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Identification, purification and characterization of a membrane-associated *N*-myristoyltransferase inhibitor protein from bovine brain

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N-Myristoyl-CoA: protein *N*-myristoyltransferase (NMT) is the enzyme that catalyses the covalent transfer of myristic acid from myristoyl-CoA to the N-terminal glycine residue of a protein substrate. Subcellular fractionation of bovine brain indicates that NMT activity was located in both the cytosolic and the particulate fraction of the cell. Removal of the particulate fraction resulted in a 2-fold enhancement of NMT activity. Reconstitution of the particulate fraction and cytosolic fraction resulted in inhibition of the elevated cytosolic NMT activity. These results indicated the existence of putative inhibitor(s) activity of NMT located in the particulate fraction of bovine brain. The inhibitor was stable to heat and was identified as a protein, on the basis of its susceptibility to the proteases trypsin and chymotrypsin. Protease degradation first required the delipidation of the particulate fraction. The inhibitor was purified to near-homogeneity by heat treatment, solvent extraction and Sephacryl S-300 gel-filtration column chromatography. The inhibitor was purified 630-fold from the particulate fraction with a 20% yield. The protein inhibitor had an apparent molecular mass of 92 kDa by gel filtration and 71 kDa by SDS/PAGE, indicating the protein is monomeric. The inhibitor did not interact directly with myristoyl-CoA and possessed no protease, thioesterase or demyristoylase activity. Purified inhibitor protein inhibited the formation of 1167 pmol of myristoyl-peptide/min per mg of protein.

INTRODUCTION

N-terminal fatty acid modification of cellular, viral and oncoproteins by myristate is catalysed by the enzyme(s) *N*-myristoyltransferase (NMT; EC 2.3.1.97). The mechanism and function of myristoylation are at present poorly understood. However, many of the known myristoylated proteins appear to be important in cellular regulation, signal transduction and virion assembly, such as cyclic-AMP-dependent protein kinase (Carr et al., 1982), pp60^{src} (Buss et al., 1984), the Ca^{2+}- and calmodulin-dependent protein phosphatase (calcineurin) (Aitken et al., 1982) and the viral gag protein precursors (Bryant and Ratner, 1990; Weaver and Panaganiban, 1990). The importance of the myristate moiety can be demonstrated in mutant proteins without the myristate modification, i.e. non-myristoylated pp60$^{v\text{-}src}$ possesses full tyrosine kinase activity but is no longer transforming (Kamps et al., 1985). NMT appears to be ubiquitous in eukaryotes, having been observed in yeast (Towler and Glaser, 1986), plants (Heuckeroth et al., 1988; Deichaite et al., 1988), invertebrate (Deichaite et al., 1988) and vertebrate tissues (Glover et al., 1988; Towler et al., 1988; Paige et al., 1989; McIlhinney and McGlone, 1990a). Characterization of NMT activity *in vivo* has indicated that myristoylation is a co-translational event, since protein-synthesis inhibitors such as cycloheximide inhibited myristoylation as well as protein synthesis (Olson and Spizz, 1986). As such, it has been thought of as a once-only event, no cycles of acylation/deacylation/reacylation being observed, with the myristoyl-glycine bond having a half-life identical with that of the polypeptide chain. More recently, however, several reports have indicated that myristoylation may also occur post-translationally (Pillia and Baltimore, 1987; Vijayarathy et al., 1989; Iozzo and Hacobian, 1990; McIlhinney and McGlone, 1990b; da Silva and Klein, 1990). In these studies myristate was demonstrated to be covalently attached via an amide bond, although in two of these studies the actual myristoylated residues were not identified (Pillia and Baltimore, 1987; Vijayarathy et al., 1989). However, the myristoylated residue of the 68 kDa protein from *Dictyostelium discoideum* (da Silva and Klein, 1990) was demonstrated to be the N-terminal glycine. Additionally, although the 68 kDa protein was stable, the half-life of the myristate moiety of the 68 kDa protein was only 15 min. At present, little is understood about the control of NMT activity.

