Determination of cholesterol at the low picomole level by nano-electrospray ionization tandem mass spectrometry

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Abstract **A mass spectrometric method for the quantification of free cholesterol in cells and subcellular membranes is presented. The method is based on a simple one-step chemical derivatization of cholesterol to cholesterol-3-sulfate by a sulfur trioxide-pyridine complex. Quantification is performed by nano-electrospray ionization tandem mass spectrometry (nanoESI-MS/MS) using a stable isotope labeled internal standard. The determination of free cholesterol is demonstrated in about 250 cells of a Chinese hamster ovary (CHO) cell line. With this method a molar ratio of free cholesterol to total phospholipids of 0.34 mol/mol in CHO cells was determined. In a subcellular membrane fraction enriched in Golgi membranes, a molar ratio of free cholesterol to total phospholipids of 0.57 mol/mol was determined. The method should be of value for quantification of other sterols as demonstrated for ergosterol and stigmasterol.—**Sandhoff, R., B. Brügger, D. Jeckel, W. D. Lehmann, and F. T. Wieland. **Determination of cholesterol at the low picomole level by nano-electrospray ionization tandem mass spectrometry. *J. Lipid Res.* 1999.** 40: **126–132.**

Supplementary key words membrane • lipid composition • ergosterol • stigmasterol • sterol • cholesterol-3-sulfate • internal standard • CHO cells • Golgi

Different subcellular organelles within a cell differ in their lipid composition (1). Protein and lipid transport between these organelles is established mainly by vesicles that bud off a donor membrane and fuse with an acceptor membrane. This process would mix the components of the various membranes, unless there is some kind of sorting process in the pathway. Hence, the lipid compositions of vesicles are likely to differ from those of their donor membranes. Such a difference can only be demonstrated by directly comparing the lipid compositions of both the vesicles and their donor membranes. One candidate for such a carrier is the COPI (coat protein complex I)-coated vesicle, available in highly purified form but at very low quantity (2). Additional candidate lipid carrier vesicles are not even characterized and cannot therefore be expected to be available in a pure form in larger quantities. Therefore a highly sensitive method is needed to analyze membrane lipid constituents.

We have established nanoESI-MS/MS (3, 4) as a useful tool for highly sensitive quantification of membrane phospholipids (5). As an extension, the use of nanoESI-MS/MS for the quantitative determination of free cholesterol at low pmol levels is described in this study. In the first step of the sample work up, [$^{13}C_2$]cholesterol is added as internal standard. This internal standardization eliminates the influence of variations in the efficiency of the extraction and derivatization steps. Quantification by nanoESI-MS/MS is achieved by comparing the ion intensities of the sulfated internal standard with the sulfated cholesterol in a precursor ion scan specific for sulfated ions (HSO_4^-) that give rise to a signal at m/z 97. As demonstrated below, the method is sensitive enough to allow the quantitative analysis of free cholesterol in as few as 250 Chinese hamster ovary (CHO) cells.

METHODS

Materials

Mass spectrometric assay. [3,4-$^{13}C_2$]cholesterol (90 atom% ^{13}C) was obtained from Cambridge Isotope Laboratories, 99% pure cholesterol from Sigma, 98% pure ergosterol, 95% pure stigmasterol, pyridine (absolute, over molecular sieve), and sulfur trioxide pyridine complex from Fluka, and 99% pure barium acetate, water, and methanol from Merck. All solvents used were of analytical grade. 1,4-Dioxane was obtained from Riedel-de Haen and stored over molecular sieve. Plastic micro test tubes (500 μl) were obtained from Eppendorf.

Fluorescence assay. Cholesterol oxidase from *Streptomyces species* (49 U/mg protein) and horseradish peroxidase (Type VI-A, 1100 U/mg solid) were obtained from Sigma, 95% ethanol for spectroscopic purpose, sodium phosphate, mono and dibasic, from Merck, and p-hydroxyphenylacetic acid from Fluka.

Cell culture

CHO cells were held in suspension culture and grown in α-minimal essential medium supplemented with 10% (v/v) heat-

Abbreviations: ESI-MS/MS, electroscopy ionization tandem mass spectrometry; CHO, Chinese hamster ovary; COPI, coat protein complex I.

