Fatty acid acylation of proteins: specific roles for palmitic, myristic and caprylic acids

Vincent Rioux*

Laboratoire de Biochimie-Nutrition Humaine, Agrocampus Ouest, INRA USC 1378, 65 rue de Saint-Brieuc, CS 84251, 35042 Rennes Cedex, France

Received 28 November 2015 – Accepted 14 December 2015

Abstract – Fatty acid acylation of proteins corresponds to the co- or post-translational covalent linkage of an acyl-CoA, derived from a fatty acid, to an amino-acid residue of the substrate protein. The cellular fatty acids which are involved in protein acylation are mainly saturated fatty acids. Palmitoylation (S-acylation) corresponds to the reversible attachment of palmitic acid (C16:0) via a thioester bond to the side chain of a cysteine residue. N-terminal myristoylation refers to the covalent attachment of myristic acid (C14:0) by an amide bond to the N-terminal glycine of many eukaryotic and viral proteins. Octanoylation (O-acylation) typically concerns the formation of an ester bond between octanoic acid (caprylic acid, C8:0) and the side chain of a serine residue of the stomach peptide ghrelin. An increasing number of proteins (enzymes, hormones, receptors, oncogenes, tumor suppressors, proteins involved in signal transduction, eukaryotic and viral structural proteins) have been shown to undergo fatty acid acylation. The addition of the acyl moiety is required for the protein function and usually mediates protein subcellular localization, protein-protein interaction or protein-membrane interaction. Therefore, through the covalent modification of proteins, these saturated fatty acids exhibit emerging specific and important roles in modulating protein functions. This review provides an overview of the recent findings on the various classes of protein acylation leading to the biological ability of saturated fatty acids to regulate many pathways. Finally, the nutritional links between these elucidated biochemical mechanisms and the physiological roles of dietary saturated fatty acids are discussed.

Keywords: Acylated proteins / N-terminal myristoylation / octanoylation / palmitoylation / saturated fatty acids

Résumé – Protéines acylées pas des acides gras : rôles spécifiques des acides palmitique, myristique et caprylique. L’acylation d’une protéine correspond à la formation co- ou post-traductionnelle d’une liaison covalente entre un acide gras actif en acyl-CoA et un résidu d’acide amíné de la protéine substrat. Les acides gras utilisés par la cellule pour acylер des protéines sont très majoritairement saturés. On parle alors, respectivement, de palmitoylation (S-acylation) lorsqu’une liaison thioester se forme entre l’acide palmitique (C16:0) et la chaîne latérale d’une cystéine, de myristoylation N-terminale lorsqu’une liaison amide intervient entre l’acide myristique et la fonction amine d’une glycine N-terminale, et d’octanoylation (O-acylation) lorsqu’une liaison ester apparaît entre l’acide caprylique (ou octanoïque, C8:0) et la chaîne latérale d’une sérine. L’acylation concerne de très nombreuses protéines (enzymes, hormones, récepteurs, oncogènes, suppresseurs de tumeur, protéines impliquées dans la transduction des signaux, protéines de structure eucaryotes et même virales) et exerce donc une grande variété de fonctions dans les régulations cellulaires. La liaison covalente de l’acide gras à la protéine est cruciale pour l’acquisition de la fonction de la protéine, en changeant son hydrophobicité, en régulant l’ancrage de la protéine à la membrane, en modifiant son adressage subcellulaire ou encore en induisant des interactions entre sous-unités protéiques. La découverte progressive de nombreuses protéines acylées, dont la fonction est régulée par l’acylation, donne donc un nouvel intérêt fonctionnel à ces acides gras saturés. L’objectif de cette revue est de synthétiser les découvertes récentes sur les différentes classes d’acylation des protéines et sur les fonctions cellulaires émergentes que cette acylation procure à certains acides gras saturés. Le lien nutritionnel entre ces mécanismes moléculaires et les apports alimentaires en acides gras saturés est finalement discuté.

