Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography B, 819 (2005) 307–313
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Novel and simple high-performance liquid chromatographic method for determination of 3-hydroxy-3-methylglutaryl-coenzyme A reductase activity

Michele Buffalini [1], Raffaella Pierleoni [1], Chiara Guidi, Paola Ceccaroli, Roberta Saltarelli, Luciana Vallorani, Sabrina Zeppa, Vilberto Stocchi *

Istituto di Chimica Biologica "Giorgio Fornaini", Università degli Studi di Urbino "Carlo Bo", via A. Saffi 2, 61029 Urbino (PU), Italy

Received 1 October 2004; accepted 18 February 2005
Available online 22 March 2005

Abstract

We present here a high-performance liquid chromatographic method for the evaluation of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase activity. The automated method was applied to fungal and mouse liver extracts and validated by the addition of mevastatin to the reaction mixture and by several intra- and inter-day assays. This method offers important advantages over those previously reported because no radiolabeled substrates or expensive techniques such as mass spectrometry are required, and the time of analysis is relatively short. Moreover, the method can be successfully applied to different biological samples; hence, it should be very useful in evaluating potential inhibitors of the HMG-CoA enzyme and investigating cholesterol metabolism, cell growth and differentiation processes.

Keywords: Mevalonolactone; Cholesterol; Mevastatin; HPLC analysis

1. Introduction

The conversion of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) to mevalonolactone, catalysed by the HMG-CoA reductase enzyme (EC1.1.1.34), is the rate-limiting step in the isoprenoid metabolism pathway in mammals, fungi, as well as plants and insects [1,2].

This key enzyme has a crucial role in the production of the large family of molecules produced by the mevalonate pathway (Fig. 1). These molecules include sterols (especially ergosterol in yeast and cholesterol in animals) involved in membrane structure; dolichol, required for protein glycosylation; haem A and ubiquinone, which participate in electron transport; isopentyladenine, found in some tRNAs; and intracellular messengers such as cytokines and gibberellins in plants and fungi, steroid hormones in animals and farnesylated mating factors in yeast [3,4].

The central role played by HMG-CoA reductase enzyme in regulating sterol biosynthesis, and the importance of normal sterol production for growth make this enzyme an attractive target for the development of new agricultural fungicides and antimycotic drugs.

Because elevated levels of HMG-CoA reductase activity have been observed in rapidly proliferating human cancer cells [5], its regulation may also play a pivotal role in tumour malignancy. Hence, the inhibition of this activity may have a therapeutic value beyond the treatment of hypercholesterolemia [6–10].

Because of its importance for sterols and the biosynthesis of secondary metabolites, the HMG-CoA reductase gene (HMGR) isolated from several organisms has been thoroughly investigated.

* Corresponding author. Tel.: +39 0722 305262; fax: +39 0722 320188.
E-mail address: v.stocchi@uniurb.it (V. Stocchi).
[1] These authors contributed equally to the manuscript.

1570-0232/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2005.02.017

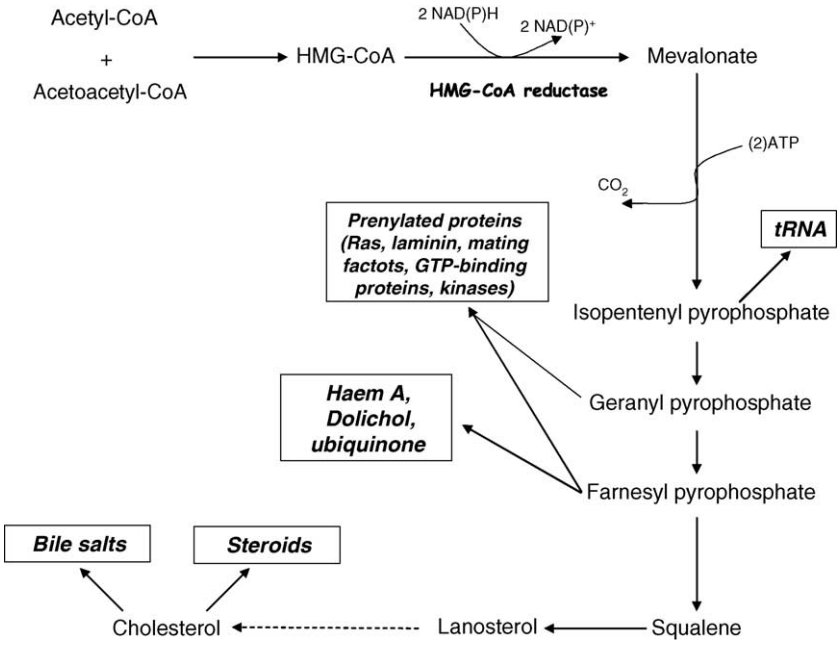

Fig. 1. Mevalonate pathway. The rate-limiting step occurs at the 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase catalysed reaction. The phosphorylation reactions are required to solubilise the isoprenoid intermediates in the pathway. Intermediates in the pathway are used for the synthesis of prenylated proteins, dolichol, coenzyme Q and the side chain of haem A.