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inactivated fetal bovine serum, 2 mm glutamine, 100 units of penicillin, and 100 units of streptomycin per ml.

Isolation of Golgi membranes

Intact Golgi membranes of CHO cells were isolated according to Beckers and Rothman (6). In order to access the degree of enrichment and contamination, the following enzyme activities were determined: cytochrome c oxidoreductase as a marker for endoplasmic reticulum (7), APDE-1 as a marker for plasma membrane (7), β-hexosaminidase as a marker for lysosomes/late endosomes (7), and sphingomyelin synthase as a marker for Golgi organelles (8, 9). According to these markers, Golgi membranes in the isolated fraction are enriched 30-fold over the homogenate.

Quantification of endogenous cholesterol sulfate and the total phospholipid concentration

Endogenous cholesterol sulfate was measured by ESI-MS/MS using dihydrocholesterol-3-sulfate as internal standard according to Metzger et al. (10). The total phospholipid content was determined according to Rouser, Fleischer, and Yamamoto (11).

Quantification of free cholesterol by a fluorescence assay

Cholesterol was extracted from CHO cells with chloroform–methanol 2:1. The extracts were dried with a stream of argon and then heated to 105°C for 20 min according to Gamble et al. (12). The samples were cooled to room temperature and 0.4 ml reagent A containing cholesterol oxidase, horseradish peroxidase, and 4-hydroxyphenylacetic acid was added according to Heider and Boyett (13). After 8 min, the samples were mixed with 0.8 ml of 0.5 m NaOH. Fluorescence instrumentation from SLM Instruments, Inc. Urbana-Champaign, IL. was used to measure the fluorescence with an exitation wave length of 365 nm and an emission wave length of 425 nm.

Standard solution

The concentration of the [3,4-$^{13}C_2$]cholesterol standard solution was calculated from ESI-MS/MS data. Therefore the signal intensities of cholesterol and standard were compared in several samples containing a defined amount of cholesterol from a 99% pure cholesterol solution of known concentration and a defined volume of the standard solution. The mass spectrometric data obtained were evaluated using the formula given under Quantification of the data.

Membrane extraction

Aliquots of subcellular membrane fractions or total CHO cells were pipetted into a 500-μl micro test tube and water was added to a total volume of 20 μl. All dioxane-containing solutions were handled with Hamilton syringes. Appropriate amounts of [3,4-$^{13}C_2$]cholesterol standard from a stock solution, 4 μm in 1,4-dioxane, were added and each sample was made up to a final volume of 120 μl with 1,4-dioxane. The samples were mixed, sonicated in an ultrasonic bath at 100% (Bransonic 12) for 10–20 sec, and dried in a vacuum concentrator. To extract cholesterol, the dried samples were dissolved in 50 μl dry 1,4-dioxane. The samples were sonicated 1–6 times for 10 sec until the pellet was resuspended. Increasing sucrose concentrations afforded more sonication steps. Then the samples were centrifuged for 20 min at 15°C and 20800 *g* in a benchtop centrifuge to pellet nonsoluble material such as sucrose or protein. The supernatants were transferred into fresh micro test tubes, using a Pasteur pipette, and dried again in the vacuum concentrator.

Commercially available Pasteur pipettes occasionally contain residual detergents that interfere with the assay. Therefore they were cleaned twice with 1,4-dioxane prior to use.

Derivatization

For conversion into cholesterol sulfate the method of Duff (14) was modified as follows. The sulfur trioxide pyridine complex solution for the sulfation reaction was freshly prepared by dissolving 25 mg sulfur trioxide pyridine complex in 5 ml of absolute pyridine, followed by sonication for about 10 seconds.

A volume of 20 μl of the reagent was added quickly to each dried sample. Test tubes were closed and sonicated for about 10 sec in an ultrasonic bath at room temperature. Reaction was allowed to take place for at least 10 min at room temperature. Then 2.1 μl barium acetate solution (314.1 mm, 1.05 equivalents) was added, and the sample was sonicated (10 sec). Hydrolysis and precipitation of barium sulfate was allowed to take place for 10 min at room temperature and 60 min at 4°C. Then 120 μl of methanol was added to the reaction mixture and the sample was centrifuged at 20800 *g* for 10 min at 15°C in a benchtop centrifuge. For mass spectrometric analysis, 10 μl of the sample was transferred into an electrospray capillary.

