Biochemical and Biophysical Research Communications **284,** 50–56 (2001)
doi:10.1006/bbrc.2001.4901, available online at http://www.idealibrary.com on IDEAL®

On the Occurrence of Multiple Isoprenylated Cysteine Methyl Ester Hydrolase Activities in Bovine Adrenal Medulla

Guido A. F. Van Dessel,*,†,[1] Hilde M. De Busser,* and Albert R. Lagrou*
†*UIA-Laboratory for Pathological Biochemistry and* **RUCA-Laboratory for Human Biochemistry, University of Antwerp, Groenenborgerlaan 171, B-2020 Antwerp, Belgium*

Received April 16, 2001

Rab proteins intervene in the controlled exocytosis of catecholamines by chromaffin cells from the adrenal medulla. These proteins are posttranslationally modified by digeranylgeranylation and carboxymethylation. Reversible carboxymethylation terminating the isoprenylation pathway may play an important role in both the functioning and the subcellular housing of small G-proteins. Controlled methylation infers a rational interplay between the two enzymes involved i.e., the protein-*S*-prenylcysteine methyltransferase and the opposing esterase. Previously we have identified a methyltransferase type III in chromaffin cells. In this paper we focus on the corresponding demethylase. The methyl ester hydrolase activity was monitored using AFCM and AGGCM as artificial substrates while *p*-nitrophenylacetate was adopted as a pseudosubstrate for nonspecific esterase action. Based on subcellular fractionation experiments, kinetic studies and screening a battery of potential effectors, including a series of metallic ions and metal chelators, multiple sulphydryl reagents and host of specific protease/esterase inhibitors, it is suggested that at least two prenylcysteine carboxymethyl esterase isoenzymes are operational in bovine adrenal medulla. These isoenzymes are distinctly different from the nonspecific esterase.

Key Words: **prenylcysteine carboxymethyl esterase; adrenal medulla; protein isoprenylation; methyltransferase type III; *S*-adenosylmethionine; aspecific esterase.**

Abbreviations used: ACM: N-acetyl-L-cysteine methylester; AdoMet: *S*-adenosyl-[^{3}H-methyl] methionine; AFCM: *N*-acetyl-*S*-Farnesyl-L-cysteine methylester; AGGCM: *N*-acetyl-*S*-geranylgeranyl-L-cysteine methylester; DMSO: dimethylsulfoxide; EDTA: ethylenediaminetetraacetic acid; EGTA: ethylene-glycol-bis(β-aminoethyl ether)*N*,*N*,*N*′,*N*′-tetraacetic acid; GTP: guanosine 5′-triphosphate; LDV: large dense vesicle; NEM: *N*-ethylmaleimide; N-fraction: nuclear fraction; pcCMT: protein-*S*-prenylcysteine-*O*-methyltransferase or carboxymethyltransferase type III; P-fraction: particulate fraction; PMSF: phenylmethylsulfonylfluoride; ROS: Rod outer segment; RSA: relative specifiec activity; TLC: thin layer chromatography; TPEN: *N*,*N*,*N*′,*N*′-tetrakis(2-pyridylmethyl)ethylenediamine.

[1] To whom correspondence should be addressed. Fax: +32-03-2180326. E-mail: guvades@ruca.ua.ac.be.

Many cellular processes are posttranslationally controlled by reversible modifications of key proteins. These modulations are preponderantly achieved by phosphorylation and dephosphorylation which are accelerated by a plethora of protein kinases and corresponding phosphatases (1, 2). Another reversible covalent modification proceeds through methylation and demethylation reactions catalyzed by respectively AdoMet-dependent methyltransferases and the opposing methylesterases.

The biological relevance of methylation and demethylation has been evidenced at first in chemotactic bacteria (3) and yeast (4). Regulated methylation has also been hypothesized to play an important role in both large G-protein coupled receptor function and small G-protein function *in vivo* (5). The latter type of methylation is catalyzed by a carboxy-methyltransferase type III and specifically methylates the C-terminal carboxyl group of proteins which have been isoprenylated previously (6).

Isoprenylation/methylation of GTP-binding proteins generally includes a tripode of posttranslational reactions with in order of sequence: an isoprenylation step, an endoproteolytic cleavage, and the methylation of the C-terminal cysteine residue (7). For other G-proteins the posttranslation modification only includes isoprenylation, while a third group of G-proteins are directly methylated after isoprenylation without intermediate endoproteolysis. In the above modification cascade only the carboxymethylation reaction is likely to occur in a reversible fashion and therefore it has been introduced as an attractive regulatory site (8).

