Stimulation of *Staphylococcus aureus* Ligase Enzyme by Magnesium Ion

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Abstract

Ligases enzymes were discovered as a member of the nucleotidyl transferase family. Here in this paper, DNA Ligase is extracted from *S. aureus* works with the cofactor NAD^+ to make a phosphodiester bond and reform between the 3'hydroxyl and 5'phosphate DNA end. *Staphylococcus aureus*-DNA Ligases Enzyme type A (SLE-A) contains two essential domains; NTase and OB- fold domain, which are the most essential domains for the enzyme function. The main aim of the study is to investigate the activity of SLE-A in the presence of magnesium ion (Mg^{+2}) by evaluating several kinetic parameters on a time course. The result showed that SLE-A has optimal activity at 500 µM of Mg^{+2}. Furthermore, the low number of Equilibrium Association Constant (K_m value) explains the binding affinity between DNA ligase of *Staphylococcus aureus* SLE-A enzyme and Mg^{+2} ion was very high and sold.

Keywords: *DNA ligases, Magnesium ion, Staphylococcus aureus, Michaelis-Menten equation.*

Introduction

Background of DNA ligase.

Fifty years ago, Lehman and other groups discovered the DNA ligases and was a turning point event in molecular biology [(1-4)]. Other DNA ligases had been lighted on since and they are presented in all domains of life [(5)]. The DNA ligase enzyme is an important component in the Ligation reaction, which also called DNA seals, DNA joins and/or DNA fixing enzyme [(6)]. All types of DNA Ligases which have the same protein fold are considered as part of nucleotidyltransferase family. For example, DNA ligases and RNA ligases [(7)]. they participated in multi processes for instance DNA repair, recombination and replication [(8)]. DNA ligase enzyme plays an important role during DNA replication: Firstly, joins Okazaki fragments, in addition it carry out a several DNA repairs like base excision, nucleotide excision, single and double-stranded repairs [(9)]. The lake or mutation of SLE-A (referees to LigA gene), resulted the loss of the ability to ligate Okazaki fragments during the replication steps [(10)]. DNA Ligases are very potent chemical material in the development of biotechnology, bimolecular and genetic engineering [(11)]. Another important implication, is that NAD^+-dependent ligases not present in mammalian cells as well as they are involved in developing antibacterial drugs "A requirement for an antibacterial enzyme target is that it should be essential for the organism and not present in the host [(12)]. The formation of a phosphodiester bond between adjacent 5 phosphate and 3' hydroxyl DNA termini is catalyzed by Ligases which known to fix several substrates.

Indeed, ligases are participated in the base

Excision DNA repair, culminating step of nucleotide and the ligation of Okazaki fragments during cell replication [(13-14)]. Importantly, NAD+ ligases play an important role in cell survival for prokaryotes [(15)]. The main characteristics of DNA ligase in prokaryotic is to present into all bacteria and all bacterial species contain

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LigA protein [16]. Kaczmarek announced in 2001, that the deletion or mutation of the LigA gene in *Staphylococcus aureus* (represent to SLE-A) lead to loss the complete growth in the bacteria [17]. The *S. aureus*-DNA ligase enzyme A (LigA) is formed of 666 signal amino acid and consist of 6 main domains with 74,993 Da molecular weight. The tertiary structure of *Staphylococcus aureus* of LigA (SLE-A) protein consists of two main domains called nuclotidyltransferase/ NTase domain and an Oligomer Binding (OB domain), which are considered as a catalytic core for all domains of life [18-19].

Magnesium Ion in DNA Ligase.

One of the most important cofactor in the ligation or the mechanism process of DNA ligases enzymes is $MgCl_2$ Ion. It considers a second cofactor in the reaction of ligase. Mg^{+2} is a co-factors that allows the ligases enzymes to join and shut a nick at the backbone of Deoxyribonucleic acid. Furthermore, LigA DNA ligase needs Mg^{+2} to draw the AMP groups that is very necessary during the mechanism of ligase and attaches with the active site of amino acid called lysine to do its work. The AMP groups comes when the ligase enzyme touches with cofactor ATP or NAD+ to form a phosphoamide -linked AMP and without the NAD^+ or ATP the reaction of ligation will be stopped completely [9].