Sample storage at −20°C exerted no noticeable influence on the cholesterol concentration as determined by the methodology introduced in this study.

Mass spectrometric analyses

Mass spectrometric analyses were performed with triple quadrupole instruments [Finnigan-MAT (San Jose, CA) model TSQ 7000 or VG micromass (Cheshire, UK) model QUATTRO II] both equipped with a nanoelectrospray source operating at an estimated flow rate of 50 to 100 nl/min.

TSQ 7000. The electrospray capillary was positioned at a distance of 0.5–1 mm before the orifice of the heated transfer capillary, which was maintained at 150°C. The spray was started by applying −600 to −900 V to the capillary. For each spectrum (mass range: 450–480 amu) 40–100 repetitive scans of 2-sec duration were averaged. All tandem MS experiments were performed with argon as collision gas at a nominal pressure of 2 mTorr. Precursor ion scans of *m*/*z* 97 were performed with a collision energy of 60 eV.

QUATTRO II. The electrospray capillary was positioned at a distance of 1–3 mm before the cone and the source temperature was set to 30°C. The spray was started by applying −800 to −1200 V to the capillary. For each spectrum (mass range: 450–480 amu) 30–50 repetitive scans of 4-sec duration were averaged. All tandem MS experiments were performed with argon as collision gas at a nominal pressure of 2×10^{-3} mbar. A collision energy of 62 eV and a cone voltage of 50 V were used for the precursor ion scan of *m*/*z* 97.

For both instruments borosilicate glass capillaries (3) from Clark Electromedical Instruments (Pangbourne, GB) of the type GC120FT-10 were pulled with a microcapillary puller (Model P-87, Sutter Instrument Co., Novato, CA) in a two-step cycle pulling procedure. Pulled capillaries were sputtered with a thin layer of gold using a Sputter Coater (BAL TEC, SCD 005).

± MS1-Spectra. Depending on the polarity of the electric tension either positively or negatively charged ions enter the first mass analyzer (1. Quadrupole), where they are separated by their mass to charge ratio (*m*/*z*) (**Fig. 1A**). In the resulting (positive or negative) total ion spectrum (±MS1) the ion intensities are plotted against the *m*/*z* ratio. In case of single charged ions, the *m*/*z* ratio directly represents the molecular mass.

± Precursor Ion Spectrum. This scan modus is used to scan the total ion mixture for a special group of ions that have in common the same product ion generated in the collision chamber (Fig. 1B). For this purpose the second mass analyzer is fixed to the *m*/*z* ratio of the product ion whereas the first mass analyzer scans a mass range of interest. The molecular ions seperated by the first mass analyzer sequentially enter the collision chamber. If such an ion is fragmented to yield the product ion of interest, it will be detected when it passes the second mass analyzer. When a product ion is detected, the instrument registers the molecular ion *m*/*z* ratio that passed the first mass analyzer at the same time. The re-

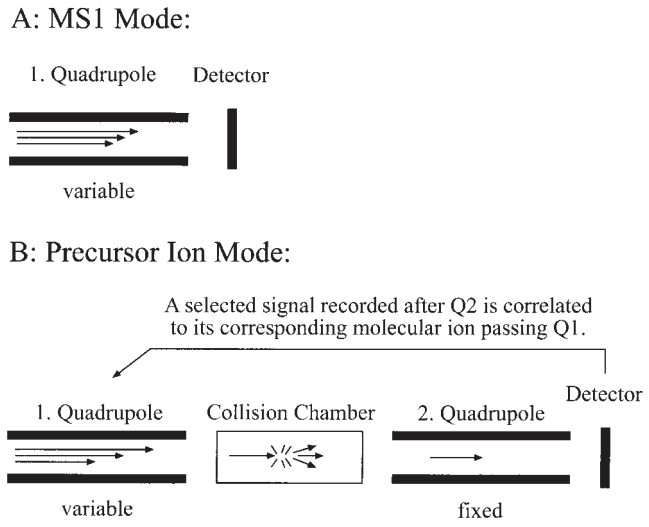

Fig. 1. Explanation of MS1- and precursor ion modes. Details are given in the text.

sulting spectrum shows only those molecular ions which have given rise to the selected product ion in the collision chamber.

