Research Article

Alteration in the amount of DNA and protein in bacterial cells by UV light and ethyl methane sulphonate

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Abstract

The biological importance of DNA is well known. There are some factors that result in its damage leading to the mutagenesis, carcinogenesis, and cell death. The microorganisms exposed to ultraviolet (UV) light and chemicals come up with some negative changes, including the reduction in the amount of DNA, leading to the decrease in the amount of protein being produced through translation. In this research *E.coli* methionine aminopeptidase gene ligated in the plasmid (pET21-*MapE*) was taken to observe the effects of UV light and ethyl methane sulphonate. As a result of the stress response, bacterial cell mass as well as amount of DNA and the protein was reduced in the bacterial culture containing plasmid (pET21-*MapE*) exposed to UV light and when treated with ethyl methane sulphonate. Furthermore, in comparison to UV light, ethyl methane sulphonate offered more reduction in the amount of DNA and protein. However, no observable changes were seen in the size of PCR amplified *E.coli* methionine aminopeptidase gene when exposed to the UV light or ethyl methane sulphonate. Giving the assumption that these factors have least effect on the stable DNA molecule when analysed by agarose gel electrophoresis but they predominantly affect the cellular machinery involved in the replication, transcription and translation processes consequently decrease in cell growth accompanying macromolecules production.

Keyword: Methionine aminopeptidase gene; UV induced damage; Ethyl methane sulphonate

Introduction

Other than some viruses DNA is key genetic material of all alive organisms. DNA is command molecule as genomic substantial, and this contains all facts regarding growth and facsimile of organism. DNA damage could result in aging of entities, cancer or some further syndromes allied to gene [1].

The major threat to change in DNA in nature is UV light. Different abrasions like nucleon threads disrupt pyrimidine glycols are induced by ultraviolet radiation conduct [2]. Chemical mutagenesis also has a great impact in the field of molecular biology. Alkylating agents were the first to be discovered as mutagens one of them is

ethylmethyl sulfonate (EMS) which has several effects on DNA because of its potency and its easy use. EMS is most commonly used as a mutagen product in plants [3]. EMS leads to mispairing alkylated G pairs with T instead of C, ultimately results in “G-C to A-T” alterations [4]. This chemical produces DNA damage which cause point mutations, insertion or deletion mutations [5]. Biological reactivity of EMS is associated to $-CH_2$ group that can be reassigned to range of nucleophilic sites [6]. It is a carcinogenic for mammals too [7]. *E.coli* has solitary one round shaped chromosome (genetic material) along with circular plasmid. It has solo chromosome of about 4,600 kb, 4,300 potential coding series, and only around 1,800 known *E.coli*. proteins Lab canvassers have utterly sequenced its chromosomal DNA [8].

Methionine aminopeptidase belongs to the M 24 family of proteins. MAP enzyme removes some essential amino acid residues from the amino-terminal of emerging polypeptides. MAP produce by *E.coli* might be an only, non-glycosylated peptide sequence embrace 264 “amino acids” and consuming a “molecular mass” of 29.33 kDa [9]. Mone *et al.* have investigated UV-induced deoxyribonucleic acid mutilation [10]. This mutilation roots cells to stifle in

ribonucleic acid synthesis and pledge nucleotide excision repair (NER). NER and dictation are familiarly amalgamated progressions [10].

This study designates three techniques that can cast off to the progression, degradation and structural properties, succeeding UV and EMS induced DNA damage in *Escherichia coli*.

Material and methods

PCR Amplification

Methionine aminopeptidase gene in the provided plasmid (pET21-*MapE*) was confirmed using specific set of primers given below in Table 1. Concentrations used for PCR were 10 X Taq buffer (1 x), $MgCl_2$ (4 mM), dNTPs (0.2 mM each), 1.5 μ l of forward primer and reverse primer (20 pmol), Genomic DNA (150 ng), Taq Polymerase (5U) in a reaction mixture of 50 μ l. Thermal cycler conditions used for pET21-*MapE* were, Initial denaturation was adjusted at 94°C for 10 min. pET21-*MapE* plasmid was amplified for 30 cycles, each cycle with 30 seconds of denaturation at 94°C, 30 seconds for primer annealing at 52°C and 30 seconds for extension at 72°C followed by final extension at 72°C for 10 min. PCR product was analyzed using 1% agarose gel electrophoresis in tris acetate EDTA (TAE) buffer (Figure 1).

Table 1. Details of the primers used in this research

Primer name	Primer sequence (5'-3')	Length	Melting temperature
Map(E)-N	CATATGGCTATCTCAATCAAGACCCC	26 bp	60.7
Map(E)-C	TTATTCGTCGTCGAGATTATCGCC	25 bp	60.6

Figure 1. 1 % agarose gel showing PCR product: Lane 1: DNA ladder, Lane 2: PCR product gene methionine aminopeptidase 795 bp

Transformation in BL 21-CodonPlus (DE3)-RIL

In 150 μ L of freshly prepared competent cells of BL 21, 2 μ L of plasmid pET21-*MapE* was added and placed in ice for 40 minutes. Heat shock was given at 42 °C for 90 seconds and again kept in ice for 5 minutes. 850 μ L of LB medium was added and incubated for 1 hour at 37 °C. Spreaded on agar plates then the plates were incubated overnight at 37 °C.

