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INHIBITION OF POLYMERASE CHAIN REACTION BY LITHIUM CHLORIDE

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ABSTRACT

Polymerase chain reaction has revolutionized the field of molecular biology. However, the technique needs careful monitoring for proper utilization. In contrast to false positives less attention has been given to false negatives. In the present study the inhibitory role of LiCl on amplification with *Taq* DNA polymerase has been studied. It was found that 30 mM concentration of LiCl conclusively inhibits amplification in a 20 µL reaction. Further the use of several PCR enhancers like BSA, DMSO and tween 20 could not help to reverse the effect. The effect of LiCl inhibition was found to be more on longer amplicons than shorter ones. $MgCl_2$ concentration up to 2.5 mM (in excess of 2.25 mM in positive or negative control) helped amplification to some extent; however, the quality of amplification was relatively poor.

Keywords: PCR, lithium chloride, inhibition, $MgCl_2$, amplification

INTRODUCTION

The polymerase chain reaction (PCR) is a relatively simple technique that amplifies a DNA template to produce specific DNA fragments in *in vitro*. Its introduction has revolutionised the molecular biology research because it needs small input for a reaction to perform [1]. A typical amplification reaction includes the target DNA, a thermo stable DNA polymerase, oligonucleotide primer (s), deoxynucleotide triphosphates (dNTPs), magnesium ions and Tris-HCl, KCl etc with a desired concentrations and pH. Once assembled, the reaction is placed in a thermal cycler to undergo denaturation, annealing and extension in cyclic manner. Though apparently the reaction seems simple, but many factors come into play during reaction, which can alter amplification results variously. It is prerequisite for any PCR to have a correct and recommended concentration of each component for desired results. Any intrinsic component of a

PCR can act as an inhibitor when it exceeds a threshold concentration. However, many chemical contaminants have either enhancing or inhibitory effect [2] and effects of many of them have been studied [3,4,5,6]. While much attention has been directed towards minimizing false-positives, relatively little attention has been given to the causes of false-negative PCRs [7] which often give intriguing and incomprehensive results. Various thermostable DNA polymerases are used for PCR depending upon their processivity, fidelity and cost-effectiveness. Among them *Taq* DNA polymerase is a widely used DNA polymerase because of its many merits. In the present study effect of lithium chloride has been studied on the polymerase chain reaction in

presence of *Taq* DNA polymerase. There is a report that lithium chloride has an inhibitory effect on *in vivo* DNA replication [8], while S. Joholm *et al.* [9] and Ptashne *et al.* [10] found its stimulatory role on replication in beta and mammary cells respectively. However, a basic difference between *in vivo* and *in vitro* amplification tends not to extrapolate such a conclusion for the later one or at least the mechanism of inhibition may differ. In *in vivo* amplification there is a complex replication mechanism working on ambient temperature in general[11] while in *in vitro* amplification there is relatively a simple mechanism which unlike *in vivo* conditions do not need an enzyme (except DNA polymerase) complex, and denaturation, annealing and extension of DNA strands are thermally performed. Lithium chloride is used in many DNA related techniques [12,13] and therefore study of its effect on any process associated with DNA amplification process is important. Further the study is important from forensic perspective. In the present study effect of different concentrations of lithium chloride on the amplification and effect of various PCR enhancers like bovine serum albumin (BSA), dimethylsulphoxide (DMSO) and tween20 and $MgCl_2$ to counter the inhibition have been studied.

MATERIALS AND METHODS

In the present study pure, unsheared DNA, free from contamination was used. For precautionary measures DNA was treated with RNase H and phenol –chloroform. Additional treatments of chloroform were given to ensure to remove any traces of phenol. Subsequently the DNA was checked for different recommended ratios [14,15,16] to ensure its fitness for PCR use. Different concentrations of lithium chloride and different sets of primers were used in a 20 µL reaction. Similarly different concentrations of enhancers were used to study counter effect on PCR inhibition by LiCl (Table 1). For a 20 µL PCR reaction the usual components (10X Taq DNA buffer), 2.25 mM $MgCl_2$, 0.2 mM of dNTP mix, 12 pM of each primer (Sigma Aldrich), 5-10 ng DNA, and 1 unit of *Taq* DNA polymerase (all supplied by Bio Basic Inc., Canada) were used. Both positive control (LiCl in the reaction) as well as negative control (LiCl absent from the reaction) was used wherever needed to ascertain reproducibility.