Materials and Methods

The Cloning of *Staphylococcus aureus* DNA Ligase.

The published open-reading frames of *S. aureus*-DNA Ligase enzyme A (SLE-A)) (belongs to LigA) was retrieved from the NCBI-PubMed database using Gene ID 45575143 and the gene size is 2004 base pair for *S. aureus* LigA. Gene was synthesised (GeneArt, ThermoFisher, UK) with a 20 bp flanking sequence containing an NdeI site and cloned into the NdeI site of pET29c. Kanamycin-resistant transformants of *S. aureus* LigA were screened by colony PCR and those showing the requisite sizes were sequenced in full on both strands (Genewiz, UK). **Figure 1** is shown a sample of *S. aureus* (SLE-A) gene was run on a 1% agarose gel. Large-scale preparations of each plasmid were made (Qiagen, UK) and stored at -20ºC in 50 µl aliquots. DNA primer sequences are as follow as (green colour refers to the NdeI site of pET29c and black colour refers to the forward and reverse primers of *S. aureus*-DNA ligase enzyme type A gene.):

LigAtop:
CATATGGCTGATTTATCGTCTCG
LigBbot:
CATATGCTAACTATTTAATTCATTTT

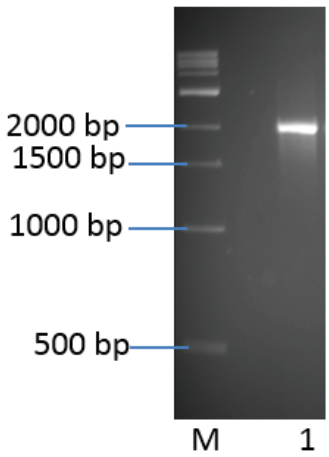


Figure 1: Cloning of *S. aureus*-DNA ligase enzyme type A.

1% of agarose gel showing the PCR cloning from *S. aureus*-DNA enzyme type A as follows: Lane M – 1 k bp DNA ladder (NEB), showing sizes of DNA between 500 bp to 10,000 bp. Lane 1 is a colony PCR products of SLE-A gene, the length of *S. aureus* SLE-A (LigA) is 2004 bp depending on the GeneBank plus the sequencing of plasmid.

The expression and purification of *Staphylococcus aureus* DNA Ligase.

For *S. aureus* LigA (SLE-A) expression, competent *E.coli* BL21 (DE3) cells was transformed with 0.5 µg plasmid (pLigA) and grown overnight at 37°C on LB-agar containing 50 µg/ml kanamycin. A single colony was picked and grown overnight in an orbital shaker at 37ºC in 5 mL LB media. A 1 mL aliquot of this starter culture was added to 500 mL fresh LB broth and grown under the same conditions until an OD_{600} of 0.6 was reached. Protein expression was induced with 1 mM isopropyl *B*-D-thiogalactoside (IPTG) and the culture incubated at 37ºC for 2 hours (LigA) until an OD_{600} ~2.0 was reached. Bacterial cultures were harvested by centrifugation at 6000 rpm for 20 minutes at 4°C and the media discarded. The cell pellet was re-suspended in 15 ml Lysis buffer (10 mM sodium phosphate, pH 7.0, containing benzamidine and PMSF protease inhibitor cocktail (Sigma-Aldrich, UK) was sonicated on ice in 10 second pulses over 3 minutes and spun at 18000 rpm for 20 minutes at 4ºC to separate supernatant and cell debris. Ligase A was subsequently purified from the supernatant using a two-step method; all steps were at 4ºC. In the first step, NaCl was adjusted to 1M in order to disrupt protein-nucleic acid interactions and ammonium sulphate then added slowly over two hours to a final concentration of 35% (w/v) in order to precipitate the enzyme. Following centrifugation at 18000 rpm for 20 minutes, the pellet was resuspended in an appropriate volume of Buffer A (10 mM sodium phosphate, pH 7.0). Salt was removed by membrane dialysis overnight in Buffer A. In the second stage, enzymes were injected onto a Hi-Trap heparin anion-exchange column (GE Healthcare, UK) in Buffer A and eluted in the same buffer with NaCl gradient to 1 M. Fractions eluting from the column were analysed by SDS-PAGE to confirm the presence and purity of the protein. Fractions containing purified LigA was pooled and dialysed into Buffer A. Ligase activity assays were then used to check for correctly-folded, functional ligase. LigA was further purified using size-exclusion chromatography (GE Healthcare, UK).