Only one HMGR gene copy has been found in animals, whereas two or more HMGR gene copies have been isolated from plants. Indeed, a single or double structural HMGR gene has been found in fungi [11].

This gene is subject to different complex metabolic regulatory mechanisms, including translational and transcriptional control [12], and enzyme activity modulation by degradation and phosphorylation [13].

At present, little is known about the metabolism of isoprenoids in mycorrhizal fungi. In a previous work, we cloned a fragment of the HMGR gene from the ectomycorrhizal fungus *T. borchii* and evaluated its expression level during the fungus life cycle [14]. The *T. borchii* HMGR gene is highly expressed in the ripe fruit body (known as the white truffle), whereas its expression is markedly lower in the mycelial tissue and the unripe fruit body. Hence, increase in the terpenic compounds synthesis may play a role in antifungal and antimicrobial processes and contribute to the flavour of the truffle ascomata.

In order to gain further insight into the metabolism of *T. borchii* terpenoids and how the HMG-CoA reductase enzyme is regulated, we have developed a new, useful, cheap assay for the assessment of HMG-CoA reductase activity.

Various approaches have been proposed for the quantification of mevalonolactone and most of them require the use of radiolabeled materials. These methods include gas chromatography (GC), colorimetric methods, gas chromatography–mass spectrometry (GC–MS) [15] and liquid chromatography–mass spectrometry (LC–MS) [16].

This paper describes the development and validation of a simple specific method for assessing HMG-CoA reductase activity.

The procedure involves high-performance liquid chromatography (HPLC), which proved to be a cheap, sufficiently sensitive, reproducible method for the detection and quantification of mevalonolactone.

2. Experimental

2.1. Reagents and apparatus

HMG-CoA, NADPH, mevalonolactone, mevastatin and other reagents for the enzyme assay were purchased from Sigma (St. Louis, MO, USA). A Gold liquid chromatographic system from Beckman (Beckman Coulter Inc., Fullerton, CA, USA) was used for mevalonolactone detection. The HPLC apparatus consisted of two Model 126 pumps and a Model 168 diode array detector. The chromatographic separations were performed using a Supelcogel Ca column (30 cm × 7.8 mm I.D.; Supelco Park, Bellefonte, PA, USA). The guard column was a Supelguard Ca/C611 (5 cm × 4.6 mm I.D.; Supelco Park, Bellefonte, PA, USA). Integration of peak areas was obtained using the 32 Karat Gold software.

2.2. Standards

Stock solutions of mevalonolactone were made by dissolving 20 mg in 1 ml of HPLC-grade water. Separate solutions

for the calibration curve and quality control samples were prepared by serial dilutions of the stock solutions with the reaction mixture (see further).

2.3. Sample preparation

The HMG-CoA reductase enzyme was obtained from the ectomycorrhizal fungus *T. borchii* Vittad. (strain MYA 1018). The mycelia were grown in Petri dishes containing dextrose potato agar (PDA) solid medium and incubated at 23 ± 1 °C in the dark for 20 days. Agar fragments, taken from the periphery of the fungal cultures in Petri dishes, were transferred to culture bottles containing 70 ml of modified Melin–Norkrans nutrient solution (MMN), pH 6.5, and incubated under the same conditions described earlier [17].

The validation experiments were carried out on CD-1 female mice (Charles River Laboratories, Milan) housed at 23 ± 1 °C with 12 h light/dark cycles, 60 ± 5% humidity and 12 air changes h^{-1}.

2.4. HMG-CoA reductase assay

T. borchii mycelia (0.5 g of dried weight) were sampled after 30 days of growth in MMN liquid medium (see earlier), washed twice with water to eliminate growth medium traces and homogenised using a Potter homogeniser (Steroglass, Perugia, Italy) with an appropriate amount (at least 500 μl) of the extraction buffer [20 mM sodium phosphate buffer, pH 7.5, 10 μM β-mercaptoethanol (β-MSH), 0.25% (v/v) Tween 20 and 10 μM phenylmethylsulphonyl fluoride (PMSF)].