Several groups have observed inhibitor activities towards NMT in a variety of cell homogenates. Although largely uncharacterized, most have been speculated to be due to cytosolic protease activities (Towler et al., 1987, 1988; McIlhinney and McGlone, 1989). McIlhinney and McGlone (1990a), however, have observed a heat-labile competitive inhibitor activity in rat brain membranes. The present paper documents the identification, purification and characterization of a heat-stable inhibitor protein of NMT activity from the particulate fraction of bovine brain. This inhibitor protein could serve as a regulator of bovine brain NMT activity.

EXPERIMENTAL

[1-^{14}C]Myristoyl-CoA (54.7 mCi/mmol) was purchased from Amersham International. All other chemicals and biochemicals were synthesized or obtained from sources described by King and Sharma (1991).

NMT purification

All procedures were performed at 4 °C, unless otherwise stated. NMT was partially purified from bovine brain. Fresh bovine brains were obtained from the local slaughterhouse and transferred to the laboratory in packed ice. Brains were homogenized in 2 vol. of buffer A (40 mM Tris/HCl, pH 7.4, containing 1 mM EDTA, 1 mM EGTA, 10 mM 2-mercaptoethanol, 1 mM benzamidine, 20 μg/ml soybean trypsin inhibitor, 0.2 mM phenylmethanesulphonyl fluoride) with a Waring blender (2 × 15 s). The homogenate was adjusted to pH 7.4 and then

Abbreviation used: NMT, myristoyl-CoA: protein *N*-myristoyltransferase.

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centrifuged at 8000 *g* for 25 min. The resulting supernatant was then centrifuged at 100000 *g* for 1 h. The supernatant obtained represented the soluble (cytosolic) fraction of the cell. The pellet obtained was resuspended in 3 vol. of buffer A. This represented the particulate (membranous) fraction of the cell.

The cytosolic fraction was applied to a DEAE-Sepharose CL-6B column (4 cm × 20 cm) equilibrated with buffer A. After application, the column was washed with 2 column vol. of buffer A, followed by stepwise elution of NMT activity with buffer A containing 400 mM NaCl. NMT activity was dialysed overnight against buffer A and applied to a phosphocellulose column (4 cm × 20 cm), equilibrated in buffer A. The column was washed with 3 column vol. of buffer A, and the NMT was eluted with a 2 litre linear salt gradient (0–1 M NaCl) in buffer B [40 mM Tris/HCl, pH 7.4, containing 0.1 mM EGTA, 10 mM 2-mercaptoethanol and 10% (w/v) sucrose]. NMT activity was eluted between 300 and 600 mM NaCl in a broad peak. Peak NMT activity was pooled and applied to a hydroxyapatite column (1.4 cm × 8 cm), equilibrated in buffer C (25 mM potassium phosphate buffer, pH 7.4, containing 0.1 mM EGTA, 10 mM 2-mercaptoethanol and 10% sucrose). The column was washed with 2 column vol. of buffer C, and the NMT was eluted with a linear phosphate gradient (25–500 mM potassium phosphate), containing 0.1 mM EGTA, 10 mM 2-mercaptoethanol and 10% sucrose. Peak NMT activity was pooled, concentrated by ultrafiltration through an Amicon PM-10 membrane, and dialysed overnight against buffer C. Partially purified NMT had a specific activity of 220 units/mg of protein. One unit of NMT activity corresponded to 1 pmol of myristoyl-peptide formed/min under our standard assay conditions. NMT was stored in buffer C at 1 mg/ml protein concentration at −20 °C. Protein concentration was determined by the dye-binding assay (Bradford, 1976) with BSA as protein standard.

NMT inhibitor assay

These were performed essentially as previously described (King and Sharma, 1991), except where detailed below. The inhibitor protein was assayed by its inhibitory activity against NMT using a peptide derived from cyclic-AMP-dependent protein kinase (Gly-Asn-Ala-Ala-Ala-Ala-Lys-Lys-Arg-Arg) as substrate. This procedure involved the comparison of NMT activity in the presence and absence of the inhibitor protein. Generally, 0.36–1.0 unit/ml NMT was incubated in the presence or absence of inhibitor protein (as indicated) and peptide substrate (463 μM) at 30 °C for 10–30 min in a final volume of 25 μl. Reactions were initiated by addition of [^{3}H]myristoyl-CoA (0.27 μM) or [^{14}C]myristoyl-CoA (1 μM), as indicated. All other conditions were as previously described (King and Sharma, 1991). One unit of inhibitor inhibits the formation of 1 pmol of myristoyl-peptide/min.