Controlled methylation infers a rational interplay between the two enzymatic activities involved respec-

0006-291X/01 $35.00

tively the methyltransferase and the corresponding demethylase. Nevertheless, in chemotactic bacteria methylation levels have been reported to be solely regulated at the level of the methylesterase by reversible phosphorylation (9).

Chromaffin cells of the adrenal medulla secrete upon activation catecholamines from LDV by controlled exocytosis (7). In this complex process many protein factors intervene including small GTP-binding proteins from the rab family. These rab proteins are the object of digeranylgeranylation and methylation, which might be both functionally and structurally important. Recently we have identified and partially characterized a methyltransferase type III in chromaffin cells (10). In this chapter we present data on the corresponding demethylation activity which was measured with the synthetic substrates AFCM and AGGCM. At this moment only scarce information is available concerning this type of esterase (11–13). From our study on the biochemical behaviour of the esterases it can be concluded that at least two isoenzymes can be demonstrated in adrenal medulla. Moreover these isoenzymes differ distinctly from a non-specific esterase activity also housing in this tissue.

MATERIALS AND METHODS

Materials. *S*-adenosyl [^{3}H-methyl] methionine (15 Ci/mmol) was purchased from Amersham (Amersham International plc, Buckinghamshire, England). Pig liver esterase and p-nitrophenylacetate were obtained from Sigma (St. Louis, MO). Protease inhibitors were from Boehringer (Mannheim, Germany). L-AGC, L-AFC, L-AFCM and L-AGGC were bought from Bachem (Switzerland). All other reagents acquired from various commercial sources were reagent grade.

Preparation of the subcellular fractions. A crude homogenate of bovine adrenal medulla was prepared as previously described and fractionated following De Duve's fractionation scheme (14). The microsomal fraction (60 min × 110,000*g*) was used as the enzyme source for the esterase assay.

Synthetic substrates. AFC and AGGC were enzymatically methylated with [^{3}H] AdoMet as outlined by De Busser *et al.* (10). The methylated products were purified from the heptane extract by preparative TLC (Silicagel Si60; eluent chloroform/ethanol 9/1; v/v). The [^{3}H- methyl] labelled AFCM or AGGCM were eluted from the silica with successively heptane (4 × 5 ml) and chloroform/ethanol (9/1; v/v; 4 × 5 ml). After evaporation these synthetic substrates were dissolved in DMSO. The specific radioactivity of both substrates amounted to 15 Ci/mmol. Cold AGCM, AFCM, and AGGCM were prepared by methyl esterification with methanolic HCl (8). L-ACM was synthesized from *N*-acetyl-L-cystine as described by Tan and Rando (12).

Esterase assay. The routine incubation mixture contained 50 nM [^{3}H-methyl] labelled AFCM (16 nM AGGCM), 150 μg protein and 50 mM Hepes buffer pH 7.4 in a total volume of 50 μl. In all experiments DMSO concentration was kept under 4% (v/v). The reaction was quenched by the addition of 2 ml of water followed by 1 ml of chloroform after 20 min incubation at 37°C. The radioactivity recovered in the aqueous upper phase was quantified. The aspecific esterase activity was monitored as previously described (15) using the substrate *p*-nitrophenylacetate.

RESULTS

When subfractionating a homogenate of adrenal medulla into a 110,000*g* sediment and a residual supernatant 50 to 60% of the AFCM and AGGCM demethylase activities were recovered in the membranous fraction together with 60 to 70% of the proteins. Most of the *p*-nitrophenylacetate esterase (60–70%) could be traced in the supernatant fraction. A more detailed subfractionation did not result into a clear-cut picture as all three esterase activities were found in respectively the N, M, L and P-fractions with a slight preference for the P-fraction. In subsequent experiments this P-fraction was used as the enzyme source. This particulate fraction of bovine adrenal medulla was shown to possess enzymatic activities capable to hydrolyse both the synthetic substrates AFCM and AGGCM, but also *p*-nitrophenylacetate, a substrate for monitoring less specific esterases, was hydrolysed at an appreciable rate. The proteinaceous nature of the intervening catalyst was confirmed by the observation that heat treatment annihilated the esterase activities. All three hydrolytic activities displayed incubation time and protein dependency (Fig. 1). For the pH-activity curves distinctly different profiles were found (results not shown). For AFCM the activity increased from pH 6.5 and reached a plateau in the pH region 7.5–9. For AGGCM a biphasic profile was registered with optima around pH 6 and 7.5, while for *p*-nitrophenylacetate the cleavage optimum was obviously centred around pH 7. The K_m- and V_{max}-values as deduced from kinetic studies and Lineweaver–Burke plots have been collected in Table 1.