The suspension obtained was then centrifuged at 18,000 × *g* for 10 min at 4 °C, and the supernatant was used as a crude extract for the enzyme assay.

Protein content was determined using the method of Lowry et al. [18]. For HMG-CoA reductase assays, 200 μg of mycelial proteins were pre-incubated for 5 min at 37 °C with 200 mM sodium phosphate buffer, pH 7.5, and 10 mM β-MSH. The reaction was then initiated by the addition of 0.24 mM NADPH and 40 μM HMG-CoA.

The complete reaction mixture (with a final volume of 1 ml) was incubated at 37 °C and stopped at fixed intervals (*T* = 5, 10, 15, 20, 30, 35 and 40 min) adding 10 μl of 6 M HCl. Controls were obtained adding cofactor, substrate and HCl (*T* = 0 min) simultaneously.

After the lactonisation of the reaction with HCl, the mixture was incubated for an additional 40 min at 37 °C to promote the generation of mevalonolactone. Then, after centrifugation at 18,000 × *g* for 2 min to remove precipitated proteins, the supernatant was neutralised with 0.1 M sodium carbonate buffer, pH 10.5.

Aliquots (200 μl) of each sample were injected onto the HPLC system described earlier. The mobile phase consisted of HPLC-grade water, and the elution was carried out at a flow rate of 1 ml min^{-1} at 37 °C. The detection was performed at 200 nm.

Sample activity was determined by calculating the concentrations of mevalonolactone in spiked samples against a calibration curve.

To validate the method, the experimental conditions described earlier were also applied to mouse liver extracts. In this case, the liver was washed twice with extraction buffer (see earlier) instead of water, and 1–2 mg of extract proteins were utilised for the HMG-CoA reductase assay.

2.5. Calibration curves

The calibration curve consisted of five different concentrations of mevalonolactone over the range 2.5–30 μg. In particular, 2.5, 5, 7.5, 15 and 30 μg of mevalonolactone standard solutions were added to 200 μl of the reaction mixture (see earlier) in which the enzyme had replaced with the extraction buffer. The curves were obtained plotting the mevalonolactone peak area versus mevalonolactone concentration in μg.

Quality control samples, used to assess precision and accuracy, contained 5, 10 and 20 μg of mevalonolactone, respectively, and were prepared and processed in the same way as calibration standards.

Intra-day precision, inter-day precision and accuracy were calculated using the data obtained during the 3-day validation. Samples were analysed on each day of the 3-day validation ($n = 6$ at each concentration).

Precision was expressed as the standard deviation of the measurement as a percentage of the mean value. Accuracy was expressed as the deviation between the true and the measured value expressed in a percentage.

2.6. Limit of quantitation

The limit of quantitation (LOQ) was defined as the lowest concentration at which the precision, expressed by the relative standard deviation, is better than 20% and accuracy, expressed by the relative difference of measured and true value, is lower than 20%. Six identical samples were analysed for the determination of LOQ.

3. Results and discussion

3.1. Chromatographic conditions and sample preparation

Previously reported methods for mevalonolactone determination have used colorimetric assays or expensive sophisticated techniques such as GC–MS or LC–MS, which usually require the radiolabeled materials [^{3}H] mevalonolactone and [^{13}C] HMG-CoA for assessing HMG-CoA reductase activity [15,16].

Moreover, the GC–MS procedures frequently involve at least one step of analyte derivatisation, whereas in LC–MS or LC–MS/MS methods complicated chromatographic systems are often used and column switching is required.

The analysis of mevalonolactone using a mixed size exclusion/ion exchange HPLC mode has not yet been developed. Even though this column has typically been used for the HPLC analysis of carbohydrates it also proved to be suitable for the determination of mevalonolactone, a cheton with a cyclic structure, comparable to those of sugars.

In addition, compared with previously reported methods [15,16,22], a simpler procedure was used for sample preparation. In fact, no microsomal fraction was analysed. Sample homogenates were centrifuged at 18,000 × *g* for 10 min at 4 °C and supernatants were used for assessing HMG-CoA reductase activity.

This method allowed us to use smaller amounts of initial material to detect mevalonolactone and is therefore suitable for analysing a wide range of samples and HMG-CoA reductase inhibitors, including statins.

Statins (mevastatin, lovastatin, etc.) are potent hypocholesterolemic agents exhibiting pleiotropic (cholesterol-independent) effects and are used in the treatment of pathologies such as cardiovascular diseases, osteoporosis, Alzheimer's disease and ischemic stroke [19,20].

According to the proposed HPLC separation, described in detail in Section 2, mevalonolactone can be detected and quantified simply using deionised water as a mobile phase instead of a buffered one.