Purification of a membrane-associated inhibitor of NMT

The particulate fraction, obtained as described in the purification of NMT, was boiled for 5 min in a boiling-water bath and precipitated material was removed by centrifugation at 30000 *g* for 30 min. The unprecipitated particulate protein in a supernatant was delipidated by extraction with 40 vol. of chloroform/methanol (2:1, v/v). The protein precipitate was collected by filtration, dried well and resuspended in 5 vol. of buffer A. Boiling and delipidation resulted in the removal of more than 99% of the particulate protein. The delipidated protein was dialysed overnight against buffer B, freeze-dried, resuspended in buffer B and further purified by Sephacryl S300 gel-filtration column chromatography. The inhibitor was applied to a Sephacryl S300 column (1.6 cm × 100 cm) equilibrated and eluted in buffer B containing 100 mM NaCl and 10% sucrose. Peak inhibitor activity was pooled and dialysed overnight against buffer B. Protein concentration was determined as described above and stored at −20 °C.

Gel electrophoresis

Non-denaturing PAGE was performed at 4 °C in the absence of SDS, modified from Laemmli (1970). Proteins were resolved with Tris/glycine electrophoresis buffer (25 mM Tris/190 mM glycine) at 30 mA/gel. The gel lane was cut into 3 mm segments, crushed and incubated overnight in 200 μl of buffer B. Gel extracts were assayed for inhibitor activity as described above.

SDS/PAGE was performed with a 4%-acrylamide stacking and a 7.5%-acrylamide resolving gel in accordance with Laemmli (1970).

RESULTS AND DISCUSSION

Identification of heat-stable inhibitor protein of NMT

Subcellular fractionation of crude bovine brain homogenate into soluble (cytosolic) and particulate (membranous) fractions resulted in a 2-fold increase in NMT activity: 184.0±12.1% (mean±S.E.M., $n = 6$) activity was recovered in the soluble fraction and 10.6±2.9% (mean±S.E.M., $n = 6$) activity was recovered in the particulate fraction. Reconstitution of the particulate and soluble fractions resulted in inhibition of the elevated soluble NMT activity to 80–86% of the homogenate activity. These results indicate the existence of an inhibitor(s) in the particulate fraction. The calculation of the actual amount of inhibitor activity in the particulate fraction was difficult, due to the presence of NMT. However, the total inhibitor activity in bovine brain can be approximated on the basis of the total NMT activities observed in the whole cell homogenate and in the corresponding soluble and particulate fractions. Homogenates yielded 1110±220 units of NMT activity/kg bovine brain (mean±S.E.M., $n = 4$), whereas the total NMT activity of the soluble and particulate fractions was 2159 units/kg of bovine brain, with 2042±417 and 117.7±3.4 units/kg (means±S.E.M., $n = 4$) in the soluble and particulate fractions respectively. These results indicate at least 1049 units of NMT inhibited in the homogenate, or 1049 units of inhibitor activity/kg of bovine brain. The inhibitor was identified as a protein, on the basis of its inactivation by trypsin and chymotrypsin. DNAase had no effect on inhibitor activity (Table 1). Proteolytic inactivation required prior removal of the lipid from the protein fraction. Delipidation of the particulate fraction resulted in transfer of the inhibitor into the soluble aqueous phase.

Purification of the heat-stable inhibitor protein

The detailed purification procedure is described in the Experimental section. The inhibitor was purified 630-fold from the bovine brain particulate fraction, in a final yield of 20%. Table 2 summarizes the data for purification of the inhibitor protein from 0.66 kg of bovine brain. NMT was effectively removed from the particulate fraction by heat treatment. The inhibitor was stable to heat treatment: boiling the particulate fraction for 2–5 min resulted in the loss of all NMT activity, but had no effect on its ability to inhibit the soluble NMT activity. The recovery of inhibitor after heat treatment was estimated to be close to 50%.