In order to investigate the requirement for the isoprenylated tail and the effect of its chain length on the esterase activity the effect of ACM and AGCM was controlled indicating that both were incompetent inhibitors of AFCM and/or AGGCM demethylation. To further explore the distinct nature of the esterases, incubations (all at pH 7) were performed in the presence of a series of potential effectors. Cations elicited a concentration-dependent effect on the esterase activities (Fig. 2). The isoprenylcysteine ester cleavage was drastically enhanced in the presence of Na^+-ions. In contrast the hydrolysis of *p*-nitrophenylacetate was not affected at all. On the other hand the effect of the inclusion of K^+-ions on the isoprenylcysteine ester cleavage was much more moderate. AFCM demethylation was activated in the presence of Ca^{2+}-ions, especially at higher concentrations (5 mM), while only minor effects were observed in the presence of Mg^{2+}-ions. The effector picture differed clearly for AGGCM demethylation: 5 mM Ca^{2+}- and Mg^{2+}-ions both caused inhibition. The *p*-nitrophenylacetate esterase activity remained unchanged in the presence of these earth alkalines. Hg^{2+}-ions drastically depleted AFCM and AGGCM demethylation, while *p*-nitrophenylacetate

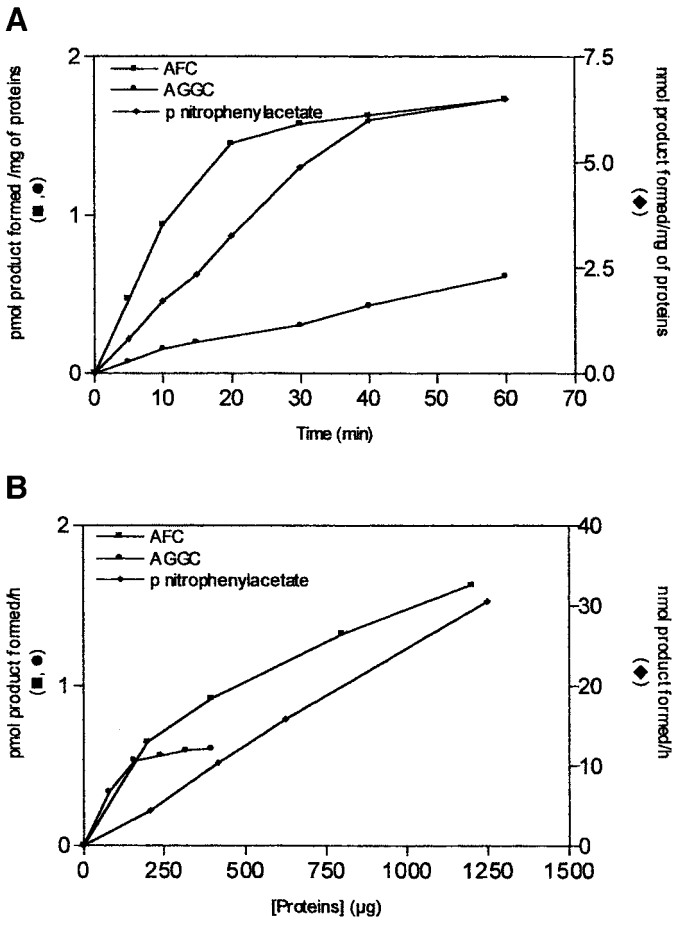

FIG. 1. Methylesterase activity as a function of time and protein concentration. Methyltransferase activity was assayed as a function of time (A) and protein concentration (B) using AFCM (■), AGGCM (●) or *p*-nitrophenyl-acetate (◆) as the respective substrates. All incubations were performed as described under Materials and Methods.

esterase activity was only slightly affected. A striking difference was found for the effect of Zn^{2+}-ions: whilst AFCM demethylation remained unaltered, the AGGCM demethylation was drastically enhanced with an optimal effect around 0.4 mM. At 1 mM Zn^{2+} concentration the velocity of *p*-nitrophenylacetate hydrolysis was nearly halved. With Mn^{2+}-ions reversed effects for AFCM and AGGCM demethylation were found i.e., activatory for AFCM and moderately inhibitory for AGGCM demethylation. Finally for *p*-nitrophenylacetate hydrolysis a 50% reduction was found with Mn^{2+}-ions.