In particular, mevalonolactone from ectomycorrhizal fungus *T. borchii* mycelial homogenate and mouse liver extract were analysed. In both cases mevalonolactone was obtained from the lactonisation of mevalonate, the product of HMG-CoA reductase enzyme induced by the addition of HCl to the reaction mixture.

All the other validation experiments were carried out using mevalonolactone standard solutions diluted to a final volume of 200 μl with the reaction mixture (Section 2).

In all cases, according to the experimental elution conditions described earlier, mevalonolactone produced a symmetrical peak with an elution time of 12.9 ± 0.2 min, which increased proportionally with the increasing amounts of exogenous mevalonolactone that were added to the reaction mixtures.

Moreover, as shown in Fig. 2, the chromatograms regarding the analyses of mevalonolactone from standard solutions re-suspended in 200 μl of the reaction mixture (A), *T. borchii* mycelia (B), or liver extracts (C) showed no interfering peaks.

In control HPLC runs, 200 μl of the reaction mixtures without the exogenous mevalonolactone or the fungal/mammalian homogenate were injected, and no peaks were found in the mevalonolactone elution time range (data not shown), thus confirming the selectivity/specificity of the HPLC procedure.

3.2. Linearity and limit of quantitation

The standard curve consisted of five standards of different concentrations of mevalonolactone ranging from 2.5 to 30 μg. Six samples were analysed for each concentration.

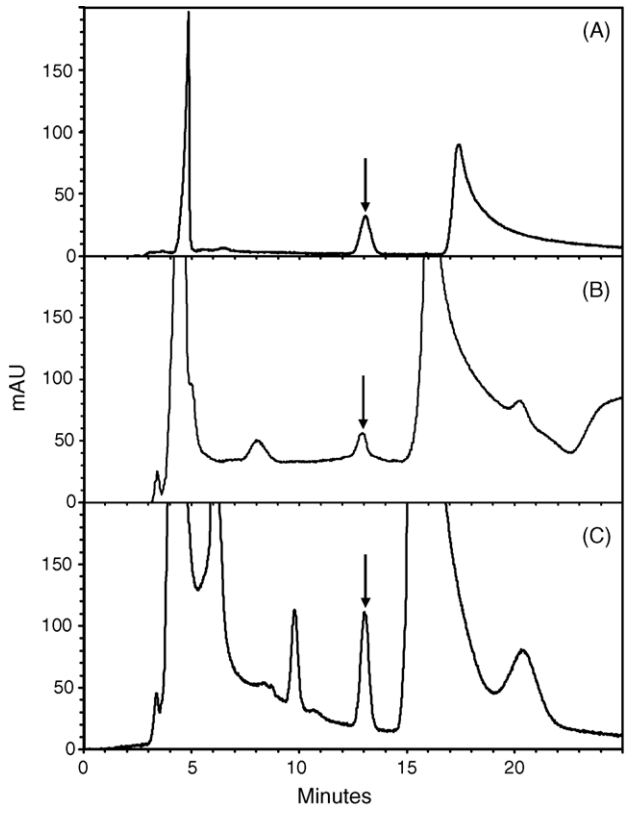

Fig. 2. HPLC chromatograms of mevalonolactone: (A) 30 μg standard solution; (B) 200 μg of total proteins from 30-day-old *T. borchii* mycelia homogenate; (C) 2 mg of protein from liver extracts incubated for 20 min after the addition of cofactor and substrate to the reaction mixture. Mevalonolactone is marked with arrows, whereas other peaks regard the reaction mixture components. No interference at the retention time of mevalonolactone (12.9 ± 0.2) is evident.

The calibration curve was calculated on each day of the 3-day validation and was linear in the studied range. The mean equation (curves coefficients ± standard deviation) of the calibration curve was $y = 3 \times 10^{-5} (\pm 0.12 \times 10^{-5})x$ and the correlation coefficient $r = 0.9987$, where x represents the mevalonolactone peak area and y represents the mevalonolactone concentrations expressed in μg. The precision was better than 5.5% and accuracy was better than 5% at all concentrations of the calibration standards.

The limit of detection (LOD) of the proposed method was 5.7 nmol of mevalonolactone injected into column in our chromatographic system. Within this concentration of mevalonolactone, the precision was better than 15% and inaccuracy was not significant (Table 1).

The limit of quantitation (LOQ) was 10 nmol of mevalonolactone corresponding to 1.25 μg/200 μl injected in the HPLC system. Lower concentrations of mevalonolactone cannot be quantified with sufficient precision and accuracy (Table 1).

