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A novel one-pot fluorescence tagging and depyrimidination strategy for quantification of global DNA methylation

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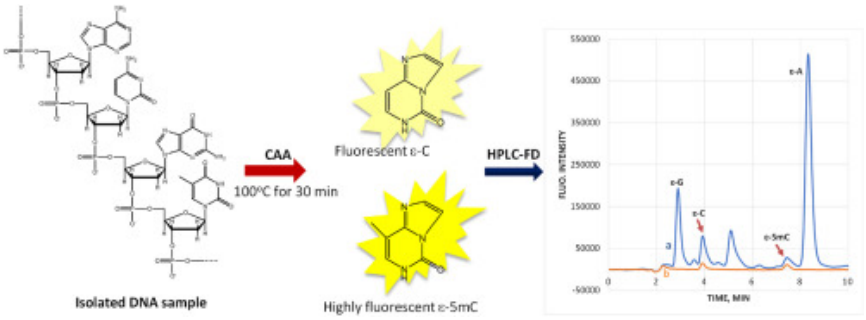
Abstract

DNA methylation is intensively studied in medical science. Current HPLC methods for quantification of global DNA methylation involve digestion of a DNA sample and HPLC determination of both cytosine (C) and 5-methylcytosine (5mC) so that percentage of 5mC in total cytosine can be calculated as DNA methylation level. Herein we report a novel HPLC method based on a one-pot fluorescence tagging and depyrimidination reaction between DNA and chloroacetaldehyde (CAA) for highly sensitive quantification of global DNA methylation. In the one-pot reaction, C and 5mC residues in a DNA sequence react with CAA, forming fluorescent etheno-adducts that are then released from the sequence through depyrimidination. Interestingly, etheno-5mC (ε-5mC) is ~20 times more fluorescent than ε-C and other ε-nucleobases resulting from the reaction, which greatly facilitates the quantification. Further, due to the tagging-induced increase in structural aromaticity, ε-nucleobases are far more separable by HPLC than intact nucleobases. The proposed HPLC method with fluorescence detection (HPLC-FD) is quick (i.e., < 1h per assay) and highly sensitive with a detection limit of 0.80nM (or 250fg on column) for 5mC. Using the method, DNA samples isolated from yeast, HCT-116cells, and tissues were analyzed. Global DNA methylation was measured to be in the range from 0.35% to 2.23% in the samples analyzed. This sensitive method allowed accurate analyses of minute DNA samples (~100ng) isolated from milligrams of tissues.

Graphical abstract



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Introduction

DNA methylation is an epigenetic modification where a cytosine (C) residue is enzymatically converted to 5-methyl-cytosine (5mC). It's well known that aberrant DNA methylation is a common feature of several conditions, including aging and cancer [[1], [2], [3], [4], [5]]. Methods for analysis of DNA methylation based on various techniques, including bisulfite qPCR and HPLC have been reported [[6], [7], [8], [9]]. Methyl-cytosine quantification by HPLC is considered the "gold standard" for assessing global DNA methylation. In HPLC-based methods [[10], [11], [12], [13], [14], [15], [16]], isolated DNA samples are first digested to nucleobases, deoxynucleosides, or deoxynucleotides by means of enzymatic or chemical treatments. A digest is then separated by HPLC with UV detection (or other detection schemes) to quantify 5mC and C simultaneously. Enzymatic digestion of a DNA sample produces deoxynucleotides if, for example, DNA degradase is used [11] or deoxynucleosides if, for example, a combination of 5′-exonuclease and 3′-nucleotidase is used [10]. An enzymatic digestion is expensive and normally takes more than 3 h to complete. To separate a DNA digest, various HPLC columns, including C_8, C_{18}, CN, diol, phenyl, and ion-exchange were tested. In most of the HPLC methods previously reported UV detection at 256, 277, or 280 nm was deployed. With UV detection these HPLC methods had limits of detection for 5mdC at the level of pmol on column. For an assay more than 5 μg of an isolated DNA sample was usually digested. To achieve better assay sensitivity and selectivity, HPLC methods with ESI-MS/MS detection were developed. The sensitivity reported (LOD for 5mdC) varied a lot, from sub-fmol [15,16] to pmol [17] on column, likely due to the differences in instrumentation and the chemical composition of HPLC mobile phases used. Widely accessible fluorescence detection (FD) in chromatography is well known for its high sensitivity, repeatability, and robustness. While HPLC-FD is among the most popular techniques used in bioanalysis, its application in DNA methylation quantification has been very limited. Precolumn derivatization must be carried out prior to HPLC-FD analyses if the analytes are not fluorescent. Since the inherent fluorescence properties of nucleobases, nucleosides, and nucleotides, are limited, fluorescent labeling of these molecules is needed for their quantification by HPLC-FD. Although many fluorescence tagging reagents are in use for fluorescently labeling of nucleic acids [18], reports on HPLC-FD analysis of nucleobases (or nucleosides/nucleotides) are few in literature. Fluorescence tagging of nucleotide phosphate with BODIPY FL EDA in capillary electrophoretic separations was reported [19,20]. To our knowledge, 2-bromoacetophenone is the only fluorescence tagging reagent reported so far for HPLC-FD determination of global DNA methylation after enzymatic digestion [21,22].