Mots clés : Acides gras saturés / myristoylation N-terminale / octanoylation / palmitoylation / protéines acylées

* Correspondence: vincent.rioux@agrocampus-ouest.fr

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.
1 Introduction

Fatty acid acylation of proteins (Towler et al., 1988) corresponds to the co- or post-translational covalent linkage of a fatty acid, activated in the form of acyl-CoA, to an amino-acid residue of the substrate protein (Fig. 1). The cellular fatty acids covalently bound to proteins are mainly saturated fatty acids (SFAs). Palmitoylation (S-acylation) corresponds to the reversible attachment of palmitic acid (C16:0) to the side chain of a cysteine residue via a thioester bond (Blaskovic et al., 2014). N-terminal myristoylation refers to the covalent attachment of myristic acid (C14:0) by an amide bond to the N-terminal glycine of many eukaryotic and viral proteins (Johnson, Bhatnagar, et al., 1994). Octanoylation (O-acylation) typically concerns the formation of an ester bond between octanoic acid (caprylic acid, C8:0) (Lemarié, Beauchamp, Legrand, et al., 2015) and the side chain of a serine residue of the stomach ghrelin peptide (Kojima et al., 1999). An increasing number of proteins (enzymes, hormones, receptors, oncogenes, tumor suppressors, proteins involved in signal transduction, eukaryotic and viral structural proteins) have been shown to undergo fatty acid acylation. The acyl moiety can mediate protein subcellular localization, protein-protein interaction or protein-membrane interaction. Therefore, through the covalent modification of proteins, these particular saturated fatty acids exhibit emerging specific and important roles in modulating protein functions (Ezanno et al., 2013).

2 Palmitic acid and protein palmitoylation

Protein S-acylation (Fig. 1) is also called palmitoylation because palmitic acid (C16:0) is the main SFA involved in this posttranslational thioester linkage with the side chain of cysteine residues (Mitchell et al., 2006), but other SFAs like myristic (Rioux et al., 2002) and lauric acids (Rioux et al., 2003) have also been found. Protein palmitoylation is catalyzed by a family of palmitoyltransferases sharing a DHHC motif and including 20 to 24 members in humans (Greaves and Chamberlain, 2011). Palmitoylation is involved in regulatory mechanisms because the association of the protein with the palmitoyl moiety is reversible and facilitates protein-membrane interactions and subcellular trafficking of proteins. Proteins that undergo this modification span almost all cellular functions. Several signal transductions depend for instance on palmitic acid, including proteins that have been shown to undergo successive myristoylation and palmitoylation, like the α subunit of many heterotrimeric G proteins (Chen and Manning, 2001).

3 Myristic acid and protein N-terminal myristoylation

Protein N-myristoylation (Fig. 1) specifically involves myristic acid (C14:0) (Beauchamp, Rioux, et al., 2009; Rioux and Legrand, 2001). Myristoyl-CoA: protein N-myristoyltransferase (NMT), the enzyme catalyzing this stable acylation, has been identified in many organisms. In mammals, two distinct NMT genes referred to as type 1 and 2 have been described (Giang and Cravatt, 1998; Rioux et al., 2006; Rundle et al., 2002). The contribution of each gene transcript to NMT expression and activity in vivo, and the specific role of each NMT isoform in cellular replication, proliferation, and other cellular processes, is however still not clearly described (Ducker et al., 2005; Selvakumar et al., 2006; Yang et al., 2005). Both isoforms seem to have a similar high substrate selectivity for myristic acid (Giang and Cravatt, 1998; Rioux et al., 2006). The myristoyl moiety has been shown to mediate protein subcellular localization, protein-protein interaction or protein-membrane interactions required for the biological activities of the myristoylated proteins (Johnson, Bhatnagar, et al., 1994). Initially described as a co-translational modification, N-myristoylation has more recently been shown to also occur as a post-translational mechanism in apoptotic cells (Martin et al., 2011), after proteolytic cleavage by caspases exposing a previously hidden N-terminal glycine residue.