This value is higher than those of other validated methods for the determination of mevalonolactone using MS techniques, according to which can quantify nanograms or picograms of analyte [16,22]. Nevertheless, the proposed

Table 1
Accuracy and precision of mevalonolactone quantification

	Calculated amount[a]	Accuracy (%)[b]	Precision (%)[c]
Intra-day ($n = 5$)			
Calibration standards[d]			
2.5	2.43 ± 0.11	−2.8	4.5
5	4.94 ± 0.1	−1.2	2.0
7.5	7.15 ± 0.29	−4.7	4.0
15	14.8 ± 0.25	−3.3	4.9
30	29.5 ± 0.34	−3.2	4.6
Quality control samples[d]			
5	4.89 ± 0.12	−2.2	2.4
10	10.12 ± 0.14	2.2	3.0
20	20.5 ± 0.21	2.5	2.0
Inter-day ($n = 5$)			
Calibration standards[d]			
2.5	2.41 ± 0.11	−3.6	4.5
5	4.88 ± 0.17	−2.4	3.5
7.5	7.66 ± 0.31	2.1	4.0
15	15.3 ± 0.5	2.0	5.2
30	28.6 ± 1.21	−4.7	4.2
Quality control samples[d]			
5	4.85 ± 0.12	−3.0	2.5
10	9.87 ± 0.46	−1.3	4.7
20	19.3 ± 1.1	−3.5	5.7

[a] Mean ± S.D. in μg.
[b] Defined as: [(measured concentration − target concentration)/target concentration] × 100%.
[c] Assessed by expressing the standard deviation of the measurement as a percentage of the mean value.
[d] Amount of mevalonolactone added per 200 μl sample in μg.

HPLC method is sensitive enough to detect variations in the quantity of mevalonolactone caused by specific inhibitors or drugs as demonstrated by the inhibition experiments described further.

3.3. Inter- and intra-day assay precision and accuracy

Intra-day assay precision of the method is illustrated in Table 1. Six sets of quality samples (low, medium and high concentration) were analysed with calibration samples in one batch (on 1 day). The precision and accuracy were better than 3% at all mevalonolactone concentrations of the samples.

Inter-day assay precision and accuracy was evaluated by analysing six sets of calibration and quality control samples on three separate days. The samples were prepared in advance and stored at 4 °C. As reported in Table 1, the precision was better than 6% and accuracy was better than 4% at all levels.

3.4. Selectivity and specificity of HMG-CoA reductase assay

Fig. 3 shows the effect of incubation time on *T. borchii* and mouse liver HMG-CoA reductase activities.

Proteins from *T. borchii* mycelium homogenates and liver extracts were added to the inactivated reaction mixture composed of phosphate buffer and β-MSH and incubated for 5 min at 37 °C. The reaction was triggered by adding NADPH and HMG-CoA and stopped at a fixed time adding 10 μl of 6 M HCl.

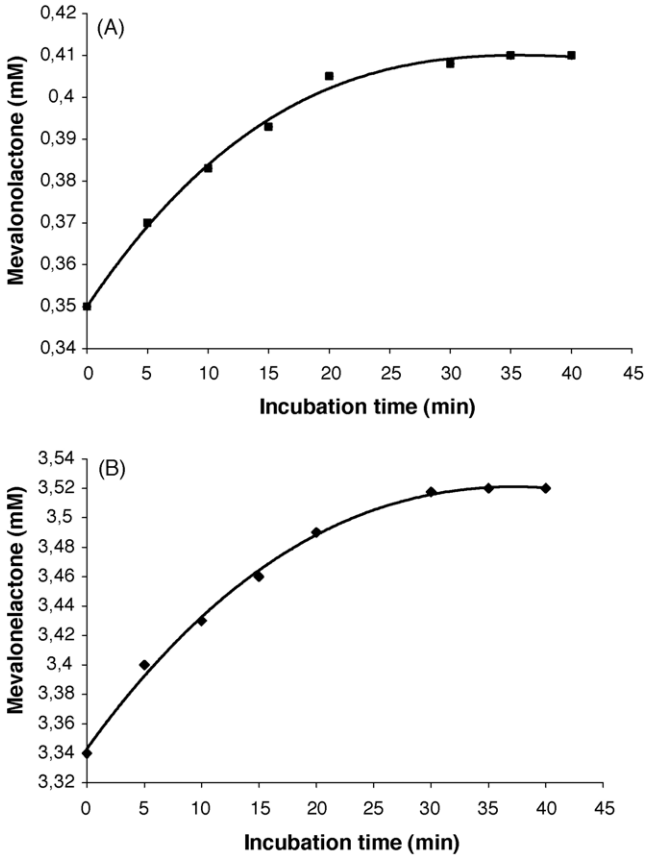

Fig. 3. Effect of incubation time on 30-day-old *T. borchii* (A) and mouse liver (B) HMG-CoA reductase activities. HMG-CoA reductase assays were performed at 0, 5, 10, 20, 30, 35 and 40 min; for controls ($T = 0$ min) cofactor, substrate and HCl were added simultaneously.