Chloroacetaldehyde (CAA) is known for long to bind to nucleobases in DNA, forming etheno-derivatives: 1,N^6-etheno-deoxyadeninosine (ε-dAdo), 3,N^4-etheno-deoxycytidine (ε-dCytid), N [2,3]-etheno-deoxyguanosine (N [2,3]-ε-dGuo), and 1,N [2]-etheno-deoxyguanosine (1,N^2- ε-dGuo) [[23], [24], [25], [26]]. It was, therefore, used as a reagent to detect unpaired adenine, cytosine, and guanine residues in nucleic acid macromolecules. Previous studies have shown that N [2,3]-ε-G and, to a lesser extent, ε-A depurinate to form apurinic sites and DNA strand breaks [27,28]. One advantage of CAA as a fluorescence tagging reagent in chromatographic analysis is that itself is not fluorescent. Using CAA for pre-column derivatization of purine related compounds such as 6-thioguanine [29], adenosine [30,31], and adenine [32] in HPLC-FD analyses was reported.

In this work we studied the fluorescence tagging reactions of C, 5mC, and oligodeoxynucleotides with CAA. From the study results, a novel analytical strategy was developed for quick and sensitive HPLC-FD quantification of global DNA methylation. The strategy takes advantage of a one-pot CAA-DNA reaction where fluorescence tagging and depyrimidination of DNA occur simultaneously. This allows both fluorescence tagging of cytosine and DNA digestion to be achieved in one procedural step, greatly shortening the time required for an assay. To separate ε-C and ε-5mC from other fluorescent compounds present in CAA-DNA reaction solution, HPLC conditions, including column selection and use of additives in the mobile phase were investigated. Analytical figures of merit were assessed for the HPLC-FD method proposed. Application of this highly sensitive method was demonstrated by analysis of DNA samples isolated from yeast, HCT cells, and pathological tissues of small sample masses.

Section snippets

Reagents and samples

Chloroacetaldehyde (~55 wt% in H_2O) was purchased from Sigma-Aldrich. PBS tablets were from Gibco. Oligodeoxynucleotides (i.e.,5′-TCA GTC GGA TAA GCA AAG AGA-3′ and 5′-TCA GTC GGA TAA GCA AAG AGA-3' ("C" stands for 5mC) were customer ordered from Sigma-Aldrich (both were of HPLC purified grade). The de-identified tissue samples were provided by Dr. Xinchun Zhou, whose protocol for sample collection was approved by Institutional Reviewed Board at University of Mississippi Medical Center…

Fluorescence tagging of cytosine and oligodeoxynucleotides with CAA

Fluorescence tagging of adenine and guanine with CAA and its application in HPLC-FD analysis were extensively reported in the literature, but study on cytosine-CAA reaction has been so far very limited [18, [27], [28], [29], [30], [31]]. To our knowledge, there is no report on using CAA for precolumn derivatization of cytosine in HPLC analysis. It was noted that tagging reactions with CAA were mostly carried out at either room temperature or 80 °C in previously reported studies. In this work, we …

4Conclusions

A sensitive HPLC-FD method has been developed for quantification of global DNA methylation. The method takes a novel analytical strategy based on a one-pot fluorescence tagging and depyrimidination reaction of

DNA with chloroacetaldehyde. The method has a detection limit of 0.8 nM (or 250 fg on column) for 5mC and is far more sensitive than most of the HPLC based methods previously reported. The method has been validated with analysis of DNA samples extracted from various biological materials,…

CRediT authorship contribution statement

Xun Liao: Conceptualization, Methodology, Data curation, Writing – original draft. **Xiaolin Bai:** Data curation. **Shuguan Wang:** Data curation. **Christany Liggins:** Data curation. **Li Pan:** Conceptualization, Data curation. **Meiyuan Wang:** Data curation. **Paul Tchounwou:** Conceptualization, Writing – review & editing. **Jinghe Mao:** Conceptualization, Data curation. **Yi-Ming Liu:** Conceptualization, Methodology, Writing – review & editing.…

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.…

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