The metal chelators EDTA and EGTA (Fig. 3) enhanced the hydrolysis of both AFCM and AGGCM. The effect of EDTA was more beneficial on the AFCM demethylation, while the activation by EGTA was more pronounced on the AGGCM demethylation. EDTA, but not EGTA, caused an inhibitory effect on the hydrolysis of *p*-nitrophenylacetate. In presence of the Zn^{2+}-

TABLE 1

Enzyme Kinetics of the Methylesterases in Bovine Adrenal Medulla

Substrate	K_m (μM)	V_{max} (nmol · mg^{-1} · min^{-1})
AFCM	0.2	5
AGGCM	0.2	2.5
p-nitrophenylacetate	770	0.8

Note. Kinetic constants were deduced from the Lineweaver–Burke plots obtained after incubation of varying concentrations of each substrate under the standard enzyme assay conditions.

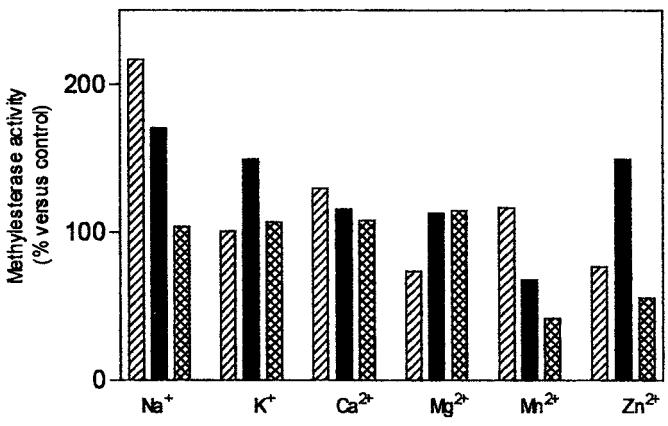

FIG. 2. Influence of cations on the methylesterase activity. The effect of cations on the esterase activity was studied using AFCM (hatched bars), AGGCM (shaded bars), or *p*-nitrophenyl-acetate (cross-hatched bars) as substrate. All cations were administered at 1 mM final concentration. Incubations were performed as described under Materials and Methods.

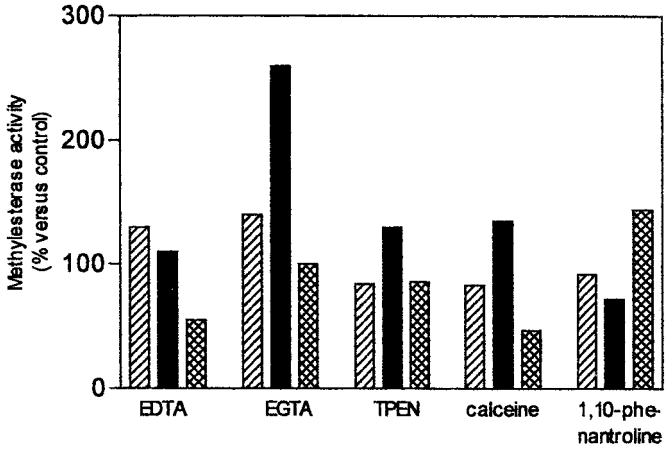

FIG. 3. Effect of various chelating agents on the methylesterase activity. Methylesterase activity was assayed using AFCM (hatched bars), AGGCM (shaded bars), or *p*-nitrophenylacetate (crosshatched bars) as substrate. EDTA, EGTA, and TPEN were administered at 1 mM final concentration, calcein, and 1,10-phenantroline were 2 mM final concentration. All incubations were performed as described under Materials and Methods.