Quantification of the data

For the quantitative measurements, baseline resolution of the molecular ion signals of sulfated cholesterol was established to obtain reproducible ion abundance data. Using negative ion nanoESI-MS the principal signals of the sulfated cholesterol and the sulfated [$^{13}C_2$]cholesterol are detected at *m/z* 465 and *m/z* 467, respectively. However, the ion signals of both compounds interfere with each other. Cholesterol sulfate has a natural +2 isotope at *m/z* 467 with an abundance of about 10% of which about half is due to the presence of ^{34}S. The sulfated [$^{13}C_2$]cholesterol standard exhibits a signal at *m/z* 465, due to the presence of nonlabeled material. Hence, the ratio of the signal intensities of I_{465} to I_{467} does not directly represent the molar ratio of cholesterol to standard. For both, the MS1- and the precursor ion data, the true molar ratio was calculated by the following equation:

$$\text{cholesterol[mol]/standard[mol]} = (Z - b)/(1 - aZ)$$

with $Z = I_{465}/I_{467}$ of the sample; $a = I_{467}/I_{465}$ of cholesterol without standard; and $b = I_{465}/I_{467}$ of standard only.

The equation was derived by the following postulates: *1*) I_{465}/I_{467} = ^{465}cholesterol sulfate [mol]/^{467}cholesterol sulfate [mol]; *2*) ^{465}cholesterol sulfate[mol] = cholesterol[mol] + b × standard[mol]; *3*) ^{467}cholesterol sulfate[mol] = a × cholesterol[mol] + standard[mol].

Values for a and b are influenced by background signals. This must be taken into account for high sensitivity analyses, with amounts of cholesterol plus standard in the piomole range, especially when the signal intensities of standard and cholesterol differ significantly.

RESULTS

Optimization of conditions

In solution the reagent for sulfation is sensitive to hydrolysis. As it is difficult to completely exclude water from the samples without a high experimental expenditure, we used an excess of reagent. However, in nanoESI-MS, inorganic salts quench signal intensities and reduce the stability of the electrospray (15). Therefore we minimized the concentration and volume of the reagent to 5 mg/ml and 20 μl, respectively. Under these conditions cholesterol sulfate was obtained in a yield of 55–60% after 20 min. After 5 min about 94% of this yield is obtained. Therefore, there is no need for prolonged incubation times.

The excess of reagent was hydrolyzed and the obtained free sulfate was precipitated by the addition of 1.05 equivalents of aqueous barium acetate. This resulted in a decrease of various background signals, which seem to result from complexes containing sulfate ions.

Dilution (1:7) of the reaction mixture with methanol further reduced the salt concentration and the sample viscosity. This resulted in a more stable electrospray and higher signal intensities for cholesterol sulfate.

Linearity

In **Fig. 2**, the molar ratio of cholesterol to [$^{13}C_2$]cholesterol standard measured is plotted versus the concentration of cholesterol in pmol per sample. The measured molar ratio of cholesterol to [$^{13}C_2$]cholesterol standard was evaluated from the signal intensity ratio I_{465}/I_{467} using the formula given under Quantification of the data. Each sample contained 20 pmol of internal standard. Nearly identical values for the MS1 scan and the precursor scan of *m/z* 97 were obtained. Linearity was obtained down to a sample to standard ratio of 0.1.

The same linearity was obtained in the range of 10 to 300 pmol of cholesterol using 200 pmol of internal standard (data not shown).

Sensitivity

To define the minimum amount of biological material that can be analyzed, samples in a range from 10000 to 250 CHO cells spiked with 40 pmol [$^{13}C_2$]cholesterol stan-

