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Cost-effective method for the preparation of uniformly labeled myristoylated proteins for NMR measurements

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ABSTRACT

Nuclear magnetic resonance (NMR) is a powerful technique for solving protein structures or studying their interactions. However, it requires molecules labeled with NMR sensitive isotopes like carbon ^{13}C and nitrogen ^{15}N. The recombinant expression of labeled proteins is simple to perform but requires quite expensive chemicals. When there is a need for special labeled chemicals, like uniformly ^{13}C-labeled myristic acid, the price significantly rises. Here we describe a cost-effective method for the recombinant expression of uniformly labeled myristoylated proteins in *Escherichia coli* demonstrated on the production of Mason-Pfizer monkey virus matrix protein. We used the ability of *E. coli* to naturally synthetize myristic acid. When grown in isotopically labeled medium the myristic acid will be labelled as well. Bacteria were co-transfected with plasmid carrying gene for yeast N-myristoyltransferase which ensures myristoylation of expressed protein. This process provided 1.8 mg of the myristoylated, doubly labeled ($^{13}C/^{15}N$)M-PMV matrix protein from 1 L of $^{15}N/^{13}C$ labeled M9 medium. The price represents approximately 50% cost reduction of conventional method using commercially available [U-^{13}C]myristic acid.

Introduction

The myristoylation, along with the palmitoylation, is one of the most common protein modifications by a fatty-acid residue [1]. It mostly occurs at the N-terminus. About 0.5–0.8% of eukaryotic proteins are myristoylated and this post-translational modification can also be found among viral and even bacterial proteins [2]. Most of them are GTP-binding proteins, protein kinases and Ca^{2+}-binding proteins [3]. The myristoylation has several functions in the modified proteins. The most common one is reversible binding to biological membranes where the myristoyl acts as a hydrophobic anchor. When a myristoylated protein is in an unbound state, the myristoyl is sequestered inside the hydrophobic cavity of the protein. However, before the protein binds to the membrane, the myristoyl is released from the pocket to facilitate the interaction with the membrane. This mechanism is called a myristoyl switch [4]. The myristoylation is necessary for correct functioning of many important proteins, e.g., recoverin (a Ca^{2+}-binding rhodopsin kinase inhibitor from eye cells) [5], Abelson kinase (a tyrosine protein kinase regulating the cell division cycle) [6], or retroviral matrix proteins [1].

Matrix protein (MA)[1] is the N-terminal domain of the retroviral Gag polyprotein.It is important for transport of Gag or immature virus-like particles (VLPs) to the plasma membrane and it is also responsible for the interaction with the membrane and other cellular components [7]. Matrix proteins of most retroviruses are myristoylated, like human immunodeficiency virus (HIV), human T-lymphotropic virus (HTLV) and Mason-Pfizer monkey virus (M-PMV) MAs [8]. Only few retroviral MAs are not myristoylated, e.g., Rous sarcoma virus (RSV) or equine infectious anemia virus (EIAV) [9].

M-PMV belongs to the D-type retroviruses which assemble VLPs in the cytoplasm of the infected cells. The VLPs are then transported to the plasma membrane, bind to it and leave the host cell through a process called budding [10]. The different location and time of the VLPs formation and budding makes M-PMV a good model system which enables to study these processes separately. Previously, we described a procedure for the preparation of myristoylated M-PMV MA [11]. For the preparation of samples for NMR experiments, bacteria are grown in minimal media with isotopically labeled sources of nitrogen ^{15}N and carbon ^{13}C. The drawback of this procedure is that for some experiments, the externally added sodium myristate must be also isotopically labeled to yield

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[1] *Abbreviations used:* MA, matrix protein; M-PMV, Mason-Pfizer monkey virus, VLP, virus-like particle; Ni-NTA, nickel-nitrilotriacetic acid; HSQC, Heteronuclear Single Quantum Coherence.

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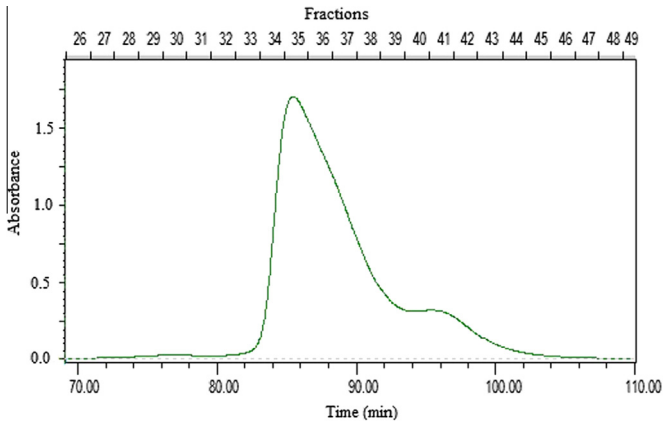

Fig. 1. Chromatogram of the myristoylated and non-myristoylated MA. The fractions 39–43 were pooled for further purification.

the protein completely enriched by carbon ^{13}C. The labeled sodium myristate is commercially available, but quite expensive. We tried to overcome the need of buying labeled sodium myristate by using the natural ability of *Escherichia coli* to synthetize the myristic acid which constitutes approximately 5% of the *E. coli* fatty acids at 37 °C [12].Therefore, the expression of proteins in *E. coli* grown in the isotopically labeled minimal media, guarantees their uniform labeling including the myristic acid.