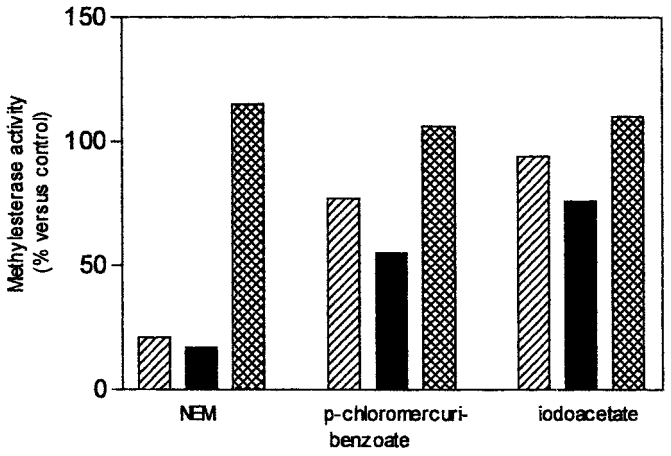

FIG. 4. Effects of suphydryl reagents on the methylesterase activity. Methylesterase activity was assayed using AFCM (hatched bar), AGGCM (shaded bar), or *p*-nitrophenylacetate (cross-hatched bar) as substrate. All reagents were 0.5 mM final concentration. Incubations were performed as described under Materials and Methods.

chelator, TPEN, the stimulatory effect of Zn^{2+}-ions on the AGGCM demethylation was tempered but not completely eliminated. Also for other metal chelators (Fig. 3) diverging effects were noted. Calceine slightly inhibited AFCM demethylation at 2 mM, while AGGCM demethylation was moderately increased; *p*-nitrophenylacetate hydrolysis decreased by about 50% in presence of this ligand. AFCM demethylation was barely influenced by 1,10-phenantroline, while AGGCM hydrolysis was limitedly inhibited. At the same time the aspecific esterase activity was distinctly enhanced. The sulphydryl reagent NEM drastically blocked the AFCM as well as AGGCM demethylation and was without any effect on the hydrolysis of *p*-nitrophenylacetate. The effects elicited by the sulphydryl reagents p-hydroxymercuribenzoate and iodoacetate on the hydrolysis of AFCM and AGGCM were much less impressive and again without any inhibitory effect on the nonspecific esterase (Fig. 4).

In order to further differentiate between the isoprenyl-dependent demethylases and the nonspecific esterase the effect provoked by a selection of specific esterase inhibitors was studied. Therefore, *p*-nitrophenol, butylacetate, F^--ions as well as a number of protease/esterase blocking agents were included in the incubation mixture. The nonspecific esterase was, as expected, strikingly inhibited by F^--ions, *p*-nitrophenol and butylacetate. On the other hand, AFCM and AGGCM demethylation were slightly enhanced in presence of these inhibitors. The effects noted upon addition of protease/esterase inhibitors have been summarized in Table 2. With the exception of aprotinin and ebelactone B, both causing a moderate decrease of 25%, *p*-nitrophenylacetate hydrolysis was nearly insensitive to all protease/esterase inhibitors tested. Moreover none of the potential inhibitors proved to be convincingly effective on the hydrolysis of the AFCM substrate. Contrary AGGCM demethylation was drastically reduced by all inhibitors tested with the exception of PMSF. The most prominent decrease (up to 70%) was observed in the presence of ebelactone B. The organophosphorus ester disopropyl phosphorofluoridate, a well known serine esterase inhibitor, was without any effect on all three esterases under study.

DISCUSSION

Although the methylation/demethylation process in the protein isoprenylation pathway has been suggested as a regulatory site because of its potential reversibility, evidence supporting the removal of the C-terminal methyl groups is still a matter of debate. In literature

TABLE 2

Effects of Various Inhibitors on the Methylesterases of Bovine Adrenal Medulla

		Substrates (% residual methylesterase activity)		
Inhibitor	Concentration (μM)	AFCM	AGGCM	*p*-nitrophenylacetate
Leupeptin	50	88	60	105
Pepstatin	12.5	94	70	95
Aprotinin	50	97	64	61
Ebelactone B	12.5	73	20	76
PMSF	50	70	117	100
p-nitrophenol	1000	128	132	19
Butylacetate	50	138	156	40
Sodiumfluoride	1000	117	144	44
L-ACM	100	99	115	ND
AFCM (cold)	100	15	130	100
AGGCM (cold)	100	75	16	ND
DFP	10	101	100	100

Note. The values were obtained under the standard enzyme assay conditions in the presence of the indicated effector. ND: not determined.