The inhibitor protein was further purified by gel filtration on a Sephacryl S-300 column. Gel filtration, in the presence of low

Table 1 Effect of hydrolases on the activity of the heat-stable inhibitor

NMT inhibitor (6.57 μg) was preincubated at 30 °C for 30 min in the presence of buffer (control), trypsin (10 μg), chymotrypsin (10 μg) or DNAase (10 μg). Parallel preincubations were performed in the absence of NMT inhibitor. Preincubations were terminated by boiling for 2 min and by addition of soybean trypsin inhibitor (0.2 mg/ml). Inhibitor activity was assayed as described in the Experimental section. Control experiments were performed with NMT in the absence of inhibitor or in the presence of soybean trypsin inhibitor. No non-specific effects of trypsin or chymotrypsin were observed. Results are expressed as units per assay or (in parentheses) as percentage of activity in the absence of inhibitor (means of 3–5 experiments).

Addition	NMT activity
None	0.54 (100.0)
Soybean trypsin inhibitor	0.59 (110.0)
Heat-treated trypsin	0.55 (101.0)
Heat-treated chymotrypsin	0.52 (96.2)
Inhibitor	0.13 (24.7)
Trypsin-treated inhibitor	0.52 (96.9)
Chymotrypsin-treated inhibitor	0.50 (91.9)
DNAase-treated* inhibitor	0.07 (13.6)

* DNAase alone appeared to inhibit NMT activity.

Table 2 Purification of NMT inhibitor protein from bovine brain

Details of the purification procedure are described in the Experimental section. During purification, inhibitor activity was assayed by using enzymically synthesized [^{3}H]myristoyl-CoA. Under optimum conditions (2 μM myristoyl-CoA, 463 μM peptide) the inhibitor exhibited a specific activity of 1167 units/mg.

Purification step	Total activity (units)	Total protein (mg)	Specific activity (units/mg)	Purification (fold)	Yield (%)
Particulate	692.3	953	0.73	1	100*
Heat treatment	361.8	134	2.7	3.7	51
Organic extraction	111.2	2.16	51.5	71	16
Sephacryl S-300	129.0	0.281	459.2	629	19

* See the Results and discussion section for calculation of total activity.

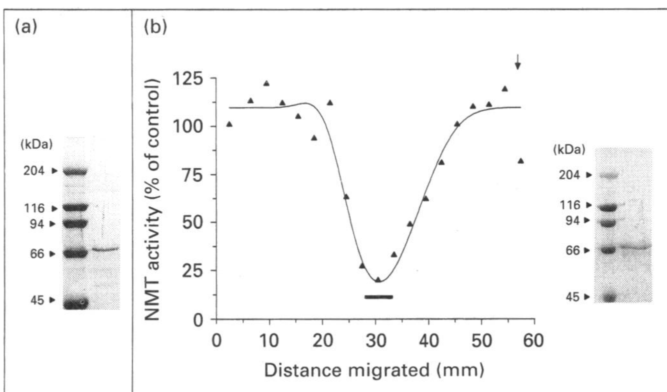

Figure 1 SDS/PAGE profile of bovine brain inhibitor protein

(**b**) Protein (1.5 μg) was loaded on to a SDS/polyacrylamide gel (7.5%) and the proteins resolved by electrophoresis at 30–35 mA. The gel was stained with Coomassie Brilliant Blue. Molecular-mass markers are indicated by arrows: myosin (204 kDa), β-galactosidase (116 kDa), phosphorylase *b* (94.7 kDa), BSA (67 kDa), ovalbumin (45 kDa). (**b**) Inhibitor protein (10.8 μg) resolved on a native 5–15% gradient gel was assayed as described in the text. The arrow indicates the position of the dye front. Insert: peak inhibitor activity was further analysed by SDS/PAGE (7.5% gel) as described in (**a**).