Fractions containing purified LigA were pooled and concentrated using spin column to ~ 1 ml. *S. aureus* LigA was finally dialysed into 1×Ligase storage buffer (30 mM Tris-HCl, pH 7.2, 1 mM DTT and 50 µg/ml BSA and 30% (v/v) glycerol). Aliquots of 50 µl were stored at -20ºC and used fresh for subsequent kinetic and binding assays.

Figure 2 is shown the large scale of expression of DNA ligase (LigA) and the final purification of the *S. aureus* SLE-A protein.

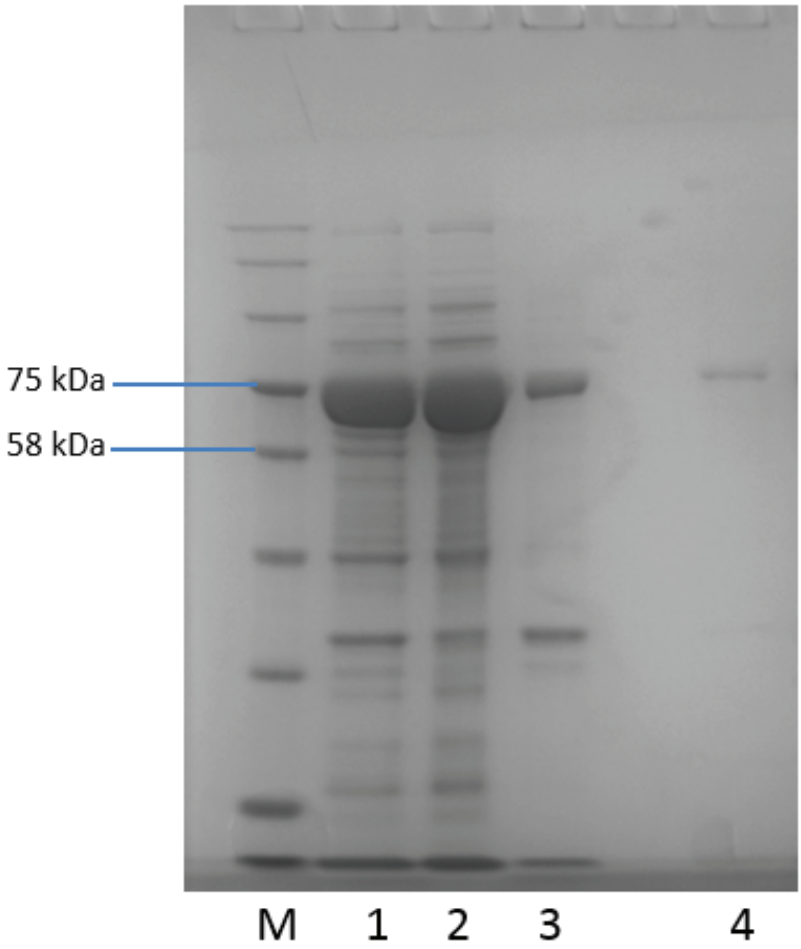


Figure 2: Large Scale Inductions of *Staphylococcus aureus*-DNA ligase enzyme type A in BL21 (DE3).

10% SDS PAGE gel shows the Large induction of *S. aureu*s SLE-A enzyme in *E. coli* BL21 (DE3) as follow: Lane M shows an NEB protein marker indicated by sizes (in kDa) pointed to the left of the gel. Lanes 1 and 2 shows the total post-induction of expressed SLE-A. Lane 3 shows the corresponding samples of *S. auerus* LigA protein that pooled from heparin column with molecular weight (size) 74,993 Da. Lane 4 shows the final purification of pure protein via using size sxclusion column.

Ligase activity and timecourse assays.