---

**Fig. 1.** Classification of the saturated fatty acid protein acylation and examples of acylated proteins. Palmitoylation (S-acylation) corresponds to the reversible attachment of palmitic acid (C16:0) to the side chain of a cysteine residue via a thioester bond. N-terminal myristoylation refers to the covalent attachment of myristic acid (C14:0) by an amide bond to the N-terminal glycine of many eukaryotic and viral proteins. Octanoylation (O-acylation) typically concerns the formation of an ester bond between octanoic acid (caprylic acid, C8:0) and the side chain of a serine residue of the stomach ghrelin peptide.
The proteins that are myristoylated all possess an N-terminal glycine residue, but the subsequent so-called “myristoylation consensus sequence” is less well-defined (Fig. 2). Computational prediction suggested that about 0.5% of all proteins in the human genome could be myristoylated (Maurer-Stroh et al., 2004). Indeed, the N-myristoylated pro tease was recently studied in human cells, leading to the identification of more than 100 N-myristoylated proteins (Thion et al., 2014). The myristoylated proteins include key components in intracellular signaling pathways, oncogenes, structural viral proteins but also common constitutive eukaryotic proteins.

Among this last category, we noticed throughout the past years that several members of the mammalian family of membrane-bound desaturases possess a potential site of myris toylation. Both isoforms of dihydroceramide Δ4-desaturase (DES1 and DES2) indeed present a site of myristoylation in rats (Fig. 2), mice and humans (Beauchamp et al., 2007; Mizutani et al., 2004; Ternes et al., 2002). DES1 catalyzes the last step of de novo ceramide biosynthesis which consists in the introduction of a trans Δ4-double bond in the carbon chain of the dihydroceramide. DES2 possesses a bifunctional Δ4-desaturase/C4-hydroxylase activity (Omae et al., 2004). The presence of the trans Δ4-double bond seems to be critical for the acquisition of the biological activities of ceramide (Bielawska et al., 1993). Indeed, ceramide is able to induce apoptosis (Garcia-Ruiz et al., 1997; Gudz et al., 1997; Siskind et al., 2002), which is not the case of its precursor dihydroceramide. We showed that both DES1 and DES2 are myristoylated and that this N-terminal modification significantly increased the activity of the recombinant DES1 when expressed in COS-7 cells (Beauchamp et al., 2007). Compared to a recombinant unmyristoylatable mutant form of DES1 (N-terminal glycine replaced by an alanine), the desaturase activity of the myristoylatable wild-type DES1 was two times higher, in the presence of myristic acid incubated with the cells. The description of this regulatory mechanism highlighted a new potential relationship between myristic acid, the saturated fatty acid capable of binding and activating the enzyme involved in the final de novo ceramide biosynthesis step, and lipoproteinosis induced through the ceramide pathway. Indeed, we subsequently showed that the myristoylation of recombinant DES1 can target part of the enzyme to the mitochondria, leading to an increase in ceramide levels (specifically in the mitochondria) which in turn leads to apoptosis in the COS-7 cell model (Beauchamp, Tekpli, et al., 2009). Finally, myristic acid also increased native DES1 activity in cultured rat hepatocytes (Ezanno et al., 2012).

A second example of a membrane-bound desaturase which has been studied for its potential myristoylation is the Δ6-desaturase (Fatty Acid Desaturase 2: FADS2) involved in essential polyunsaturated fatty acid synthesis (Aki et al., 1999; Cho et al., 1999; D’Andrea et al., 2002). Several years ago, myristic acid was shown to trigger a specific and dose-dependent increasing effect on Δ6-desaturase activity in cultured rat hepatocytes (Jan et al., 2004) whatever the substrate used to measure this enzyme activity (oleic acid, linoleic or α-linolenic acid). Because the FADS2 enzyme exhibits an N-terminal glycine residue (Fig. 2), the increase in the activity of Δ6-desaturase by myristic acid was first postulated to be mediated by N-myristoylation. However, bioinformatic predictions indicated and biological experiments confirmed that FADS2 is not myristoylated (Beauchamp et al., 2007). Nevertheless, FADS2 is believed to cooperate with NADH-cytochrome b5 reductase (NCh5R) in the endoplasmic reticulum membrane (Beauchamp et al., 2004) and this last enzyme is also known (Fig. 2) to be N-terminally myristoylated (Borgese et al., 1996; Colombo et al., 2005; Ozols et al., 1984). The hypothesis according to which the myristoylation of NADH cytochrome b5 reductase could account for the increased Δ6-desaturase activity was therefore proposed (Riou et al., 2011). Although its linkage with myristic acid is not absolutely required for its association with endoplasmic reticulum membranes (Strittmatter et al., 1993), myristoylation of NCh5R may modify the transfer by lateral diffusion of electrons from NCh5R to the heme of cytochrome b5 and then to the terminal desaturase. It may also change the interaction between NCh5R and the desaturase. Moreover, it may modify the conformation of the whole complex, as the analysis of the relative contribution of the myristoyl moiety in membrane binding in a model of phospholipid vesicles suggests (Strittmatter et al., 1993). In such a hypothesis, not only the Δ6-desaturase but also all the membrane-bound desaturases which are associated with NCh5R would be affected by this regulatory mechanism. This regulation may explain the effect of dietary myristic acid on the overall conversion of α-linolenic acid to longer highly unsaturated fatty acids, like eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids, shown in rat nutritional experiments (Legrand et al., 2010; Rioux et al., 2005, 2008).