In the case of control samples, substrate–cofactor mixture and HCl were added together ($T = 0$) (see Section 2). In both cases, the concentration of mevalonolactone increased linearly for about 15 min and then reached a plateau between 20 and 40 min. Hence, in subsequent experiments, HMG-CoA reductase activity was evaluated stopping the reaction 20 min after the addition of substrate–cofactor mixture.

In the case of mouse liver extracts, the specific activity of the HMG-CoA reductase enzyme, defined as the quantity of mevalonolactone produced in the time unit per milligram of protein, was 131.77 pmol mg^{-1} of protein min^{-1}. This value is comparable to the result reported in Ohashi et al. [21], which was determined using a well-established radiolabeled assay, and thus supports the reliability of our procedure.

To ascertain the selectivity/specificity of the method the effect of a well-known HMG-CoA reductase inhibitor, mevastatin, was tested.

Statins are one of the most widely prescribed classes of drugs in the world because of their excellent cholesterol-lowering effect and overall safety profile. They reduce plasma cholesterol by inhibiting the limiting enzyme in the choles-

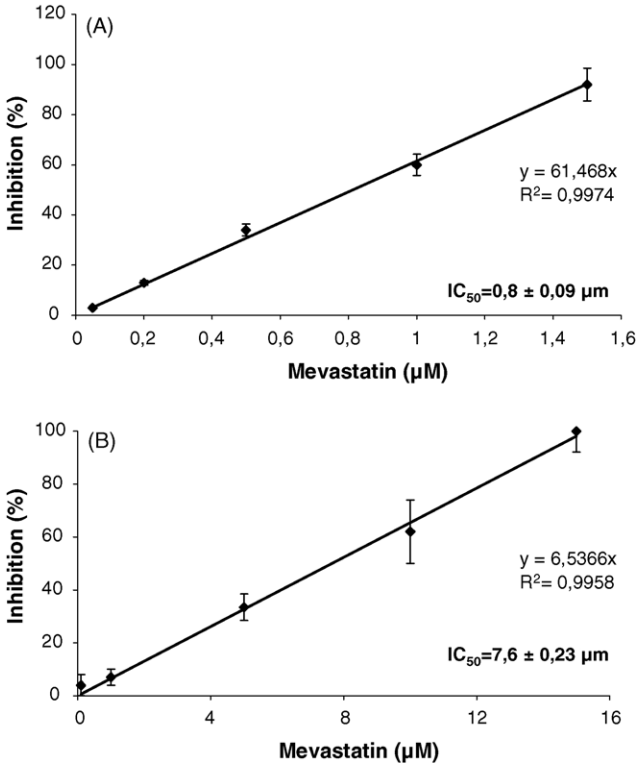

Fig. 4. Inhibition of 30-day-old *T. borchii* mycelial (A) and mouse liver (B) HMG-CoA reductase activity by mevastatin. Different concentrations of mevastatin, in the range 0.1–20 μM, were pre-incubated with the inactivated reaction mixture before adding the cofactor to start the reaction (see Section 2). Values are the mean of six independent experiments.

terol biosynthetic pathway (HMG-CoA reductase), which prevents de novo synthesis of cholesterol. The pleiotropic effect of statins is well documented [19,20], but data suggest that they may be involved in many more processes than originally thought [22,23]. Many patent applications have attempted to cover combination therapies, broadening statins' potential applications to almost all known diseases. Unfortunately, many of the new claims are not well substantiated and biological data are lacking.

In our experiment, the inhibitor was pre-incubated with the inactivated reaction mixture (buffer, β-MSH and fungal/mouse liver homogenate) without substrate and cofactor for 5 min at 37 °C before starting the reaction. Time zero (controls) and $T = 20$ min samples obtained as described earlier were injected onto HPLC and analysed.

The tested compound determined a marked inhibition of HMG-CoA reductase activity in both fungal and liver extracts, leading to a significant decrease of mevalonolactone peak areas. In order to better characterise the inhibitory action of this compound increasing concentrations of the statin were tested.