Three HPLC-purified oligonucleotides (Fisher Scientific, UK) were used:

20Top **5'-HEX-ATCTCGCGTATGGGCCTTCG-3'**

30Top **5'-P-CTGCTCACAGGACACCTGGTATACGTAATG-3'**

50Bot **5'-CATTACGTATACCAGGTGTCCTGTGAGCAGCGAAGGCCCATACGCGAGAT-3'**

These were mixed in a ratio of 1:1:1 at a concentration of 10 µM in 50 µl of 1×Ligase reaction buffer (30 mM Tris-HCl, pH 7.5, 1 mM DTT and 50 µg/ml BSA) and annealed by cooling in a PCR machine from 100ºC to 4ºC at 0.1ºC/min. The resulting 50mer oligoduplex contained a single off-centre nick between the 20Top and 30Top strands with a phosphoryl group on the 5'-side of the nick; a hexachlorofluorescein (HEX) group at the 5-end of the 20Top strand permitted the DNA to be quantitated. Kinetic assays on LigA was carried out in 1× ligase reaction buffer. The concentration of nicked oligoduplex was 1000 nM in most experiments. Each timecourse (50 µl) contained nicked oligoduplex, 1×Ligase buffer, the requisite cofactor(s) and 40 nM of LigA. Each reaction of time course was containing a single nick of six $MgCl_2$ samples ranging from (0.1, 1, 10, 50, 100, and 500 µM) on a denaturing PAGE 15%. All the samples were incubated at 16 °C. The time points were prepared for 5 times as follow (0 time, 3 mins, 5 mins, 10 mins, 20 mins and 60 mins) and all the tubes were heated at 100 °C for 2 mins. Shortly, the intensity of the 50TOP bands were measured by using computer software ImageGauge. The data were plotted with % intensity against time revealing the initial rate.

Data Analysis.

Products of timecourse reactions were run on 40 cm long, 0.4 mm thick 15% (w/v) denaturing polyacrylamide gels containing 1×TBE (89 mM Tris-borate, 2 mM EDTA) and 40% urea. Gels were pre-run at 60 W for 30 minutes until warm. Samples (10 µl) were heated at 90ºC for 2 minutes, loaded and run at 50 W for ~90 minutes. The HEX-labelled DNA strands were visualised by excitation at 600 nm using a fluorescence imager (FLA-5000, Fuji, Japan). The digitised images were quantified using ImageJ (imagej.nih.gov) by firstly removing background (50-pixel 'rolling-ball' average) and then integrating the area under each peak. These data were used to calculate the fraction of counts distributed between the 20mer substrate and 50mer product bands in each lane; these were multiplied by the DNA concentration to give the amount of each in nM. Initial rates (V_0) were determined by the gradient of the first 20% of the reaction. Rate data were fit to the Michaelis-Menten equation (Grafit v5, Erithacus Software, UK) and V_{max} and K_d parameters were elucidated by plotting their V_0 values for each timecourse and fitting them by using Michaelis- Menten equation

as follow $V_0 = V_{max} + [S]/ K_m + [S]$

The definition of K_d is the concentration of substrate that gives half maximum rate. It was obtained from the Michaelis- Menten equation fit above as well.

Results

Nick-joining ligation activity.

All proteins purified in this study have been proved to join nicks in double stranded nucleic acid in different conditions and terms. The purified of LigA protein of *E coli* DNA ligase have been confirmed to join nicks in double stranded nucleic acids with varying efficiencies in presence of NAD+. In order to achieve the ligation experiment and how the ligation was affected by varying concentrations of Mg^{+2}, a double-stranded DNA substrate (dsDNA) was synthesised from Fisher Scientific, UK with a single nick on the top strand. Three HPLC purification of oligonucleotides were synthesised as follow: the length of first top oligonucleotide was 50 bases contains from two top strands (20 and 30 bases) and both of them are complementary to adjacent the 50 base bottom strand. The 20 top single strand attached fluorescent HEX group and the nick was made on the top strand between 30 top and 20 top. The complementarity in the bottom and top strands location, the two top strands (30 and 20) was adjacent to each other. The nick on the top strand (50 oligonucleotide) was at 3'-hydroxyl group of the 20 base strand and 5'-phosphate group of the 30 base strand.

When the buffer has the nicked DNA substrate and SLE-A (LigA) protein of the appropriate concentration and conditions, the nick on the top strand will be sealed by the proteins.