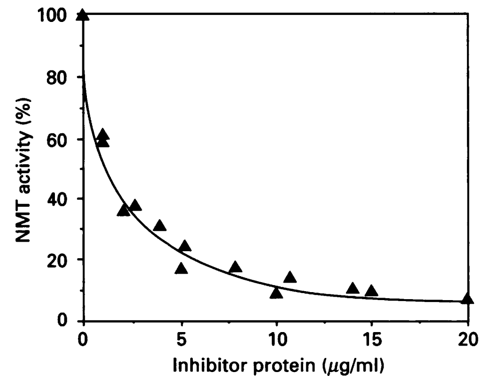

Figure 2 Dose-dependent inhibition of NMT activity by inhibitor protein

Bovine brain NMT (0.36 unit/ml) was incubated with delipidated inhibitor protein (0–20 μg/ml) in the presence of peptide derived from cyclic-AMP-dependent protein kinase (463 μM) at 30 °C for 30 min. The reactions were initiated by addition of 0.27 μM myristoyl-CoA. Results are expressed as percentages of control (NMT activity in the absence of inhibitor).

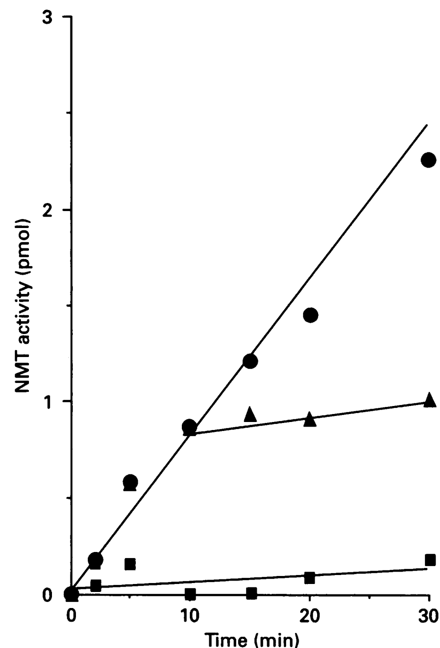

Figure 3 Time-dependent inhibition of NMT by inhibitor protein

Bovine brain NMT (0.36 unit/ml) was incubated with peptide derived from cyclic-AMP-dependent protein kinase (463 μM) in a final volume of 250 μl. The reactions were initiated by addition of 0.27 μM myristoyl-CoA. At times 0 min (■) or 10 min (▲), inhibitor protein (19.2 μg/ml) was added to the incubations; control incubations were performed by addition of buffer (●). Samples (15 μl) of the reaction mixture were assayed at the indicated times as described in the Experimental section. The results are expressed as pmol of myristoyl-peptide formed as a function of time (mean of two experiments).

(100 mM) or high (500 mM) NaCl concentration, indicated that the inhibitor had an apparent molecular mass of 92 kDa (mean of two determinations). SDS/PAGE of the peak activity from Sephacryl S-300 indicated a single major protein band of 71 ± 0.6 kDa (mean ± S.E.M. of two determinations) (Figure 1a). A similar result was also obtained from non-denaturing PAGE. Inhibitor protein (10.8 μg) was resolved on a 5–15% non-denaturing gradient gel at 4 °C as described in the Experimental section. The gel was cut into 3 mm segments, and each gel slice was crushed and incubated overnight in 200 μl of buffer B at