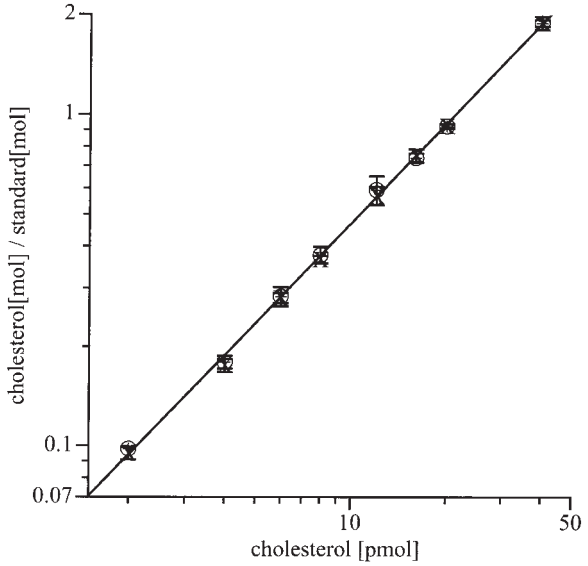

Fig. 2. Dependence of the signal ratio of cholesterol to the standard on the concentration of cholesterol, using 20 pmol of internal [$^{13}C_2$]cholesterol standard per sample; n = 3. ⊕: − MS1 scan, X: − precursor ion scan of *m/z* 97.

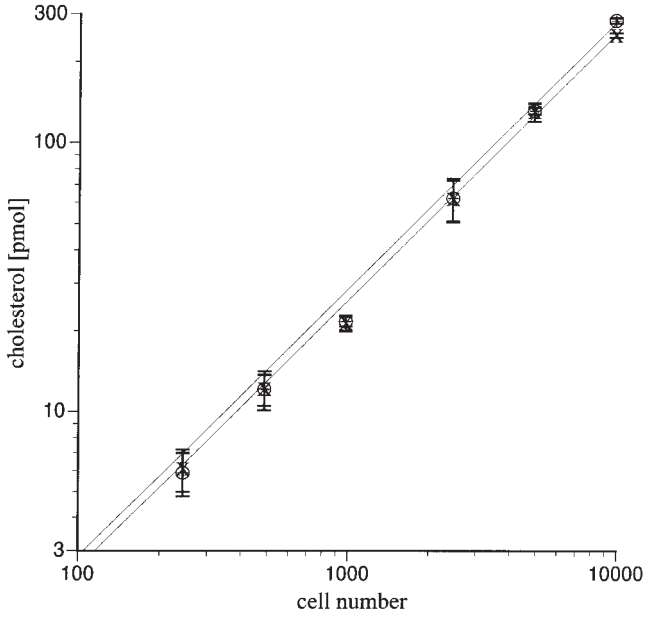

Fig. 3. Quantification of cholesterol in CHO cells. The cholesterol content is plotted vs. the CHO cell number of the samples. Samples were independently extracted and derivatized, containing 40 pmol of internal [$^{13}C_2$]cholesterol standard each. ⊕: − MS1 scan, X: − precursor ion scan of *m*/*z* 97; n = 3, upper straight line: −MS1, lower straight line: − precursor ion scan, both lines were forced through zero.

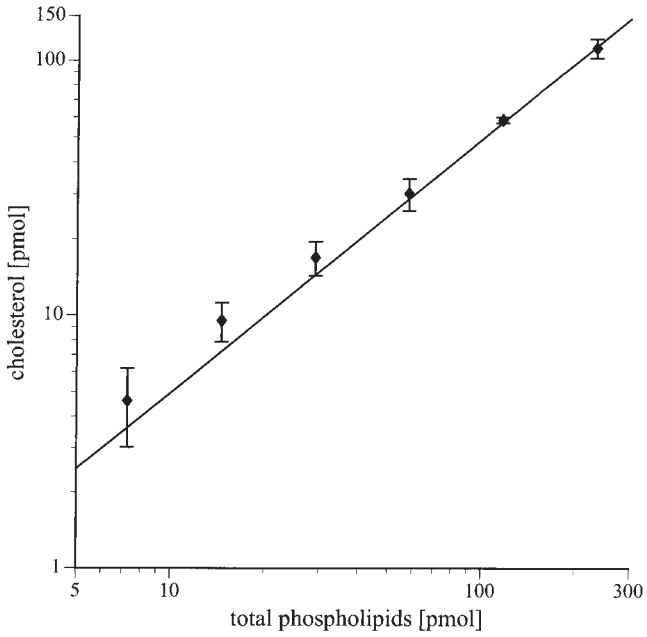

Fig. 4. Quantification of cholesterol in a membrane fraction enriched in CHO Golgi. The cholesterol content is plotted versus the total phospholipid content of the samples. Samples were independently extracted and derivatized, containing 40 pmol of internal [$^{13}C_2$]cholesterol standard each. Precursor ion scan of *m*/*z* 97; n = 6, straight line was forced through zero.