The samples were taken at specific time points and transferred to the stop buffer to stop the ligase protein its work. During electrophoresis, the denaturing gel separate the double stranded structure (50 base top and bottom) and causes to unfold the dsDNA into the linear chain. The dsDNA substrates will be migrated through the gel as single stranded. Therefore, the size of DNA ligation is easy to distinguish as the distance migrated by the DNA. Once the products sDNA of 20 and 30 base strands ligated will be migrated less position on the

denaturing gel, comparing to only the 20 base strand, which will be stayed at the bottom of the gel. The visible light to the ligated products in the denaturing gel was easy to follow due to the attached of fluorescent HEX group at 20 top strand.

The Effect of Mg^{+2} ion on the *S. aureus* DNA ligase .

To assess whether ligation of such nucleic acids may be a substantial value of these enzymes, at least in biochemical condition, all these experiments have been characterized the ligation of the dsDNA with different protein of *S. aureus* SLE-A (LigA)across a range of independent variable of Mg^{+2} ion. All the experiments were carried out at 16 ^{0}C (except temperature) and 1 µM of oligonucleotide substrate in total volume of 50 µl of 1X DNA ligase buffer and the mixture of reaction were completed with free water. The concentration of enzymes that used in all these experiments was 40 nM. To check how was influenced by different Mg^{+2} ion concentrations; the process of ligation was performed in the existence of Mg^{+2} concentrations from 0.1, 1, 10, 50, 100, and 500 µM. At low of Mg^{+2} ion, initial average increases slowly till over 10 µM (does not show), where is initial rate rises rapidly around 50 µM and the loop of curve begins to rise until 500 µM. The value for V_{max} gained by organized initial average in front of Mg^{+2} ion concentrations. The V_{max} obtained by Mg^{+2} ion concentrations which was µM/min and the K_m (the [$MgCl_2$] that given half V_{max} rate) was as well in µM. To examine the V_{max} and K_m (shown as K_d at the figure) for Mg^{+2}, using six Mg^{+2} concentrations, these initial rate values (in % per minute) were calculated against the concentration of Mg^{+2} in each experiment and fit the curve to a Michaelis-Menten equation by using the software Grafit. The initial rate and the measurement of the intensity were explained by details in the data analysis in the Material and Method above. **Figures 3** is an example of gels showing the ligation of DNA ligase of S. *aureus* SLE-A with different concentration of Mg^{2+} that appeared by bands of DNA in the denaturing gel. The plot for the $MgCl_2$ ion shows below in **Figure 4**. The V_{max} (capacity) of Mg^{+2} ion was 3.9 %/min where is the K_m (is shown as K_d) was 5.2 µM.

