Analytica Chimica Acta 981 (2017) 1–10

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Determination of formylated DNA and RNA by chemical labeling combined with mass spectrometry analysis

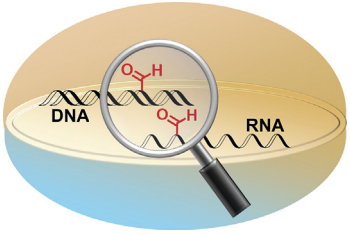
Han-Peng Jiang, Ting Liu, Ning Guo, Lei Yu, Bi-Feng Yuan*, Yu-Qi Feng

Key Laboratory of Analytical Chemistry for Biology and Medicine (Ministry of Education), Department of Chemistry, Wuhan University, Wuhan 430072, China

HIGHLIGHTS

- Chemical labeling-MS analysis was developed for study of nucleic acid formylation.
- The detection sensitivities of formylated nucleosides increased by 307–884 folds.
- Six formylated nucleosides from both DNA and RNA were detected.
- Three formylated nucleosides were firstly discovered in human cells and tissues.
- Significant increase of two formylated nucleosides in thyroid carcinoma tissues.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 24 January 2017
Received in revised form
25 May 2017
Accepted 1 June 2017
Available online 19 June 2017

Keywords:
DNA and RNA formylation
Chemical labeling
In-tube solid-phase microextraction
Ultra high performance liquid chromatography - tandem mass spectrometry analysis
Cancer

ABSTRACT

Nucleic acids carry diverse chemical modifications that exert critical influences in a variety of cellular processes in living organisms. In addition to methylation, the emerging DNA and RNA formylation has been reported to play functional roles in various physiological processes. However, the amounts of formylated DNA and RNA are extremely low and detection of DNA and RNA formylation is therefore a challenging task. To address this issue, we developed a strategy by chemical labeling combined with in-tube solid-phase microextraction - ultra high performance liquid chromatography - electrospray ionization - tandem mass spectrometry (in-tube SPME-UPLC-ESI-MS/MS) analysis for the sensitive determination of DNA and RNA formylation. Using the developed method, we were able to simultaneously measure six formylated nucleosides, including 5-formyl-2′-deoxycytidine (5-fodC), 5-formylcytidine (5-forC), 5-formyl-2′-deoxyuridine (5-fodU), 5-formyluridine (5-forU), 2′-*O*-methyl-5-formylcytidine (5-forCm) and 2′-*O*-methyl-5- formyluridine (5-forUm), from DNA and RNA of cultured human cells and multiple mammalian tissues. The detection limits of these formylated nucleosides improved by 307–884 folds using Girard's P (GirP) labeling coupled with in-tube SPME-UPLC-ESI-MS/MS analysis. It was worth noting that 5-forU, 5-forCm and 5-forUm which have not been detected in human sample before, were discovered in cultured human cells and tissues in the current study. In addition, we observed significant increase of 5-forC and 5-forU in RNA ($p = 0.027$ for 5-forC; $p = 0.028$ for 5-forU) and 5-fodU in DNA ($p = 0.002$) in human thyroid carcinoma tissues compared to normal tissues adjacent to the tumor using synthesized stable isotope GirP (d_5-GirP)-assisted quantification. Our results indicated that aberrant DNA and RNA formylation may contribute to the tumor formation and development. In addition, monitoring

* Corresponding author.
E-mail address: bfyuan@whu.edu.cn (B.-F. Yuan).

http://dx.doi.org/10.1016/j.aca.2017.06.009

of DNA and RNA formylation may also serve as indicator for cancer diagnostics. Taken together, the developed chemical labeling combined with in-tube SPME-UPLC-ESI-MS/MS analysis can facilitate the in-depth functional study of DNA and RNA formylation.

1. Introduction

Nucleic acids contain diverse chemical modifications that exert critical influences in a variety of cellular processes in living organisms [1]. DNA cytosine methylation (5-methylcytidine, 5-mdC) has long been known to be an important epigenetic mark that plays important regulatory roles in biological systems [2–4]. In addition to DNA, reversible RNA modification has recently been proposed to represent another realm for biological regulation [5]. For example, N^6-methyladenosine (m^6A) on RNA molecules was discovered with potential functions on the control and regulation of gene transcription and protein translation [6,7].

In addition to DNA methylation, DNA formylation has been recently discovered [8]. 5-Formyl-2′-deoxycytidine (5-fodC) has been identified as an intermediate in the Ten-Eleven Translocation (Tet) proteins mediated DNA active demethylation [8]. Bachman et al. later further found 5-fodC was a stable modification in DNA and can alter the structure of DNA double helix [9,10]. Moreover, 5-fodC exhibited distinct distribution pattern from those of 5-mdC and has been identified as having more protein binders than 5-mdC [11,12]. Therefore, it is possible for 5-fodC in DNA exerts functional roles that go beyond being a DNA demethylation intermediate. Besides DNA, 5-formylcytosine (5-forC) was also found existence in mitochondrial transfer RNA (tRNA) for methionine ($mt\text{-}tRNA^{Met}$) in mammals [13]. The 5-forC modification cloud effect protein synthesis and a lack of 5-forC at $mt\text{-}tRNA^{Met}$ has pathological consequences [13]. Therefore, DNA/RNA formylation is considered as an additional epigenetic modification in addition to DNA/RNA methylation.