dard were independently extracted and derivatized (**Fig. 3**). In samples of 244 ± 3 cells, free cholesterol was quantified to 6.1 ± 1.1 pmol. An overall mean ± SD of 24.8 ± 1.7 fmol/cell (±6.9% SD) for the cellular concentration of free cholesterol was obtained (values from precursor ion scan of *m*/*z* 97). The resulting ratio of cholesterol to total phospholipids of 0.34 is consistent with data (0.36 and 0.34 mol/mol) from the literature (7, 16).

As a control, cholesterol stearate was submitted to the same procedure, which did not result in a detectable signal for cholesterol sulfate. Subsequently, this assay was applied to decreasing amounts of a membrane suspension 30-fold enriched in CHO Golgi membranes (**Fig. 4**). Again each sample, after the addition of 40 pmol of internal standard, was independently extracted and derivatized. The total phospholipid content was used as a measure for the amount of membranes.

An overall mean ± SD of 0.57 ± 0.07 mol/mol (±12.4% SD) for the molar ratio of cholesterol to phospholipids was obtained (values from precursor ion scan of *m*/*z* 97).

Comparison of the ESI-MS/MS assay with an enzymatic fluorescence assay

In order to determine interassay variability, the cholesterol concentration in CHO cells was quantified by *i*) nanoESI-MS/MS and *ii*) an enzymatic fluorescence assay with cholesterol oxidase (13). The results are compared in **Table 1**. With the nanoESI-MS/MS assay, the concentration of free cholesterol was determined to be 22.1 ± 1.3 fmol/cell (±5.9% SD) (values from MS1- and precursor ion scan of *m*/*z* 97) and with the fluorescence assay to 19.3 ± 4.1 fmol/cell (±21.5% SD).

Sterol specificity of both assays

Three sterols with a 3-β-hydroxyl group, cholesterol, ergosterol, and stigmasterol, were analyzed separately and as a mixture both by nanoESI-MS/MS and by an enzymatic fluorescence assay (**Table 2**). As these sterols all differ in molecular weight, they are detected without mutual interference of their ion signals by ESI-MS [**Fig. 5**, pictograms according to (17)]. Therefore this analytical principle can be used to measure sterol mixtures as they are given by biological samples. However, the individual response factors of the sterols are not identical. Using [$^{13}C_2$]cholesterol as an internal standard, this must be taken into account for the quantification of other sterols than cholesterol.

TABLE 1. Comparison of the mass spectrometric with an enzymatic fluorescence assay

Method	Cell Number	Free Cholesterol
		fmol/cell[a]
ESI-MS/MS	976	22.3 ± 1.5
	1952	21.8 ± 1.3
Enzym.-fluor.	2440	21.4 ± 5.6
	4880	16.0 ± 3.3
	9760	20.1 ± 0.4

[a] Mean ± SD (n = 3).

TABLE 2. Comparing ESI-MS/MS and a fluorescence assay with respect to their sterol specificity

	ESI-MS/MS Assay				Enzyme Fluorescence Assay				
	Sample				Sample				
Sterol	1	2	3	4	1′	2′	3′	4′	
	pmol				*pmol*				
Set values [pmol]									
Cholesterol	208			208	208			208	cholesterol
Ergosterol		224.6		224.6		224.6		224.6	ergosterol
Stigmasterol			262.9	262.9			262.9	262.9	stigmasterol
Measured values									
Cholesterol	209[ab]			207[ab]					
Ergosterol		156[ab]		156[ab]	235[ac]	83[ac]	175[ac]	447[ac]	Σ sterols
Stigmasterol			211[ab]	213[ab]					

[a] Mean of two independent experiments.
[b] Relative response factors of ergosterol and stigmasterol compared to the [$^{13}C_2$]cholesterol standard are not taken into account.
[c] Values were calculated from a cholesterol calibration line.