UV and chemical treatment

UV light treatment: A single bacterial colony (*E.coli* BL codon plus) containing plasmid pET21-*MapE* was inoculated into 5 ml LB medium containing 100 μ g/ml of ampicillin. The culture was incubated overnight in shaking incubator at 100 rpm and 37 °C. Next day 100 μ l from this culture medium was added to 7 test tubes having 5 ml LB medium 100 μ g/ml ampicillin and 0.08 mM lactose. One was considered as blank or uninduced while the remaining 6 tubes were exposed to UV light for 5 min, 10 min, 15 min, 20 min, 25 min and 30 min respectively and then kept in shaking incubator for overnight at 37°C. These tubes were used for plasmid isolation and expression check. Each test tube was divided in two equal parts, one half of every test tube sample was used for plasmid

isolation (gel electrophoresis) and other half was used for expression check using 12% SDS PAGE.

Chemical exposure: Ethyl methyl sulphonate was used for chemical treatment with a varying concentration 0 μ g/5ml, 5 μ g/5ml, 10 μ g/5ml and 15 μ g/5ml, 20 μ g/5ml, 25 μ g/5ml. These test tubes kept at room temperature. After treating chemically all test tubes were kept in shaking incubator for overnight at 37°C. These tubes were used for plasmid isolation and expression check. Each test tube was divided in two equal parts, one half of every test tube sample was used for plasmid isolation (gel electrophoresis) and other half was used for expression check using 12% SDS PAGE.

Results

The gene methionine aminopeptidase present in the provided plasmid was confirmed by PCR (Figure 1). After getting confirmation about the presence of the methionine aminopeptidase gene present in the plasmid, the next step was to expose the gene (PCR product) directly to the UV light. For that 5 μ l each of the PCR product was taken into 7 PCR tubes.

Plasmid DNA from the UV exposed culture was isolated from each sample and 5 μ l of each sample when run on the agarose gel, indicated that with the increase in the time

of UV exposure the amount of the DNA was decreased (Figure 3 and 4). After exposing the bacterial culture to the UV light for different times, expression of the methionine aminopeptidase as well as decline in the amount of bacterial proteins was observed with the increase in exposure time keeping the induction concentration constant (Figure 5). Plasmid DNA from the EMS treated culture was isolated from each sample and 5 μ l of each sample was run on the agarose gel which indicated that with the increase in the amount of EMS the amount of the DNA

was decreased (Figure 6 and 7). After exposing the bacterial culture to the EMS for different times, expression of the methionine aminopeptidase with the decline in the amount of bacterial proteins was observed in response to the increase in exposure time keeping the induction concentration constant (Figure 8). Overall in this research it was concluded that the amount of DNA and the protein was reduced in the bacterial culture induced to UV light and treated with EMS.

Figure 3. 1% Agarose gel showing plasmid DNA isolated from the bacterial culture exposed to UV light. Lane 1: DNA ladder, Lane 2: Plasmid from the bacteria exposed to UV light for 5 minutes, Lane 3: Plasmid from the bacteria exposed to UV light for 10 minutes, Lane 4: Plasmid from the bacteria exposed to UV light for 15 minutes, Lane 5: Plasmid from the bacteria exposed to UV light for 20 minutes, Lane 6: Plasmid from the bacteria exposed to UV light for 25 minutes, Lane 7: Plasmid from the bacteria exposed to UV light for 30 minutes

Figure 4. Change in the amount of DNA on exposure to UV light

Figure 5. 12% SDS PAGE showing expression of methionine aminopeptidase from the bacteria exposed to UV light: Lane 1: Protein from unexposed bacteria, Lane 2: Protein from bacteria exposed to UV light for 5 minutes, Lane 3: Protein from bacteria exposed to UV light for 10 minutes, Lane 4: Protein from bacteria exposed to UV light for 15 minutes, Lane 5: Protein from bacteria exposed to UV light for 20 minutes, Lane 6: Protein from bacteria exposed to UV light for 25 minutes, Lane 7: Protein from bacteria exposed to UV light for 30 minutes

Figure 6. 1 % Agarose gel showing plasmid DNA isolated from the bacteria treated with ethyl methyl sulphate: Lane 1: DNA ladder, Lane 2: Plasmid from the bacteria not treated with EMS, Lane 3: Plasmid from the bacteria treated with 5 $\mu\text{g/ml}$ EMS, Lane 4: Plasmid from the bacteria treated with 10 $\mu\text{g/ml}$ EMS, Lane 5: Plasmid from the bacteria treated with 15 $\mu\text{g/ml}$ EMS, Lane 6: Plasmid from the bacteria treated with 20 $\mu\text{g/ml}$ EMS, Lane 7: Plasmid from the bacteria treated with 25 $\mu\text{g/ml}$ EMS, Lane 8: Negative control