These two detailed examples show that through the myristoylation of the dihydroceramide Δ4-desaturase (DES) and of the NADH-cytochrome b5 reductase, myristic acid may therefore be considered as one of the regulators of cellular bioactive lipid concentration such as ceramide and polyunsaturated fatty acids.

4 Caprylic acid and ghrelin octanoylation

Fifteen years ago, caprylic acid (C8:0) was surprisingly found attach to the ghrelin (Fig. 1) purified from rat stomach (Kojima et al., 1999), but only recently the presence of the octanoyl moiety appeared crucial for this peptide hormone (Lemarié, Beauchamp, Le grand, et al., 2015). Ghrelin is a 28 amino acid peptide expressed in the digestive tract and mainly in the stomach. Its octanoylated form binds to the growth hormone secretagogue receptor (GHSR-1a) located in the pituitary gland and hypothalamus (Howard et al., 1996).
Octanoylated ghrelin is therefore suspected to regulate many relevant biological processes including the secretion of the growth hormone (GH), the modulation of appetite and food intake, the secretion of the hypothalamic neuropeptides involved in the regulation of appetite, the essentiality of energy intake and by inhibiting energy expenditure and fat catabolism (Tschöp et al., 2000). However, as demonstrated in recent studies showing that ghrelin-null mice did not exhibit altered food intake nor altered expression of hypothalamic neuropeptides involved in the regulation of appetite, the essentiality of endogenous ghrelin in the regulation of food intake appeared controversial (Albarran-Zeckler et al., 2011).

5 Impact of dietary SFAs on cellular protein acylation

Because of their potential dual origin (diet and endogenous synthesis), the impact of dietary SFAs on the regulation of the protein acylation processes is still questioned. More specifically, human data reporting the balance between intake and de novo synthesis of SFAs are not available. In addition, the optimal cellular concentration of saturated fatty acyl-CoAs required for each type of protein acylation is not known and data available suggest that the free cytosolic concentration of fatty acyl-CoA esters is in the low nanomolar range (Faergeman and Knudsen, 1997).

Palmitic acid is universally found in natural fats, representing 15–25% of total fatty acids. Therefore, due to its high dietary level (30–38 g/day in humans) (Katan et al., 1994) and well-described predominant synthesis by the Fatty Acid Synthase (Singh et al., 1984), palmitic acid intracellular concentration may not be considered as the rate-limiting molecular for the palmitoylation mechanism.

Concerning myristic acid, its endogenous biosynthesis (Rioux et al., 2007) appeared very low in cultured rat hepatocytes. If it is also the case in humans, the diet is therefore the main source (4–8 g/day) for this particular fatty acid (Wolk et al., 2001) which represents about 10% of SFAs in ruminant milk fat. Very low dietary intakes of myristic acid may likely lead to insufficient intracellular concentration of myristoyl-CoA to ensure the proper activity of N-myristoyltransferase (NMT), when considering the whole pool of myristoylatable proteins. One may therefore wonder about the risk of total eviption of dairy products as the unique source of dietary myristic acid. In yeast, studies analyzing the
activity of NMT have suggested that the enzyme was able to use both exogenous and endogenous myristic acid as substrate (Duronio et al., 1991, 1992; Johnson, Knoll, et al., 1994). The requirement for myristic acid suggests that in certain cases, it could be the rate-limiting molecule in this mechanism or that competition could occur. In addition, the mechanism by which myristic acid initially esterified in the TAG or PL is used for myristoylation is unknown, too.