The fluorescence assay is based on the enzymes cholesterol oxidase and horseradish peroxidase. Cholesterol oxidase is known to be nonspecific for cholesterol (18, 19). Therefore, the fluorescence assay cannot distinguish cholesterol from the other sterols, because all three sterols are oxidized. However, using a standard protocol for cholesterol, the incubation time was too short for the quantitative oxidation of ergosterol and stigmasterol (Table 2).

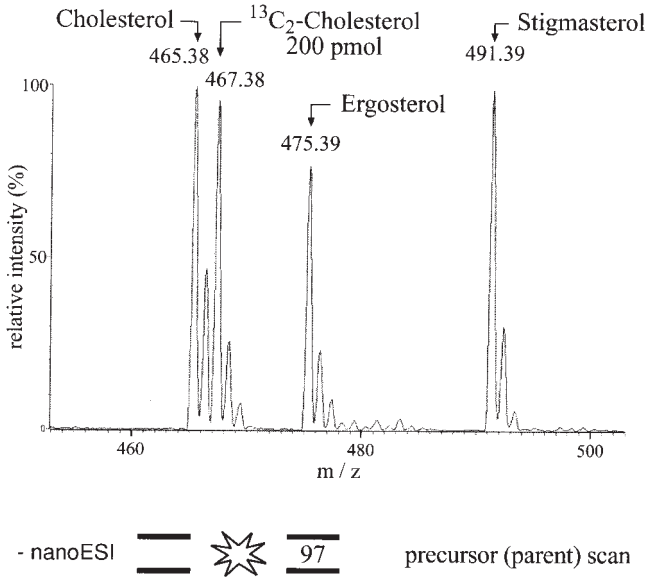

Fig. 5. ESI-MS/MS analysis of a sulfated sterol mixture. Precursor ion scan of *m*/*z* 97. The *m*/*z* values in the spectrum correspond to the sulfated sterols. The analysis represents one of two measurements of sample 4 described in Table 2. Definitions of pictograms referring to (17): Ions are produced by a nanoESI-source. The first quadrupole scans a variable *m*/*z* range. Ions that passed the first quadrupole collide with argon in the collision chamber and the resulting product ions go on to the second quadrupole. The second quadrupole is fixed to a *m*/*z* of 97. Only ions with this *m*/*z* value can pass this quadrupole to be detected.

Quantification of ergosterol with [$^{13}C_2$]cholesterol as internal standard

[$^{13}C_2$]cholesterol (200 pmol/sample) was used as an internal standard for the quantification of varying amounts of ergosterol from a stock solution (**Fig. 6**). With the instrumental parameters used, sulfated ergosterol has a relative molar response factor of 70% compared to the concentration of sulfated internal [$^{13}C_2$]cholesterol standard. A linear titration curve was obtained in the range of 0 to 440 pmol of ergosterol.

DISCUSSION

Ceramides (20) and endogenous cholesterol sulfate (10) have already been successfully profiled by ESI-MS/MS, and phospholipids by nanoESI-MS/MS (5). Using an internal standard added prior to extraction of the biological sample, we quantified phosphatidylcholine, sphingomyelin (5), and endogenous cholesterol sulfate (10). Cholesterol itself is hardly detectable by ESI-MS due to the absence of a charged functional group. Therefore we converted cholesterol to cholesterol sulfate, which in contrast to cholesterol exhibits a high ionization efficiency in the negative ion mode (10) due to its sulfate ester group. The chemical conversion of hydroxyl groups with pyridine sulfur trioxide complex is nonspecific (14). Hence other sterols will also be converted into the corresponding sterol sulfates as shown for ergosterol and stigmasterol. This renders the derivatization method applicable for sterols, e.g., as demonstrated for ergosterol in Fig. 6.

We have shown for sulfated cholesterol that both the MS1 scan and the specific precursor ion scan of *m*/*z* 97 provide identical and straight calibration lines (Fig. 2B). Nevertheless, higher specificity is reached with the precursor scan, as only sulfate-ester-containing ions contribute to the detected ion signals. For biological samples this is of general advantage. Therefore, cholesterol concentrations from biological samples presented here were obtained exclusively using the precursor ion scan mode.