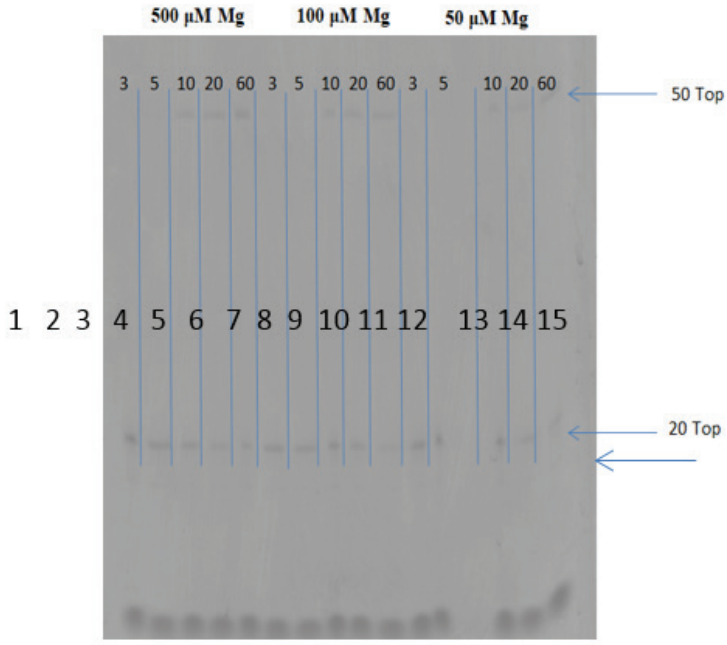
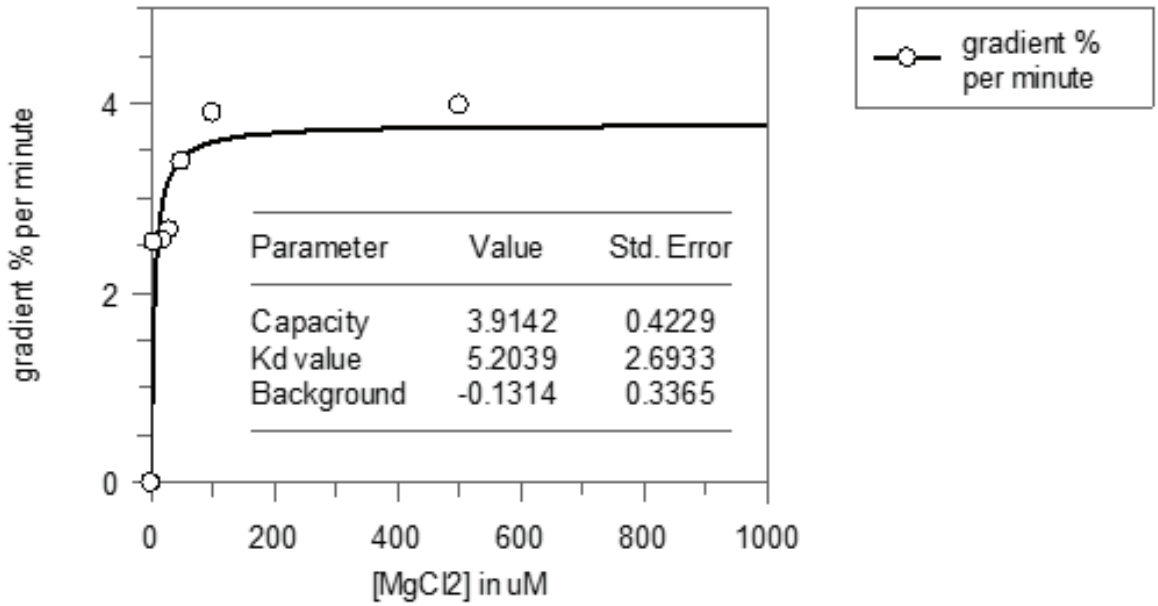


Figure 3 is the denaturing PAGE gel of three of the six MgCl2 Ion concentrations tested.

Denaturing PAGE gel that was got from this study is labelled as following lanes: 1-6 which is corresponded to the following time points 3,5,10,20 and 60 minutes, at 500 µM of Mg+2 ion as an example of this gel, at the top of gel are 50 mer (50 Top) and the bottom of gel are 20 mer (20 Top). The Bromophenol blue is the colour which is used to check the bands on the denaturing gel.