The amount of 5-fodC and 5-forC in DNA and RNA are extremely low and usually occur at a frequency of several modifications per million nucleosides [8,14,15]. Due to the importance of 5-fodC in DNA and 5-forC in RNA, the in-depth understanding of the functional roles of 5-fodC and 5-forC requires appropriate and sensitive analytical strategy. So far, some methods were developed to determine nucleoside modifications, such as sequencing-based techniques [16–19], thin-layer chromatography detection [20], immunohistochemistry [21], capillary electrophoresis (CE) with UV [22] and laser-induced fluorescence (LIF) [23] detection, high-performance liquid chromatography (HPLC) with UV [24], fluorescence detector (FLD) [25] and mass spectrometry (MS) detection [26]. However, these developed methods either lack enough detection sensitivity for low-abundant nucleoside modifications, or cannot offer quantitative determination of the modifications. Hence, the determination of DNA and RNA formylation is still a challenging task. Moreover, whether other types of endogenous DNA and RNA formylation exist in mammals besides 5-fodC and 5-forC is seldom explored due to the lack of sensitive analytical methods.

Owing to the inherent sensitivity and qualitative capability, liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS) has been widely used in the analysis of trace level compounds [27]. However, analysis of 5-fodC and 5-forC by LC-ESI-MS is challenging since the abundance of 5-fodC in DNA and 5-forC in RNA is extremely low and the ionization efficiency of 5-fodC and 5-forC in electrospray ionization (ESI) is also usually low. In this respect, an easily ionizable moiety can be introduced to target analytes to enhance the ionization efficiency for the sensitive detection by LC-ESI-MS [28]. In our previous studies, we used 2-bromo-1-(4-dimethylamino-phenyl)-ethanone and 2-bromo-1-(4-diethylaminophenyl)-ethanone to label the cytosine modifications and this strategy can efficiently increase the ionization efficiency of nucleosides [29–31]. Since the formylated DNA and RNA carry the aldehyde group that can be easily derivatized, we can label the formylated DNA and RNA to bring in an easily ionizable group to increase the detection sensitivity during mass spectrometry analysis.

In the current study, three labeling reagents including Girard's P (GirP) reagent, Girard's T (GirT) reagent and 4-(2-(trimethylammonio)ethoxy)benzenaminium halide (4-APC) (Fig. 1A) that

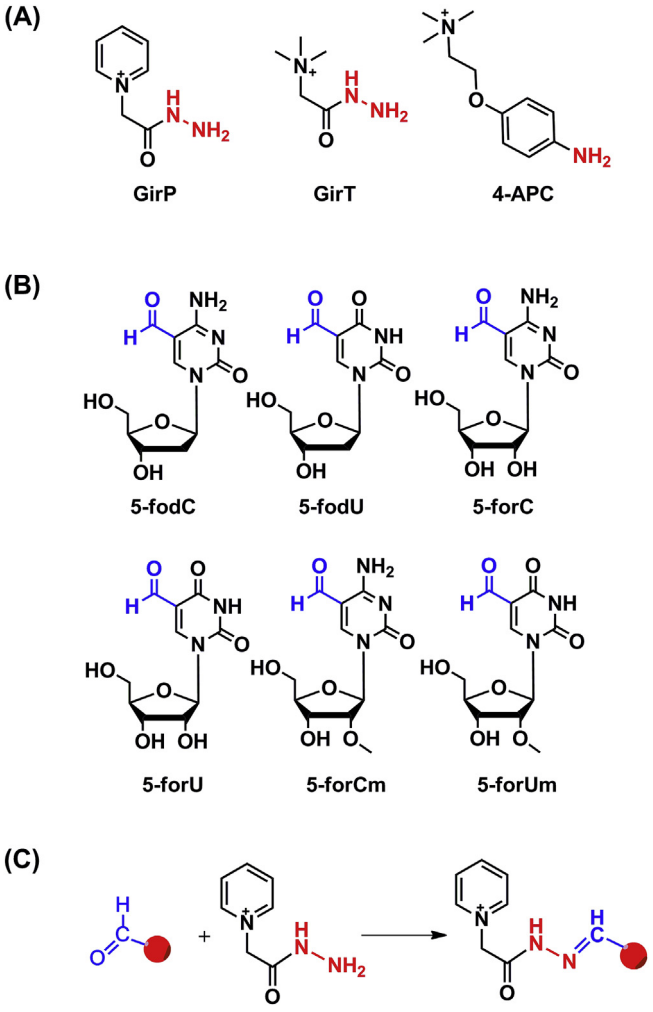


Fig. 1. (A) Chemical structures of the three labeling reagents. (B) Chemical structures of six formylated nucleosides. (C) Labeling reaction between formylated nucleosides and GirP reagent.