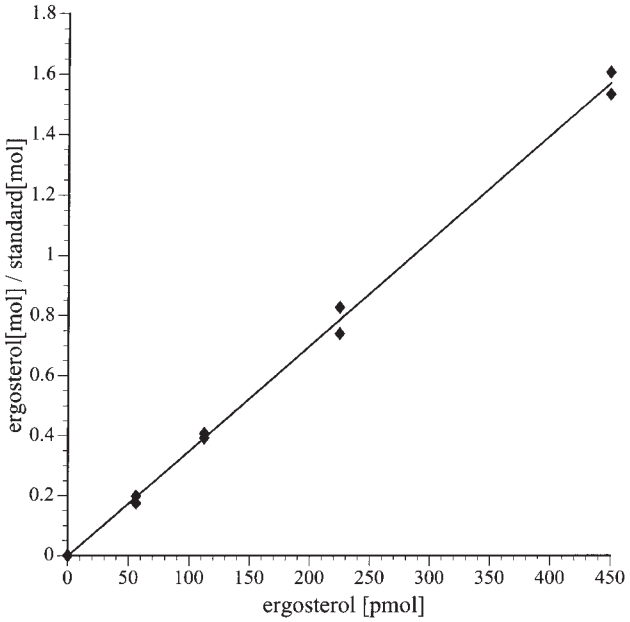

Fig. 6. Dependence of the signal ratio of ergosterol to [$^{13}C_2$]cholesterol standard (200 pmol) on the concentration of ergosterol. Precursor ion scan of m/z 97. For the same molar concentration, a relative response factor of 70% compared to the same concentration of the standard signal was obtained from the curve.

With the assay presented, free cholesterol could be quantified in biological membranes down to approximately 5 pmol of total free cholesterol. This corresponds to a sensitivity about 10-fold higher than an enzymatic fluorescence method (12, 13).

The assay was applied to compare the cholesterol concentrations of purified COPI-coated vesicles with their donor Golgi membranes. In both CHO cells and rat liver, a reduced cholesterol concentration in the COPI-coated vesicles of 36 and 60%, respectively, was obtained compared to the donor Golgi membranes. This demonstrates a segregation of cholesterol from these vesicles (Brügger, B., R. Sandhoff, W. Nickel, J. B. Helms, K. Gorgas, W. D. Lehmann and F. T. Wieland, unpublished results).

The specificity of the nanoESI-MS/MS assay for a given sterol is obtained by derivatization, mass to charge resolution, and precursor ion scanning of m/z 97. Thus individual sterols can be quantified in complex mixtures. In contrast, the specificity of the fluorescence assay depends on cholesterol oxidase that is reported to oxidize sterols bearing a 3-β-hydroxyl group (18, 19), with preferences for, but no discrimination between, individual sterols. A further advantage of the nanoESI-MS/MS assay is the addition of an internal standard prior to the preparation, because variations due to nonquantitative extraction or derivatization during sample work up are accounted for.

In some cells (e.g., keratinocytes), cholesterol sulfate is an endogenous constituent of their membranes. In the case of CHO cell membranes the measured concentration of endogenous cholesterol sulfate was below the detection limits and compared to cholesterol by a factor of at least 500 lower (data not shown). Taking into account the SD of the assay presented, this amount is negligible.

In biological samples, containing non-negligible amounts of endogenous cholesterol sulfate, the endogenous cholesterol sulfate concentration can be determined prior to derivatization. To determine the free cholesterol concentration, the concentration of endogenous cholesterol sulfate must be subtracted from the total cholesterol sulfate content obtained after derivatization. However, in this case, two internal standards with different mass shifts are required, as e.g., dihydrocholesterol-3-sulfate and [$^2H_6$]cholesterol. The internal standard for endogenous cholesterol sulfate is needed to determine the intensity due to endogenous cholesterol sulfate in the mass spectrum of the derivatized sample. This value must be subtracted from the total intensity of the signal at 465 m/z (sulfated cholesterol and endogenous cholesterol sulfate). With the minimal volume needed for one measurement and the total volume of one assay, the measurement of one sample can be repeated many times.

Here we have demonstrated the use of nanoESI-MS/MS for the determination of free cholesterol at the low picomole level. The methodology presented extends the analytical methods towards an accurate determination of cholesterol in small amounts of biological samples such as several hundred cells or preparations of subcellular structures such as transport vesicles.

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