In particular, different concentrations of mevastatin, ranging from 0.1 to 20 μM were used. As shown in Fig. 4, the tested compound inhibited the production of mevalonolactone in a concentration-dependent manner, and a concentration of mevastatin of about 2 μM in *T. borchii* mycelia homogenate and 15 μM in liver extracts completely inhibited reductase activity.

The calculated IC_{50} values, corresponding to the inhibitor concentration that inhibits the enzyme activity by 50% at a given substrate value (providing a useful parameter for pharmacologists), were 0.8 ± 0.09 and 7.6 ± 0.23 μM for fungal and liver tissue extracts, respectively.

The mammalian IC_{50} value was similar to the value reported in Park et al. [24], confirming the specificity and the applicability of the proposed non-microsomal HPLC method. In the method of Park et al. [24], the same enzyme was extracted from rat liver, and mevalonolactone was evaluated using a LC–MS mevalonolactone assay.

Considering the pervasiveness of cardiovascular disease and the aging population in Western societies, the discovery of new serum cholesterol-lowering drugs represents an important area of research for all pharmaceutical companies. Simple, reproducible, sensitive methods for assessing HMG-CoA reductase activity, such as the proposed HPLC non-microsomal procedure, may be a useful tool for researchers in this field.

Moreover, the availability of such procedures will improve our knowledge of pharmacokinetic drug interactions, including relevant quantitative data for potential effectors, to ensure safe and proper use of the cholesterol-lowering drugs.

To evaluate the stability of mevalonolactone as a function of time and temperature, the above-mentioned samples were also stored at 4 °C and room temperature and tested over a period of 5 days. Mevalonolactone from both fungal and mammalian extracts was found to be stable for the entire period at 4 °C and for 48 h at room temperature.

3.5. Applications

The proposed method was used to determine 3-hydroxy-3-methylglutaryl-coenzyme A reductase activity in *T. borchii* mycelial tissue because little information is currently available on the metabolism of the isoprenoids in ectomycorrhizal fungi.

Mycelia were collected after 30 days (active growth phase) and 60 days (stationary growth phase) of growth on MMN liquid media [17] and HMG-CoA reductase activity was evaluated by determining mevalonolactone formation.

In 30-day-old mycelia, the specific activity of HMG-CoA reductase was 760 pmol mg^{-1} of protein min^{-1}. On the contrary, no activity was detected on mycelia collected after 60 days of growth.

According to these data, the HMG-CoA reductase activity of the investigated fungus is differentially regulated by the cell growth phase. We therefore hypothesise a pivotal role of this enzyme in hyphae formation and differentiation.

This behaviour is similar to what we previously observed for many enzymes of the glycolitic and pentose phosphate pathways [25]. Indeed, both metabolic pathways proved to

be more active during the exponential growth phase then in the stationary phase, as shown by the marked decrease of the specific activity of most of the enzymes involved.

In the case of the enolase enzyme, the biochemical data have also been confirmed by molecular analyses evaluating the expression level of the corresponding mRNA [26].

It stands to reason that in the initial vegetative growth phase, biosynthetic pathways are strongly induced because the formation and differentiation of the hyphae require the synthesis of new membranes and cell walls, as well as a higher energy supply to meet the cell demand. In another previous work, a fragment of *T. borchii* HMGR gene was cloned and its expression evaluated both in mycelial tissue and ripe or unripe fruit bodies [14]. In this study, HMGR gene expression was found to be up-regulated in the ripe ascocarps, whereas it was lower in the unripe ones and the mycelial tissue. This suggests involvement of terpenic compounds, and thus of HMG-CoA reductase enzyme in antimicrobial and antifungal processes and in truffle aroma formation. In order to more thoroughly investigate the physiological role of *T. borchii* HMG-CoA reductase, further analyses of the enzymatic activity either during ascocarp maturation or in the ectomycorrhizal tissues are in progress.

4. Conclusions

A simple HPLC analysis using a size exclusion/ion exchange for the detection and quantification of mevalonolactone, the indirect product of HMG-CoA reductase enzyme activity (produced by the mevalonate lactonisation), was developed and validated.

Among the advantages of the proposed HPLC method over previous methods is that it does not require sophisticated and very expensive techniques, such as LC–MS or GC–MS and radiolabeled materials [15,16,21].

Furthermore, this method requires limited time of analysis (15–20 min for each run) and smaller amounts of initial material because of the less complicated non-microsomal procedure used for sample preparation.

The proposed method has a higher limit of quantitation compared with previously reported methods but it provides a precise, accurate and reproducible quantification of mevalonolactone and is sensitive enough to evaluate potential HMG-CoA reductase inhibitors.

It is also clear, based on the data discussed in this paper, that this method can be successfully applied to a wide range of biological samples, from fungi to mammals.