Figure 7. Change in the amount of DNA on treatment with EMS

Figure 8. 12% SDS PAGE showing expression of methionine aminopeptidase from the bacteria treated to EMS: Lane 1: Protein from the bacteria not treated with EMS, Lane 2: Protein from the bacteria treated with 5 µg/ml EMS, Lane 3: Protein from the bacteria treated with 10 µg/ml EMS, Lane 4: Protein from the bacteria treated with 15 µg/ml EMS, Lane 5: Protein from the bacteria treated with 20 µg/ml EMS, Lane 6: Protein from the bacteria treated with 25 µg/ml EMS, Lane 7: Protein from the bacteria treated with 30 µg/ml EMS

Discussion

UV energy is record risky and mutagenic factor of the solar emission continuum. Effects of UV light include the enhancement of apoptosis that is the programmed cell death. Stoppage of transcription and hindrance in the cell cycle transition that is the passage of one stage to the other to complete cell cycle [11]. DNA repair is also accounted in the after effects of the UV radiations. Here in this study UVC light (254 nm) was used. Undeniably, photo chemical responses are extra proficient contained by DNA at this wavelength, which near to immersion extreme of the pyrimidine and purine nucleobases [12].

DNA is a very stable molecule and it is very hard to change during vital processes. Although it is a stable molecule but stability of DNA is not absolute as there exists many factors from living organisms to environment that could cause damage. DNA damage is actually known as some abnormal

changes in the structure. As molecular structure of DNA undergoes structural change during its damage which is considered as some compassionate kind of chemical indemnities. Maximum DNA mutilation can be reinstated by the restoration system in entity leaving behind very little unrepaired genetic material mutilation. However even the small unrepaired damage causes magnificent effect on the organism [13].

Bioanalytical technologies such as electrophoresis are used for the identification of the damage done by ultraviolet rays to the DNA. In this research *E.coli* methionine aminopeptidase gene was used as experimental DNA. After the course of UV light induction when the electrophoresis was performed there was not observable change in the amount of DNA. The mutation if any that had taken place did not appear on the agarose gel. The fragments of DNA molecule that were broken down as

a result of stress into small pieces were small enough in size and concentration that did not appear on the agarose gel.

As the experiment was done *in vitro* next step was to perform the same experiment *in vivo* where the DNA was able to replicate using plasmid pET21-*mapE*. BL bacterial cells were transformed with the plasmid pET21-*MapE* and grown in the LB medium using ampicillin as an antibiotic. The plasmid DNA was induced to UVC light (254 nm) Then the transcription and translation examination were performed. Minor changes in the concentration of DNA on the Agarose gel as well as the amount of protein on SDS PAGE were observed. The reason was that UV light causes the formation of thymine cyclobutane dimmers but overall concentration remains the same. But as the duration of induction was increased the decrease in the concentration of the DNA as well as protein amount was observed as shown in the Figure 4 and 5 respectively.

Next goal was to check the amount of DNA and protein after exposing the plasmid containing bacteria to the mutagenic chemical Ethyl methanesulfonate (EMS). Nucleophilic mono and di substitution reaction mechanisms were followed by EMS in alkylation of cellular and nucleophilic sites. In this study after treating the plasmid containing bacteria to the EMS (5µg/ml, 10µg/ml, 15µg/ml, 20µg/ml, 25µg/ml and 30 µg/ml,) the gradual decrease in the concentration of DNA and Protein was observed as shown in Figures 7 and 8 respectively. The same findings were reported by Xingkui Tao et al., who determined the optimal concentration of EMS mutation for *E.coli* cells six EMS concentration gradients (0.1% , 0.2%, 0.3%, 0.4%, 0.5%, 0.6%). Their results showed that the survival rate of the cells was significantly different under the different concentrations of EMS. In the same

processing time, the EMS concentration was increased, but the suspension cells survival rate decreased gradually [14]. Reason was that in the presence of EMS the bacterial cells come in stress therefore mRNA production decreased leading to the reduction of protein amount.

Conclusion

Overall in this research it was concluded that the amount of DNA and the protein turned out to be reduced in the bacterial culture induced to UV light and treated with EMS. However when DNA was directly induced to UV light no observable change in the amount of DNA was seen. This is because DNA is quite stable molecule and exposure to UV light (5-30 minutes) cause no effect on its amount. In comparison to UV and chemical method more reduction in the amount of DNA and protein was observed in the experiment when bacteria were treated with EMS. Where the sudden decrease in the amount of DNA was observed with only 5 µg/ml EMS.

Authors' contributions

Conceived and designed the experiments: A Arif, Performed the experiments: A Arif & S Kanwal, Analyzed the data: A Arif, Contributed reagents/ materials/ analysis tools: B Khan, Wrote the paper: MAU Khan.

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