Discussion

Because of the NAD^+-dependent DNA ligase is very essential enzyme for DNA replication and repair, it had been reported as a prospective broad-spectrum antibacterial target, since there are a high conserved phylogenetic and distinctly different from the Eukaryotic DNA ligases [20-22]. Thus, prokaryotic NAD^+-dependent ligases is present as a promising drug target for antimicrobial therapy comparing to ATP-dependent in human [23-24]. In 1973s, Modrich and Lehman were referred that magnesium ion increase the activity of ligase protein. Magnesium ion is very important component in the step1 and step2 of the ligation mechanism of ligases enzyme in general. Moreover, in the same study showed that the magnesium ion is being involved to increase the rate of phosphodiester synthesis and DNA adenylation in the system of mechanism of ligases enzymes [25]. The obtained results in this paper from the investigation the effect of Mg^{+2} ion on *Staphylococcus aureus*-DNA Ligases enzyme type A (SLE-A) activity showed that 500 µM of Mg^{+2} ion had the best on ligases's activity for fixing the nick in the DNA between the 20 mer and 30 mer, and transfer them to 50 mer. Comparing this result with different study by using ammonium sulphate ion to determine how much this ion affected to the *E. coli* DNA Ligase activity (LigA), and the result was showed that the best concentration of ammonium sulphate was as well 500 µM, which is indicated the similarity between these ions activity on different enzymes [26]. Moreover, the results can be concluded that DNA ligases of *E. coli* and *Staphylococcus aureus* are structurally similar. The K_m value (equilibrium association constant) in this study was 5.2 µM, which is explained the binding affinity between DNA ligase of *Staphylococcus aureus* SLE-A and $MgCl_2$ ion was slightly similar to the K_m value for NAD^+ cofactor for *S. aureus* LigA (2.75 µM) that found by Gul *et al,* 2004 [27]. However, different result by Sriskanda & Shuman 2001 who obtained the K_m value for *E. coli* DNA ligase enzyme LigA for singly-nicked DNA substrate and was 12.2 nM, which is completely different comparing with this study [7]. Generally, it was mentioned above in the discussion that the main objective in the biology and medical sciences is to find a target in the bacteria to be as novel drug target for antimicrobial therapy, and DNA ligase is one of them since there are no similarity to the eukaryotic DNA ligases. However, magnesium ions here did not inhibit the activity of *Staphylococcus aureus*-DNA Ligases enzyme type A (SLE-A), but increase the rat of reaction. Therefore, Due to the importance of the DNA ligase enzyme, further study would be involved to identify more compounds that would be able to inhibit the activity of DNA ligase (SEL-A) but not inhibit the activity of eukaryotic DNA ligases by blocking the communication sites between the

magnesium ions (Mg^{+2}) and the ligase of bacteria, and eventually control the growth of them.

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References

1- Olivera BM, Hall ZW, Anraku Y, Chien JR, Lehman IR. On the mechanism of the polynucleotide joining reaction. InCold Spring Harbor Symposia on Quantitative Biology 1968 Jan 1 (Vol. 33, pp. 27-34). Cold Spring Harbor Laboratory Press.

2- Gefter ML, Becker A, Hurwitz J. The enzymatic repair of DNA. I. Formation of circular lambda-DNA. Proceedings of the National Academy of Sciences of the United States of America. 1967 Jul;58(1):240.

3- Gellert M. Formation of covalent circles of lambda DNA by E. coli extracts. Proceedings of the National Academy of Sciences of the United States of America. 1967 Jan;57(1):148.

4- Zimmerman SB, Little JW, Oshinsky CK, Gellert M. Enzymatic joining of DNA strands: a novel reaction of diphosphopyridine nucleotide. Proceedings of the National Academy of Sciences of the United States of America. 1967 Jun;57(6):1841.

5- Le D, Hua X, Huang L, Gao G, Lu H, Xu Z, Tian B, Hua Y. Biochemical characterization of two DNA ligases from Deinococcus radiodurans. Protein and peptide letters. 2008 Jul 1;15(6):600-5.

6- Dwivedi N, Dube D, Pandey J, Singh B, Kukshal V, Ramachandran R, Tripathi RP. NAD+□ Dependent DNA Ligase: A novel target waiting for the right inhibitor. Medicinal research reviews. 2008 Jul;28(4):545-68.

7- Sriskanda V, Shuman S. A second NAD+-dependent DNA ligase (LigB) in Escherichia coli. Nucleic acids research. 2001 Dec 15;29(24):4930-4.

8- Nandakumar J, Nair PA, Shuman S. Last stop on the road to repair: structure of E. coli DNA ligase bound to nicked DNA-adenylate. Molecular cell. 2007 Apr 27;26(2):257-71.

9- Shuman S. DNA ligases: progress and prospects. Journal of Biological Chemistry. 2009 Jun 26;284(26):17365-9.

10- Poidevin L, MacNeill SA. Biochemical characterisation of LigN, an NAD+-dependent DNA ligase from the halophilic euryarchaeon Haloferax volcanii that displays maximal in vitro activity at high salt concentrations. BMC Molecular Biology. 2006 Dec 1;7(1):44.

11- Sriskanda V, Shuman S. Role of Nucleotidyl Transferase Motif V in Strand Joining byChlorella Virus DNA Ligase. Journal of Biological Chemistry. 2002 Mar 22;277(12):9661-7.