Because of the pivotal role played by the HMG-CoA reductase enzyme in cholesterol metabolism, cell growth and differentiation processes, the proposed method may also provide useful insights into its regulatory pathway and allow us to verify the inhibitory action of new potential inhibitor compounds.

References

[1] J. Chappell, F. Wolf, J. Proulx, R. Cuellar, C. Saunders, Plant Physiol. 109 (1995) 1337.
[2] M.E. Basson, M. Thorsness, J. Rine, Proc. Natl. Acad. Sci. U.S.A. 83 (1986) 5563.
[3] J.D. Conolly, R.A. Hill, Dictionary of Terpenoids, Chapman & Hall, New York, 1992.
[4] S. Woitek, S.E. Unkles, J.R. Kinghorn, B. Tudzynski, Curr. Genet. 31 (1997) 38.
[5] M.G. Caruso, M. Notarnicola, M. Santillo, A. Cavallini, A. Di Leo, Anticancer Res. 19 (1999) 451.
[6] S. Rao, D.C. Porter, X. Chen, T. Herliczec, M. Lowe, K. Keyomarsi, Proc. Natl. Acad. Sci. U.S.A. 96 (1999) 7797.
[7] L. Altucci, R. Addeo, S. Cicatiello, M.G. Dauvois, M. Parker, M. Truss, M. Beato, V. Sica, F. Bresciani, A. Weisz, Oncogene 12 (1996) 2315.
[8] W. Wang, R.J. Macaulay, Int. J. Cancer 82 (1999) 430.
[10] P.M. Ghosh, N. Ghosh-Choudhury, M.L. Moyer, G.E. Mott, C.A. Thomas, B.A. Foster, N.M. Greenberg, J.I. Kreisberg, Oncogene 18 (1999) 20.
[11] S. Woitek, S.E. Unkles, J.R. Kinghorn, B. Tudzynski, Curr. Genet. 31 (1997) 38.
[12] T.F. Osborne, J. Biol. Chem. 266 (1991) 13947.
[13] R. Sato, J.L. Goldstein, M.S. Brown, Proc. Natl. Acad. Sci. U.S.A. 90 (1993) 9261.
[14] S. Zeppa, C. Guidi, A. Zambonelli, L. Potenza, R. Pierleoni, L. Vallorani, C. Sacconi, V. Stocchi, Curr. Genet. 42 (2002) 161.
[15] B.H. Wollen, P.C. Holme, W.J. Northway, P.D. Martin, J. Chromatogr. B 760 (2001) 179.
[16] M. Jemal, A. Schuster, D.B. Whigan, Rapid Commun. Mass Spectrom. 17 (2003) 1723.
[17] R. Molina, For. Sci. 25 (1979) 585.
[18] O.H. Lowry, A.L. Rosebrough, R.J. Farr, J. Randall, J. Biol. Chem. 193 (1951) 265.
[19] M. Takemoto, J.K. Liao, Arterioscler. Thromb. Vasc. Biol. 21 (2001) 1712.
[20] B. Wolozin, W. Kellman, P. Ruosseau, G.G. Celesia, G. Siegel, Arch. Neurol. 57 (2000) 1439.
[21] K. Ohashi, J. Osuga, R. Tozawa, T. Kitamine, H. Yagyu, M. Sekiya, S. Tomita, H. Okazaky, Y. Tamura, N. Yahagi, Y. Iizuka, K. Harada, T. Gotoda, H. Shimano, N. Yamada, S. Ishibashi, J. Biol. Chem. 31 (2003) 42936.
[22] N. Nath, S. Giri, R. Prasad, A.K. Singh, I. Singh, J. Immunol. 172 (2004) 1273.
[23] S. Parvathy, M. Ehrlich, S. Pedrini, N. Diaz, L. Refolo, J.D. Buxbaum, A. Bogush, S. Petanceska, S. Gandy, J. Neurochem. 90 (2004) 1005.
[24] E.J. Park, D. Lee, Y.G. Shin, D.D. Lantvit, R.B. van Breemen, A.D. Kinghorn, J.M. Pezzuto, J. Chromatogr. B 754 (2001) 327.
[25] R. Saltarelli, P. Ceccaroli, L. Vallorani, A. Zambonelli, B. Citterio, M. Malatesta, V. Stocchi, Mycol. Res. 102 (1998) 403.
[26] E. Polidori, R. Saltarelli, P. Ceccaroli, M. Buffalini, R. Pierleoni, F. Palma, P. Bonfante, V. Stocchi, Fungal Genet. Biol. 41 (2004) 157.