03	nd[c]
DTT	0.43	0.14	0.46	0.05
Cysteine	0.57	1.04	0.10	nd[c]
GSH	**1.00**	**1.00**	**1.00**	**1.00**
γ-Glutamylcysteine	1.22	1.27	1.60	2.02
Homocysteine	1.50	1.25	0.83	nd[c]
Cysteinylglycine	1.66	0.85	0.83	nd[c]

[a] Optimized HPLC conditions as in section 2.3.

[b] Defined as: $\frac{\text{peak area of the considered thiol adduct}}{\text{thiol molar quantity injected}} \times \frac{\text{GSH molar quantity injected}}{\text{peak area of GSH adduct}}$

[c] nd=Not detected.

Table 2
Main validation parameters of the cellular thiols assay using the HPLC system coupled with post-column NPM reaction (I), thiol-selective OPA reaction (II) and heterobifunctional OPA reaction (III)

Thiols	Linearity range (six concentration levels) (μM)	Correlation coefficient (r)	C.V. (%) ($n=5$)	
			(a)	(b)
I				
GSH	0.16–6.50	0.9890	4.2	4.9
Cysteine	0.41–16.5	0.9992	1.6	4.7
Cysteinylglycine	0.28–11.0	0.9991	4.2	5.4
II				
GSH	0.16–6.50	0.9999	1.6	2.2
Cysteine	0.41–16.5	0.9998	4.4	5.4
Cysteinylglycine	0.28–11.0	0.9997	2.4	3.5
γ-Glutamylcysteine	0.14–5.50	0.9999	2.5	3.4
III				
GSH	0.16–6.50	0.9999	0.5	1.9
γ-Glutamylcysteine	0.14–5.50	0.9999	2.3	2.8

[a] Tested at the highest concentration of the linearity range.
[b] Tested at the lowest concentration of the linearity range.

and V79HGGT cells (14.0±1.2 nmol mg protein^{-1}). Cysteine (0.85±0.2 nmol mg protein^{-1}) was only detected in V79HGGT cells. No other thiols were found at a concentration higher than the detection limit. When the culture medium was supplemented with GSH and glycylglycine (medium B), no modification of the intracellular thiols content was observed in V79cl cells. On the contrary, the contents of GSH (21.0±1.4 nmol mg protein^{-1}) and cysteine (11.2±0.9 nmol mg protein^{-1}) increased in V79HGGT cells. Moreover, cysteinylglycine appeared at a concentration of 5.15±0.15 nmol mg protein^{-1}. Chromatograms corresponding to the determination of intracellular thiols in V79HGGT are presented in Fig. 4.

The second application concerns the study of the relationship between the intracellular GSH content and the enzymes involved into its synthesis (γ-glutamylcysteine and GSH synthetases). Their specific activities were measured in the crude cytosolic fractions (free of GGT activity) of both cell lines. The determination of γ-glutamylcysteine synthetase activity consists in measuring γ-glutamylcysteine as the reaction product in the presence of DTT, L-cysteine and L-glutamic acid, whereas the determination of GSH synthetase activity consists in measuring GSH as the reaction product in the presence of

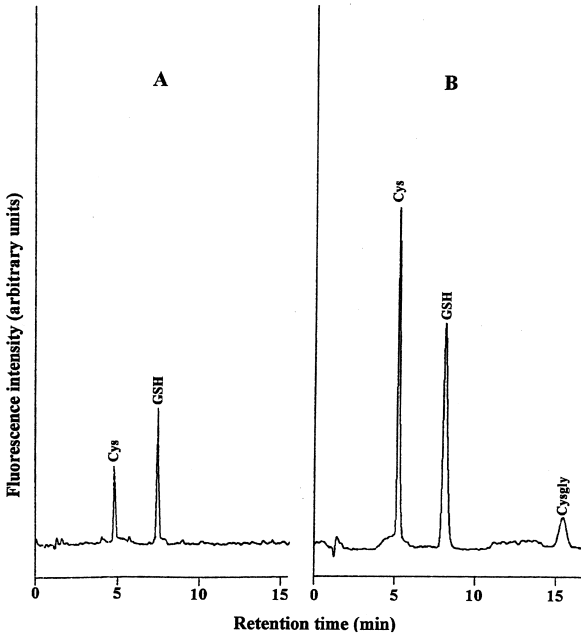

Fig. 4. Chromatograms corresponding to the analysis of intracellular thiols in V79HGGT cells. (A) Culture under standard conditions, (B) culture in the presence of 2.5 mM GSH and 25 mM glycylglycine. Optimized HPLC conditions as in section 2.3; post-column NPM reaction ($\lambda_{ex}=342$ nm, $\lambda_{em}=389$ nm) Cys, cysteine; GSH, glutathione; Cysgly, cysteinylglycine.