12- Korycka-Machala M, Rychta E, Brzostek A, Sayer HR, Rumijowska-Galewicz A, Bowater RP, Dziadek J. Evaluation of NAD+-dependent DNA ligase of mycobacteria as a potential target for antibiotics. Antimicrobial agents and chemotherapy. 2007 Aug 1;51(8):2888-97.

13- Lahiri SD, Gu RF, Gao N, Karantzeni I, Walkup GK, Mills SD. Structure guided understanding of NAD+ recognition in bacterial DNA ligases. ACS Chemical Biology. 2012 Mar 16;7(3):571-80.

14- Lehnman IR. DNA ligase: structure, mechanism, and function. Science. 1974 Nov 29;186(4166):790-7.

15- Wilkinson A, Day J, Bowater R. Bacterial DNA ligases. Molecular microbiology. 2001 Jun;40(6):1241-8.

16- Sriskanda V, Shuman S. A second NAD+-dependent DNA ligase (LigB) in Escherichia coli. Nucleic acids research. 2001 Dec 15;29(24):4930-4.

17- Kaczmarek FS, Zaniewski RP, Gootz TD, Danley DE, Mansour MN, Griffor M, Kamath AV, Cronan M, Mueller J, Sun D, Martin PK. Cloning and functional characterization of an NAD+-dependent DNA ligase from Staphylococcus aureus. Journal of Bacteriology. 2001 May 15;183(10):3016-24.

18- Lee JY, Chang C, Song HK, Moon J, Yang JK, Kim HK, Kwon ST, Suh SW. Crystal structure of NAD+-dependent DNA ligase: modular architecture and functional implications. The EMBO Journal. 2000 Mar 1;19(5):1119-29.

19- Georlette D, Blaise V, Bouillenne F, Damien B, Thorbjarnardóttir SH, Depiereux E, Gerday C,

Uversky VN, Feller G. Adenylation-dependent conformation and unfolding pathways of the NAD+-dependent DNA ligase from the thermophile Thermus scotoductus. Biophysical journal. 2004 Feb 1;86(2):1089-104.

20- Benson EL, Tomich PK, Wolfe ML, Choi GH, Hagadorn JC, Mutchler VT, Garlick RL. A high-throughput resonance energy transfer assay for Staphylococcus aureus DNA ligase. Analytical biochemistry. 2004;2(324):298-300.

21- Gul S, Brown R, May E, Mazzulla M, Smyth MG, Berry C, Morby A, Powell DJ. Staphylococcus aureus DNA ligase: characterization of its kinetics of catalysis and development of a high-throughput screening compatible chemiluminescent hybridization protection assay. Biochemical Journal. 2004 Nov 1;383(3):551-9.

22- Chen XC, Hentz NG, Hubbard F, Meier TI, Sittampalam S, Zhao G. Development of a fluorescence resonance energy transfer assay for measuring the activity of Streptococcus pneumoniae DNA ligase, an enzyme essential for DNA replication, repair, and recombination. Analytical biochemistry. 2002 Oct 15;309(2):232-40.

23- Brötz-Oesterhelt H, Knezevic I, Bartel S, Lampe T, Warnecke-Eberz U, Ziegelbauer K, Häbich D, Labischinski H. Specific and potent inhibition of NAD+-dependent DNA ligase by pyridochromanones. Journal of Biological Chemistry. 2003 Oct 10;278(41):39435-42.

24- Nandakumar J, Nair PA, Shuman S. Last stop on the road to repair: structure of E. coli DNA ligase bound to nicked DNA-adenylate. Molecular cell. 2007 Apr 27;26(2):257-71.

25- Chauleau M, Shuman S. Kinetic mechanism and fidelity of nick sealing by Escherichia coli NAD+-dependent DNA ligase (LigA). Nucleic acids research. 2016 Mar 18;44(5):2298-309.

26- Alomari A. *Biophysical and Kinetic Analysis of Escherichia coli DNA Ligase Activity and Inhibition* (Doctoral dissertation, University of Portsmouth).

27- Gul S, Brown R, May E, Mazzulla M, Smyth MG, Berry C, Morby A, Powell DJ. Staphylococcus aureus DNA ligase: characterization of its kinetics of catalysis and development of a high-throughput screening compatible chemiluminescent hybridization protection assay. Biochemical Journal. 2004 Nov 1;383(3):551-9.

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