Concerning finally caprylic acid, no endogenous biosynthesis of this fatty acid has been described in animals except in the lactating mammary gland (Fernando-Warnakulasuriya et al., 1981). On the other hand, natural food sources of caprylic acid are restricted to specific vegetable oils and milk products. Caprylic acid is abundant in coconut oil (6–10% of FAs, with C8:0 mainly in sn-1 and -3 positions on the triglycerides, TG) and in palm kernel oil (2–5% of FAs). Milk is the only natural source of animal caprylic acid with strong differences between mammalian species. C8:0 represents about 0.5% of FAs in human milk (Jensen, 1996), but is higher in cow milk (1–2%) (Jensen et al., 1990), in goat milk (3%) (Alonso et al., 1999), in rat milk (5–6%) (Fernando-Warnakulasuriya et al., 1981) and reaches up to 15–18% in rabbit milk (Perret, 1980). Caprylic acid is primarily esterified in sn-3 position of the TGs in cow (Jensen et al., 1990), rat (Staggers et al., 1981) and human (Jensen, 1996) milks. In western countries, dietary MCFAs represent less than 2% of total dietary energy and caprylic acid is only a minor part of these MCFAs in milk fat (1–2% of cow milk FAs).

Part of caprylic acid coming from dietary medium chain TG (MCTs) can be early released during digestion through the action of preduodenal lipase (Clark et al., 1969), leading to its potential and yet not clearly quantified direct absorption by the stomach mucosa (Lai and Ney, 1998; Perret, 1980). Dietary caprylic acid is therefore suspected to directly provide GOAT enzyme with octanoyl-CoA co-substrates (Fig. 3) necessary for the acyl modification of ghrelin. Indeed, ingestion by mice of either MCFAs or MCTs increased the stomach concentration of acylated ghrelin (Nishi, Hiejima, Hosoda, et al., 2005), without changing the total ghrelin amounts. Nishi et al. detected heptanoylghrelin (Nishi, Hiejima, Hosoda, et al., 2005) or decanoylghrelin (Nishi et al., 2013) in the stomachs of mice fed with triheptanoin or tricaprin, confirming that at least part of the ingested MCFAs was directly used for ghrelin acylation. These results are consistent with the hypothesis of gastric absorption of MCTs but part of the caprylic acid present in the stomach may also come from intestinal absorption followed by uncompleted uptake by the liver. In ruminants, the ingestion of MCFAs during 2 weeks by lactating dairy cows increased the plasma acylated ghrelin concentrations (Fukumori et al., 2013). In cachectic patients, a 2-week administration of acylated ghrelin, whereas acylated ghrelin remained stable, suggesting a specific decreased degradation of acylated ghrelin in obese (Takagi et al., 2013). In humans, it has also been shown that the GOAT enzyme was present in the blood, which could modify the balance between de-acylation and re-acylation (Goebel-Stengel et al., 2013). For all these reasons, the concentration of both the acylated and unacylated plasma ghrelin may not simply reflect the stomach concentration (Nishi, Hiejima, Mifune, et al., 2005b).

6 Conclusion

Focusing on fatty acid acylation of proteins, this review reports new knowledge on cellular and physiological functions of individual SFAs. This review particularly emphasizes that palmitic, myristic and caprylic acids, through their capacity to acylate different proteins, have important and specific roles for which they cannot be a substitute for each other and that cannot be assumed by other fatty acids. For this reason, like for other physiological and pathophysiological aspects (Legrand, 2013; Legrand and Rioux, 2015) not detailed in the present review, SFAs should no longer be considered as a single group in terms of structure, metabolism and functions.

Acknowledgements. The author acknowledges Pr. Philippe Legrand (Head of the laboratory of Biochemistry and Human Nutrition, Agrocampus Ouest-INRA USC 1378, Rennes, France), his former
References


Lemarié F, Beauchant E, Legrand P, Rioux V. 2015. Revisiting the metabolism and physiological functions of caprylic acid (C8:0) with special focus on ghrelin octanoylation. *Biochimie*


Cite this article as: Vincent Rioux. Fatty acid acylation of proteins: specific roles for palmitic, myristic and caprylic acids. OCL 2016, 23(3) D304.