there is an obvious poverty of papers focussing on the demethylase which is involved in the turnover of the methylgroup on the C-terminal isoprenylcysteine residues. In addition the study of this methyl turnover is encumbered by interference from nonspecific proteases and esterases. For example, the vacuolar protease carboxypeptidase Y from bakers yeast (16) readily catalyzes the hydrolysis of AFCM. Also pig liver esterase powers the hydrolysis of the methylester of AFCM [17; Van Dessel *et al.* unpublished observation]. Indirect evidence for methylgroup turnover on mammalian small GTP-binding proteins came from the observation that GTPγS promotes both the translocation to the membrane and the methylation of a series of proteins including rac2, (18) rap1 (19), and G25K (20). An interesting feature with regard to G25K was that the methylated form was found to be membrane associated (21), while the unmethylated form remained soluble and associated with GDP (20, 22). In *Saccharomyces cerevisiae* (16) no nonvacuolar methyl esterase activity could be demonstrated using AFCM as a substrate. In intact cells of the same yeast strain however, a slow turnover of the products of the STE14 isoprenylcysteine methyltransferase was observed. Although it could not be decided whether the demethylation resulted from ester hydrolysis or peptide degradation (23). In intact mammalian cells Gutierrez *et al.* (11) also failed to detect methylgroup detachment from p21 H-ras over a 2 h period. Hrycyna and Clark (5) argue that this type of esterase activity might depend upon protein sequences or conformations upstream from the modified cysteine residue at the C-terminus. In addition these authors speculate that the role of the methylation/demethylation modification might vary depending on the nature of the proteins. If the modification is solely structural, no turnover at all is expected as in the case of ras proteins. Alternatively, if the methylation status is physiologically relevant, a dynamic role for methylation/demethylation is most likely.

The occurrence of enzymatic hydrolysis of AFCM by a membrane associated demethylase has been shown in bovine retinal rod outer segments (8). The specificity of the demethylase was inferred from the stereoselectivity, the requirement for the isoprenoic chain and the specific inhibition by ebelactone B (12). This methylesterase activity processing AFCM and AGGCM was also hypothetized to mediate the partial loss of methylgroups from the methylated γ-subunit of transducin. However a later contradictory study by Fukada *et al.* (24) claimed that transducin was kept fully methylated in ROS membranes. As outlined before, the search for specific methylesterase was compromised by the ability of nonspecific proteases and esterases to accelerate the hydrolysis of methylesters *in vitro*.

In this study we have investigated the occurrence of (a) specific demethylase(s) in bovine adrenal medulla using AFCM and AGGCM as artificial substrates to mimic the endogenous isoprenylated/methylated proteins. The presence of a specific esterase in this tissue was already suspected in a previous paper from the observation that an enhancement of methylation could be provoked by including a specific isoprenylated protein methylesterase inhibitor (10). *p*-Nitrophenylacetate was adopted as a substrate for monitoring nonspecific esterase activities.

From fractionating a bovine adrenal medulla homogenate into a particulate and a soluble fraction it can be concluded that both AFCM and AGGCM demethylase activities are for the greater part membrane associated contrary to the *p*-nitrophenylacetate esterase activity. However from the relative high recovery in the supernatant compared to the percentage methyltransferase type III found in the particulate fraction, it seems that the membrane association of the demethylase is much less firm than that of the corresponding transferase (10). Nevertheless it cannot be excluded that this supernatant demethylase still resides in microvesicular structures. Upon differential pelleting no unambiguous decision could be made with regard to a specific intracellular localization. Indeed demethylase activities could be demonstrated in all subcellular fractions with a tendency of somewhat higher RSA values in respectively N- and P-fractions.

The kinetics and the sensitivity of the demethylases toward heat denaturation sustained the proteinaceous nature of the catalysts involved. The K_m values of AFCM and AGGCM were nearly equal, but differed by several orders of magnitude with the K_m-value of the nonspecific esterase (nM vs μM). The K_m values of AFCM and AGGCM demethylase reported by Tan and Rando (12) in rod outer segment membranes are much more elevated. The V_{max} values on the other hand were much lower in adrenal medulla. From the lack of inhibition by either L-ACM or L-AGCM it can be concluded that the demethylases catalyzing the hydrolysis of AFCM or AGGCM in adrenal medulla require substrates ornamented by a isoprenoid tail with appropriate chain length. In mixed substrate experiments ([^{3}H-methyl] AFCM in presence of cold AGGCM and vice versa) both substrates minimally affected each other's demethylation tempting to conclude that we are dealing with two isoenzyme types displaying different chain length specificity. The absence of inhibition when including *p*-nitrophenylacetate is suggestive that none of the two isoenzyme activities must be ascribed to nonspecific esterase action. This hypothesis is further validated by the diverging profiles of the pH-activity curves. Optimal AFCM demethylation was found in the neutral pH range as previously reported by Tan and Rando (12) for AFCM hydrolysis in rod outer segment membranes and by Dunten *et al.* (13) for the G25K methyl esterase in rabbit brain. The pH-activity curve for AGGCM hydrolysis characterized by two op-