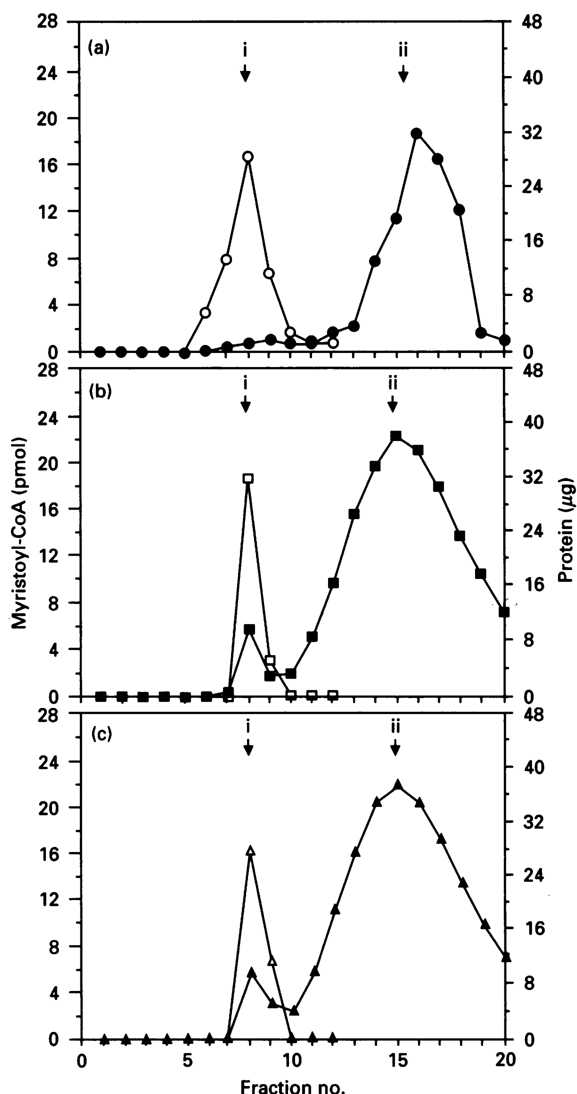

Figure 4 Inhibitor protein does not bind myristoyl-CoA

Myristoyl-CoA (3.29–4.5 μCi, 84–150 pmol) was incubated in (**a**) the presence of inhibitor protein (22.2 μg), (**b**) the presence of NMT (1.0 unit/ml) or (**c**) the presence of NMT (1.0 unit/ml) and inhibitor protein (6.22 μg). The incubation mixtures were applied to a Sephadex G-25 column (7 mm × 270 mm), equilibrated and eluted with buffer B, and 0.5 ml fractions were collected and assayed for radioactivity (●, ■, ▲) and protein (○, □, △). Arrows indicate the elution of (i) inhibitor protein or NMT alone, and (ii) myristoyl-CoA alone.

4 °C. The resulting solutions were assayed for inhibitor activity as described in the Experimental section. Peak inhibitor activity (indicated by the solid bar in Figure 1b) was pooled, freeze-dried and analysed by SDS/PAGE (7.5% gel). The inhibitor activity resolved into a single protein band of 71 kDa (Figure 1b). We conclude therefore that the inhibitor protein is a monomeric protein of 71 kDa. The inhibitor protein was estimated to be 75% pure, on the basis of densitometric scanning of the Coomassie-Blue-stained gel (Figure 1a).

Mode of action of inhibitor protein

The NMT activity was markedly inhibited by the inhibitor protein in a dose-dependent fashion (Figure 2). In a standard NMT assay reaction, increasing amounts of inhibitor protein resulted in increasing inhibition of NMT, with 1 mg of inhibitor protein inhibiting the formation of 1167 pmol of myristoyl-peptide/min. The contribution of inhibition due to lipid in the particulate fraction was investigated by using the total lipid extract. The particulate fraction was extracted as described in the Experimental section. The total lipid extract was dried under N_2, redissolved in methanol, and assayed for inhibition. Incubation of the lipid mixture under the standard NMT assay conditions resulted in half-maximal inhibition of the NMT activity at 700 μg of lipid/ml (M. J. King and R. K. Sharma, unpublished work), whereas under identical assay conditions half-maximal inhibition of NMT activity by the protein fraction was 1.4 μg of protein/ml (IC_{50} 19.7 nM; Figure 2). Therefore, in terms of concentration the inhibitor protein was 500-fold more potent than the total lipid extract in the inhibition of the NMT reaction. Inhibition of the NMT reaction was rapid, with no requirement for any preincubation; complete inhibition occurred within 5 min (Figure 3).

There are several possible mechanisms to explain NMT inhibition: (1) the sequestration of myristoyl-CoA, (2) the sequestration of the peptide substrate, (3) thioesterase activity, the breakdown of the myristoyl-CoA substrate, (4) proteolytic degradation, (5) demyristoylation of the myristoyl-peptide, or (6) direct interaction of the inhibitor with NMT.