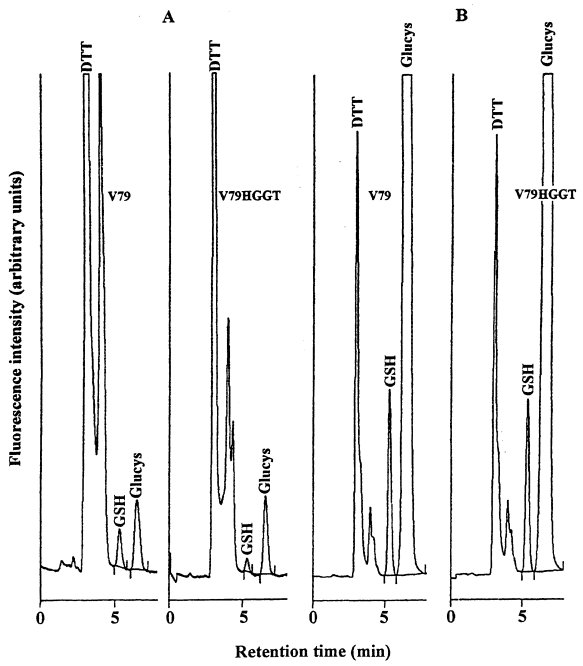

Fig. 5. Chromatograms corresponding to the determination of γ-glutamylcysteine (A) and GSH (B) synthetase activities in V79cl and V79HGGT cell lines. Optimized HPLC conditions as in section 2.3; post-column heterobifunctional OPA reaction (λ_{ex} = 340 nm, λ_{em} = 440 nm) GSH, glutathione; Glucys, γ-glutamylcysteine; DTT, dithiothreitol.

DTT, γ-glutamylcysteine and glycine. The HPLC system coupled with the post-column heterobifunctional OPA reaction is of particular interest in this case since γ-glutamylcysteine and GSH could be assayed without interference of other thiols added to the culture medium in excess as substrate (cysteine or γ-glutamylcysteine) or additive (DTT) (Fig. 5). γ-Glutamylcysteine synthetase activities in V79cl and V79HGGT cells were 0.79±0.08 and 1.06±0.10 nmol min^{-1} mg $protein^{-1}$, respectively and GSH synthetase activities were 14.5±1.9 and 12.9±1.6 nmol min^{-1} mg $protein^{-1}$, respectively.

4. Discussion

HPLC techniques devoted to the separation of underivatized thiols usually relies upon a reversed-phase ion-pairing process. Presently, the capacity factors of thiols increase in agreement with the carbon content of the different apolar stationary phases tested: 12, 14 and 21% for Spherisorb, Nucleosil and LiChrospher, respectively [25]. The best compromise between a short overall elution time (less than 15 min) and a good resolution between thiols (R_s>1.5) was obtained on Spherisorb ODS-2 and eluting conditions were further optimized using this column (Fig. 2).

The two thiol-selective reagents, NPM and OPA, tested for post-column derivatization coupled with spectrofluorimetric detection have to be used under different conditions. Since NPM is unstable in aqueous solution, it is prepared in a water miscible organic solvent such as acetonitrile and added to the mobile phase after this latter has been alkalinized using either a borate buffer [13] or a triethylamine solution [14,15]. Consequently, this reaction needs to be applied in a hyphenated HPLC system including a two-way post-column device. OPA could be used in two modes (Fig. 1). In the case of heterobifunctional analytes containing an amino and a thiol group, OPA was used without co-reagent. Since some structural compliances, especially concerning the steric hindrance and the distance between the amino and thiol groups have to be met to obtain a fluorescent adduct [16], very few analytes can be detected by this way. So this reaction gives a high degree of selectivity for GSH and γ-glutamylcysteine vs. other endogenous thiols [7,8,11,15]. It has already been applied in HPLC systems either in pre- [7,11] or post-column [8,15,18] mode and its advantages vs. monobromobimane, a widely-used thiol selective reagent, have been previously discussed [7,8].

Experimental conditions for the thiol-selective OPA reaction involving OPA in the presence of an amino compound as a second reagent were surveyed. Since chemical interaction is possible between the amino reagent and OPA, they have to be mixed just before the reaction with the thiols occurs and two approaches can be considered: use of a two-way post-column device [16] or addition of the amino compound to the mobile phase [17]. In this case, it should not react with thiols nor modify their chromatographic behaviour. Glycine fulfils these requirements and has been retained in the present method. Isoindole adducts obtained with thiols show important variations in their fluorescence response. In fact, the fluorescence quantum yield of these deriva-

tives is known to be very sensitive to their structural environment, especially to carbonyl and amino groups present in the lateral chains close to the fluorophore, which explains the low response obtained for cysteine.

The incubation of V79HGGT cells in the presence of an excess of GSH shows the increase of GSH, cysteine and cysteinylglycine intracellular content. GGT catalyzes the breakdown of the extracellular GSH into glutamic acid and cysteinylglycine; the latter can undergo further cleavage and all the resulting amino acids enter the cell to be used in the GSH synthesis. Since V79cl cells exhibit no GGT activity, the extracellular GSH is not metabolized and the intracellular thiol content is unchanged.

GSH and γ-glutamylcysteine are the reaction products of GSH and γ-glutamylcysteine synthetases respectively. Only a few methods have been reported for the measurement of the activity of these enzymes [7, 24]. Their usual specific activities in cultured cells are relatively low (within the range 1 to 20 nmol min^{-1} mg $protein^{-1}$), 1000-fold less than in red blood cells, for example. So, the HPLC system coupled with the post-column heterobifunctional OPA reaction is a helpful analytical tool for this purpose since it monitors γ-glutamylcysteine and GSH with high selectivity vs. other thiols, especially the excess of substrate and DTT.

In conclusion, the developed isocratic HPLC conditions coupled with the NPM-derivatization seems the best method for the measurement of the intracellular thiols: high sensitivity, good response, separation of all the thiols and a low response for DTT often used in excess as a reducing reagent for thiol studies. The thiol-selective OPA reaction is a possible alternative (simpler ease-of-use, global lower cost in terms of reagent and solvent consumption) but two important drawbacks exist: poor limit of detection for cysteine and relatively high response for DTT. The heterobifunctional OPA post-column reaction presents the same advantages as the thiol-selective OPA reaction but a higher selectivity, thus it is well-suited to the measurement of the reaction products of the two enzymes involved in the GSH synthesis.

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