tima has not yet been referred to in literature. The maximum at the slight acidic pH corresponds to the pH optimum reported for the lysosomal protease cathepsin B which was also capable of liberating the methylgroup from methylated G25K (13). The second maximum around pH 7 is similar to the pH optimum found by others for the demethylation of isoprenylated/methylated substrates (12, 13).

In order to further differentiate between the catalytic activities a number of effectors was screened as potential activators/inhibitors of AFCM, AGGCM and *p*-nitrophenylacetate hydrolysis. The diverging effects upon inclusion of alkalines and earth alkalines further confirmed the different protein nature of the three esterase activities. A same conclusion could be drawn from the effects observed when including the transition metal ions Mn^{2+} and Zn^{2+}. The varying metal ion dependency was additionally confirmed by the effects induced by chelating agents. Taking together, again all these data point to the occurrence of three individual esterases in bovine adrenal medulla. The privileged role of Zn^{2+}-ions in the mevalonate pathway has been already highlighted in a previous paper (10). With regard to the blocking capacity of sulphydryl reagents both AFCM and AGGCM hydrolysis were inhibited at comparable rates, while the nonspecific esterase remained unaltered. Pronounced inhibition could only be achieved by NEM and mercurous ions.

In a final series of experiments the issue of group specific protease/esterase inhibitors was addressed. Of all inhibitors tested only ebelactone B and PMSF inhibited the AFCM demethylation to a moderate extent. The nonspecific esterase was also mildly affected, but to a different degree. For AGGCM hydrolysis a much more substantial inhibition of the demethylation rate was registered especially when including the serine esterase inhibitor ebelactone B. The divergent effects elicited by serine specific protease/esterase inhibitors is intriguing, but probably results from the accessibility of the reagents to the active centre of the enzymes. A similar observation has been made by other research groups (12, 13).

As a final conclusion we were able to demonstrate the presence of AFCM, AGGCM and *p*-nitrophenylacetate esterase activity in a P-fraction of bovine adrenal medulla. From the data presented in this paper we suggest that these three enzyme activities must be ascribed to three different protein factors. Obviously a definitive conclusion awaits purification of this enzyme triplet. Furthermore the imperative questions about the endogenous proteins in the adrenal medulla that are object of C-terminal methylation/demethylation and whether control of this posttranslation modulation is involved in the regulation of GTP binding protein activity and controlled exocytosis remain unanswered.

ACKNOWLEDGMENTS

The authors are indebted to Mrs. S. Plompen for skillful technical assistance and to Mrs. G. Naessens for the layout of the manuscript. This work was partially financed by RAFO Grant 1VDESGKP98 and Rosa Blanckaert grant.