Material and methods

Bacteria and media

E. coli BL21 (DE3) strain was used for the production of the recombinant protein. The inoculum prepared in the Luria–Bertani medium (LB, Sigma–Aldrich) contained ampicillin (100 mg/l) and kanamycin (50 mg/l). M9 medium with [U-^{15}N]NH_4Cl,D-[U-^{13}C]glucose and the same amount of the antibiotics as in the LB medium was used [13]. ISOGRO-$^{13}C/^{15}N$ Powder-Growth Medium supplement (Sigma–Aldrich) was added to the production M9 medium to ensure bacterial growth conditions as in LB medium.

Expression of protein

The inoculum was prepared in 50 ml of the LB medium induced from the glycerol stock stored at −140 °C using the two-plasmid expression system according to Prchal et al. [11]. After 8 h of cultivation at 37 °C in 250 ml shaked flask,cells were pelleted at 8000*x* RCF for 10 min and re-suspended in $^{15}N/^{13}C$ labeled M9 medium and cultivated additional 12 h at 37 °C. Then, 1 L of the production M9 medium was inoculated to OD_{590} 0.1. At OD_{590} 0.5 isopropyl-β-D-thiogalactopyranoside (IPTG) was added to the final concentration of 0.4 mM and the cultivation continued for another 4 h at 37 °C. Then the bacteria were pelleted by centrifugation at 8,000x RCF for 10 min. The supernatant was discarded and the pel-

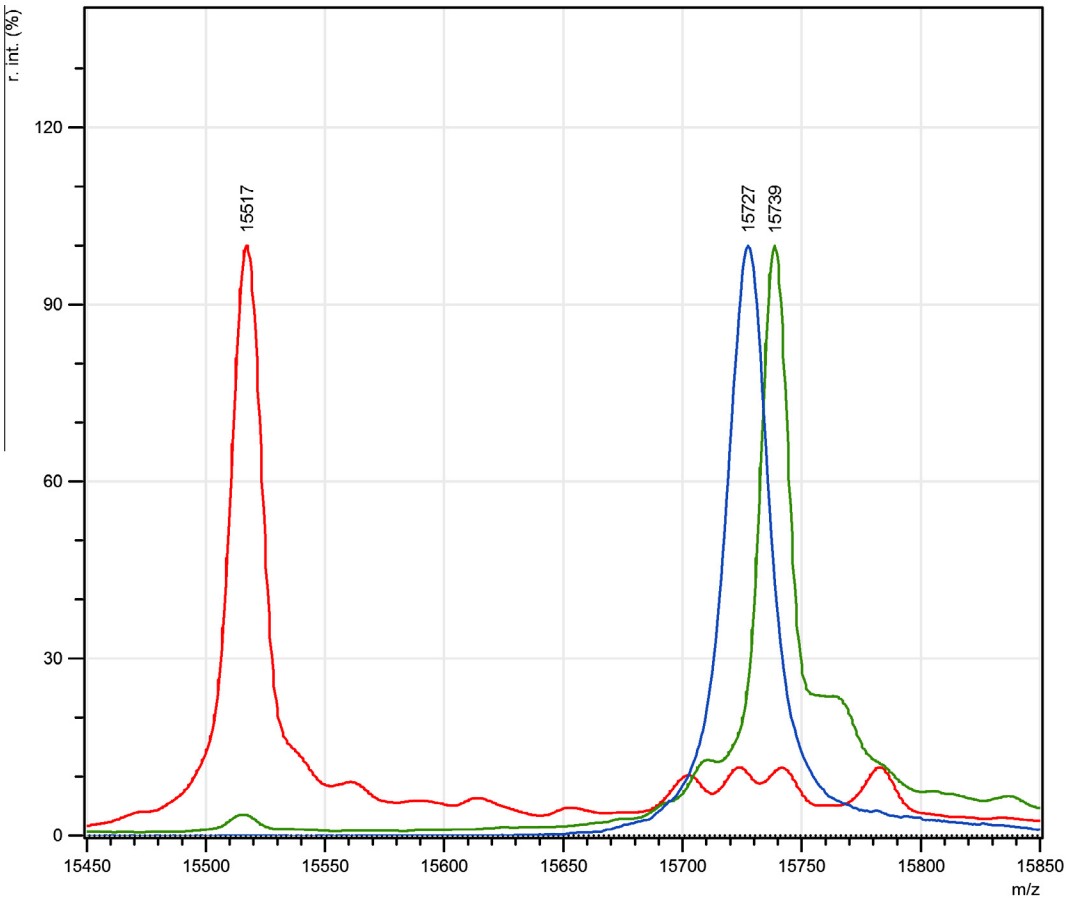

Fig. 2. MALDI-TOF/TOF spectrum of the purified uniformly $^{15}N/^{13}C$ labeled myristoylated MAwith labeled myristoyl (green, 15,739 *m/z*),$^{15}N/^{13}C$ labeled non-myristoylated MA (red, 15,517 *m/z*) and $^{15}N/^{13}C$ labeled myristoylated MA with non-labeled myristoyl (blue, 15,727 *m/z*).Difference between mass of the myristoylated proteins is 12 *m/z* which correspond to the mass change of ^{13}C uniformly labeled myristoyl (100% ^{13}C labeled myristoyl represents 224 Da, non-labeled represents 210 Da). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)