To examine whether the inhibitor protein interacted with myristoyl-CoA, radiolabelled myristoyl-CoA was incubated with inhibitor protein in the presence or absence of NMT at 30 °C for 30 min, followed by Sephadex G-25 gel-filtration column chromatography (Figure 4). The concentration of inhibitor protein was 4 times the K_i with respect to the NMT activity. The elution profile of radiolabelled myristoyl-CoA incubated with inhibitor alone shows the inhibitor protein being eluted in the void volume, well separated from the radioactivity (Figure 4a). On the other hand, incubation of radiolabelled myristoyl-CoA with NMT alone resulted in some radioactivity being eluted in the void volume, associated with the protein peak (Figure 4b). Incubation of radiolabelled myristoyl-CoA with both NMT and excess inhibitor protein resulted in an elution profile identical with that of radiolabelled myristoyl-CoA and NMT alone, i.e. there was no increase or loss in radioactivity associated with the protein peak (Figure 4c). These results indicate that the inhibitor protein does not bind myristoyl-CoA, nor does it prevent myristoyl-CoA from binding to NMT. Sequestration of peptide is an unlikely mechanism, owing to the concentration of peptide compared with that of the inhibitor (high μM versus low nM).

Previously, NMT inhibitor activity observed in yeast and rat tissues has been attributed to thioesterase or protease activity

Table 3 Effect of inhibitor protein on myristoyl-CoA concentration

[^{3}H]Myristoyl-CoA was generated as previously described (King and Sharma, 1991). The myristoyl-CoA (40 μl) was incubated with inhibitor protein (10.8 μg) for 20 min at 30 °C and then extracted with 3 × 4 vol. of heptane, and the radioactivity in the aqueous and organic phases was determined by liquid-scintillation counting. Control experiments were performed with [^{3}H]myristic acid. The extractions were 98% efficient. Results are depicted as the proportion of radioactivity (c.p.m.) recovered in each phase (mean ± S.E.M. of two experiments).

		Proportion of radioactivity in phase	
Addition	Inhibitor	Aqueous	Organic
Myristoyl-CoA	−	0.82 ± 0.01	0.18 ± 0.01
	+	0.86 ± 0.0002	0.14 ± 0.0005
Myristic acid	−	0.02 ± 0.001	0.98 ± 0.001
	+	0.02 ± 0.001	0.98 ± 0.001

(Towler et al., 1987, 1988; McIlhinney and McGlone, 1989, 1990a). To test whether the inhibitor protein possesses thioesterase activity, [^{3}H]myristoyl-CoA was incubated in the presence or absence of inhibitor protein. The mixture was extracted with 3×4 vol. of heptane, and the radioactivity in the aqueous and organic phases was determined; 82–86 % of the radioactivity remained in the aqueous phase (Table 3). Control experiments with [^{3}H]myristic acid resulted in 98 % of the radioactivity being extracted into the organic heptane phase. These results demonstrate that the inhibitor protein does not possess any thioesterase activity.

To investigate if the inhibitor protein possessed any protease activity, two approaches were used. First, inhibition was rapid, requiring no preincubation (Figure 3); a 10 min preincubation of inhibitor protein with either the peptide substrate or NMT resulted in no increase in inhibitor activity. Secondly, inhibitor protein (19.2 μg/ml) added 10 min after the initiation of the transferase reaction resulted in full inhibition, identical with that observed with inhibitor added before the initiation of the transferase reaction (Figure 3). This experiment also argues against the inhibitor exerting its effect by a demyristoylation of the radiolabelled myristoyl-peptide. If the inhibitor protein possessed any demyristoylase activity, a time-dependent loss of radioactivity recovered in the assay would be observed in Figure 3. We conclude therefore that the inhibitor protein may act directly on NMT.