REFERENCES

1. Hunter, T. (1987) A thousand and one protein kinases. *Cell* **50,** 828–829.
2. Walton, K., and Dixon, J. (1993) Protein tyrosine phosphatases. *Annu. Rev. Biochem.* **62,** 101–120.
3. Stock, J., Surette, M., McCleary, W., and Stock, A. (1992) Signal transduction in bacterial chemotaxis. *J. Biol. Chem.* **267,** 19753–19756.
4. Hrycyna, C., Sapperstein, S., Clarke, S., and Michaelis, S. (1991) The *Saccharomyces cerevisiae* STE14 gene encodes a methyltransferase tat mediates C-terminal methylation of a a-factor and RAS proteins. *EMBO J.* **7,** 1696–1709.
5. Hrycyna, C., and Clark, S.. (1993) Modification of eukaryotic signalling proteins by C-terminal methylation reactions. *Pharmacol. Ther.* **59,** 281–300.
6. Rando, R. (1996) Chemical biology of isoprenylation/methylation. *Biochim. Biophys. Acta* **1300,** 5–16.
7. Partoens, P., Slembrouck, D., De Busser, H., Vaughan, P., Van Dessel, G., De Potter, W., and Lagrou, A.. (2000) Neurons, chromaffin cells, and membrane fusion. *Subc. Biochem.* **34,** 323–378.
8. Pérez-Sala, D., Tan, E., Canada, F., and Rando, R. (1991) Methylation and demethylation reactions of guaninenucleotide-binding proteins of retinal rod outer segments. *Biochemistry* **88,** 3043–3046.
9. Stock, J., and Simmons, S. (1988) Methylation, demethylation at glutamate residues in membrane chemoreceptor proteins. *Adv. Exp. Med. Biol.* **231,** 201–212.
10. De Busser, H., Van Dessel, G., and Lagrou, A. (2000) Identification of prenylcysteine carboxymethyltransferase in bovine adrenal chromaffin cells. *Int. J. Biochem. Cell Biol.* **32,** 1007–1016.
11. Gutierrez, L., Magee, A., Marshall, C., and Hancock, J. (1989) Posttranslational processing of p21 ras is two-step and involves carboxyl methylation and carboxy-terminal proteolysis. *EMBO J.* **8,** 1093–1098.
12. Tan, E., and Rando, R. (1992) Identification of an isoprenylated cysteine methylester hydrolase activiry in bovine rod outer segment membranes. *Biochemistry* **31,** 5572–5578.
13. Dunten, R., Wait, S., and Backlund, P., Jr. (1995) Fractionation and characterization of protein C-terminal prenylcysteine methylesterase activities from rabbit brain. *Biochem. Biophys. Res. Commun.* **8,** 174–182.
14. Van Dessel, G., Lagrou, A., Hilderson, H., and Dierick, W. (1993) Characterization of the *in vitro* conversion of dolichol to dolichoate in bovine thyroid. *Biochim. Biophys. Acta* **1167,** 307–315.
15. Petit, K., Miserez, B., De Block, J., Van Dessel, G., and De Potter, W. (1992) The presence of phospholipase A2 in bovine adrenal medulla. *Biochim. Biophys. Acta* **1125,** 150–156.
16. Hrycyna, C., and Clark, S. (1992) Maturation of isoprenylated proteins in *Saccharomyces cerevisiae.* Multiple activities catalyze the cleavage of the three carboxy-terminal amino acids from farnesylated substrates *in vitro. J. Biol. Chem.* **267,** 10547–10464.
17. Park, S., Howald, W., and Cashman, J. (1994) *S*-oxidative cleavage of farnesylcysteine and farnesylcysteine methylester by the flavin-containing monooxygenase. *Chem. Res. Toxicol.* **7** 191–198.

18. Philips, M., Pillinger, M., Staud, S., Volker, C., Rosenfeld, M., Weissmann, G., and Stock, J. (1993) Carboxymethylation of RAS-related proteins during signal transduction in neutrophils. *Science* **259,** 977–980.
19. Huzoor-Akbar, F., Winegar, D., and Lapetina, E. (1991) Carboxyl methylation of platelet rap-1 proteins is stimulated by guanosine 5′-(3-*O*-thio)triphosphate. *J. Biol. Chem.* **266,** 4387–4391.
20. Backlund, P., Jr. (1992) GTP-stimulated carboxyl methylation of a soluble form of the GTP-binding protein G25K in brain. *J. Biol. Chem.* **267,** 18432–18439.
21. Yamane, H., Farnsworth, C., Xie, H., Evans, T., Howald, W., Gelb, M., Glomset, J., Clarke, S., and Fung, B. (1991) Membrane-binding domain of the small G protein G25K contains an *S*-(all-transgeranylgeranylcysteine methylester at its carboxyl terminus. *Proc. Natl. Acad. Sci USA* **88,** 286–290.
22. Backlund, P., Jr. (1993) Carboxyl methylation of the lower weight GTP-binding protein G25K: Regulation of carboxyl methylation by rho GDI. *Biochem. Biophys. Res. Commun.* **196,** 534–542.
23. Hrycyna, C., Yang, S. M., and Clarke, S. (1994) Protein carboxyl methylation in *Saccharomyces cerevisiae:* Evidence for STE14-dependent and STE14-independent pathways. *Biochemistry* **33,** 9806–9812.
24. Fukada, Y., Matsuda, T., Kokame, K., Takao, T., Shimonishi, Y., Akino, T., and Yoshizawa, T. (1994) Effect of carboxyl methylation of photoreceptor G protein gamma-subunit in visual transduction. *J. Biol. Chem.* **269,** 5163–5170.