In conclusion, we have observed a potent protein inhibitor of NMT located in the particulate fraction of bovine brain. This inhibitor protein possesses no protease, thioesterase or demyristoylase activity. Inhibitor(s) of NMT activity have been observed by Towler et al. (1987, 1988) and McIlhinney and McGlone (1989, 1990a) in yeast and rat liver and brain. McIlhinney and McGlone (1990a) reported that an inhibitor activity located in rat brain membranes was heat-labile and thus possibly enzymic, exhibiting protease or CoA hydrolase/transferase activity. An alternative explanation proposed was that the observed inhibition was due to lipid vesicles sequestering or diluting the added label. Our results clearly demonstrate that the bovine brain NMT inhibitor is a protein and that the inhibitor protein exerts its activity via different mechanisms from those proposed by McIlhinney and McGlone (1990a). The inhibition observed with the protein fraction was 500-fold more potent than the lipid phase, suggesting that the protein inhibitor may be involved in the regulation of NMT activity. Although the apparent molecular mass appears large for a heat-stable protein (71 kDa by SDS/PAGE), Ingebritsen (1989) has described a heat-stable protein inhibitor of phosphotyrosyl-protein phosphatase activity from bovine brain with an apparent molecular mass of > 500 kDa. The physiological role of the NMT inhibitor protein is at present unknown. However, it may serve to regulate the myristoylation reaction.

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REFERENCES

Aitken, A., Cohen, P., Santikarn, S., Williams, D. H., Calder, A. G., Smith, A. and Klee, C. B. (1982) FEBS Lett. **150**, 314–318

Bradford, M. M. (1976) Anal. Biochem. **72**, 248–254

Bryant, M. and Ratner, L. (1990) Proc. Natl. Acad. Sci. U.S.A. **87**, 523–527

Buss, J. E., Kamps, M. P. and Sefton, B. M. (1984) Mol. Cell. Biol. **4**, 2697–2704

Carr, S. A., Biemann, K., Shozo, S., Parmelee, D. C. and Titani, K. (1982) Proc. Natl. Acad. Sci. U.S.A. **79**, 6128–6131

da Silva, A. M. and Klein, C. (1990) J. Cell Biol. **111**, 401–407

Deichaite, I., Casson, L. P., Ling, H. P. and Resh, M. D. (1988) Mol. Cell. Biol. **8**, 4295–4301

Glover, C. J., Goddard, C. and Felsted, R. L. (1988) Biochem. J. **250**, 485–491

Heuckeroth, R. O., Towler, D. W., Adams, S. P., Glaser, L. and Gordon, J. I. (1988) J. Biol. Chem. **263**, 2127–2133

Ingebritsen, T. S. (1989) J. Biol. Chem. **264**, 7754–7759

Iozzo, R. V. and Hacobian, N. (1990) Biochem. Biophys. Res. Commun. **172**, 905–912

Kamps, M. P., Buss, J. E. and Sefton, B. M. (1985) Proc. Natl. Acad. Sci. U.S.A. **82**, 4625–4628

King, M. J. and Sharma, R. K. (1991) Anal. Biochem. **199**, 149–153

Laemmli, U. K. (1970) Nature (London) **227**, 680–685

McIlhinney, R. A. J. and McGlone, K. (1989) Biochem. J. **263**, 387–391

McIlhinney, R. A. J. and McGlone, K. (1990a) J. Neurochem. **54**, 110–117

McIlhinney, R. A. J. and McGlone, K. (1990b) Biochem. J. **271**, 681–685

Olson, E. N. and Spizz, G. (1986) J. Biol. Chem. **261**, 2458–2466

Paige, L. A., Chafin, D. R., Cassady, J. M. and Geahlen, R. L. (1989) Anal. Biochem. **181**, 254–258

Pillia, S. and Baltimore, D. (1987) Proc. Natl. Acad. Sci. U.S.A. **84**, 7654–7658

Towler, D. W. and Glaser, L. (1986) Proc. Natl. Acad. Sci. U.S.A. **83**, 2812–2816

Towler, D. W., Eubanks, S. H., Towery, D. S., Adams, S. P. and Glaser, L. (1987) J. Biol. Chem. **262**, 1030–1036

Towler, D. W., Adams, S. P., Eubanks, S. H., Towery, D. S., Jackson-Machelski, E., Glaser, L. and Gordon, J. I. (1988) J. Biol. Chem. **263**, 1784–1790

Vijayarathy, C., Bhat, N. R. and Avadhani, N. G. (1989) J. Biol. Chem. **264**, 7772–7775

Weaver, T. A. and Panganiban, A. T. (1990) J. Virol. **64**, 3995–4001

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