RESULTS AND DISCUSSIONS

The polymerase chain reaction is arguably the most important biotechnological innovation to date, and is rapidly becoming a standard technique in a molecular biology research. Since its conception new and innovative applications for PCR have been and are being developed at exponentially increasing rate. Although PCR continues to remain a backbone of molecular biology research but its promise will not be completely fulfilled until improved. One of the problems with PCR that is less discussed is the reaction inhibition. This may be total or partial and can manifest itself as complete reaction failure [2]. Many metal ions e.g., nickel bind to replication proteins and alter the process of replication [17]. Negative [8,18,19] or positive [9,10] effects of lithium ions on DNA replication in a number of biological systems in *in vivo* conditions have been reported. In the present study in a 20 µL reaction, 30 mM lithium chloride invariably inhibits PCR (Figure 1). In certain cases inhibitory concentration was 25 mM. It has also been found that with decreasing concentration of lithium chloride, the amplification quality goes on increasing (Figure 1). It is known that lithium ions bind to nucleic acid bases, leading to an increased H-bond stability [8], which would interfere with DNA replication (*in vitro* or *in vivo*). Alternatively, it has been also described that lithium ions are powerful competitors of mono- and divalent cations, mainly sodium, magnesium and calcium [20]. These cations are regulators of cell proliferation, acting on enzymes involved in initiation of DNA synthesis. Therefore, enzymes related to DNA replication could be the targets for the activity of lithium ions. Mg^{2+} ions form complexes with dNTPs, primers, DNA templates and DNA polymerase in a polymerase chain reaction. In the present case it seems that mode of inhibition of the PCR is mainly due to the interference of LiCl with Mg^{2+} ions i.e. it inhibits availability of Mg^{2+} ions to bind with dNTPs, DNA and as cofactor for *Taq* DNA polymerase.

In the absence of adequate free magnesium, *Taq* DNA polymerase is inactive. *Taq* DNA polymerase is more sensitive to Mg^{2+} concentration (http://www.promega.com/paguide/chap1.htm). In the absence of adequate free magnesium ions, *Taq* DNA polymerase is inactive. Thus with the increased concentration of Mg^{2+} ions (in the form of $MgCl_2$) the inhibition of PCR by LiCl gets countered to some extent (Figure 2), however, the amplification quality still remains poor. Increasing concentration of $MgCl_2$ above a certain concentration (Figure 2) again suppresses the reaction probably due to any other factor and so again leads to reaction failure. There was no effect of length and GC % content of primer on inhibition. In amplification of multiple amplicons in a reaction, it was found that inhibition effect was more for amplicons with increased size (Figure 3). The lesser effect of inhibition on shorter fragments is probably because they out compete longer ones for resources (Mg ions).

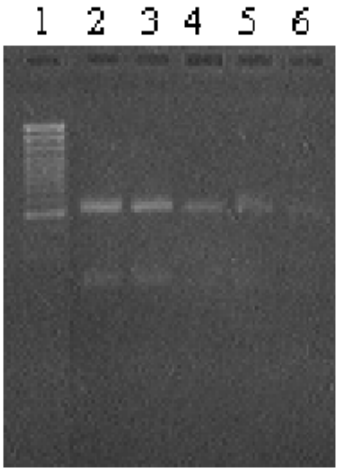

Figure 1

Figure 1. ***Amplification results with different concentrations of LiCl.1: DNA marker, 2: No LiCl, 3: 10 mM, 4: 15 mM, 5: 25 mM, 6: 30 mM.***

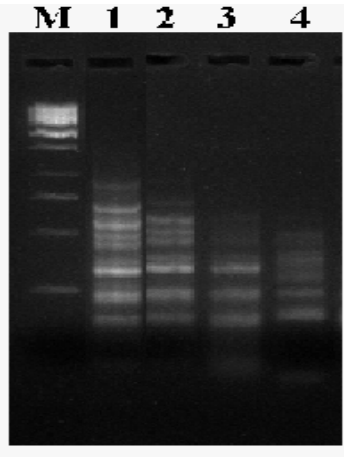

Figure 2

Figure.2. ***Showing counter effect of different concentrations of $MgCl_2$ on inhibition of PCR by LiCl. $MgCl_2$ concentration of 2.25 mM in positive and negative control.***
N: Negative control (No LiCl),M : DNA Marker, P: positive control (30 mM LiCl), 1: $MgCl_2$ of 3.0 mM conc., 2: $MgCl_2$ 3.75 mM conc., 3: 5.25 mM conc., 4: 7.5 mM conc.

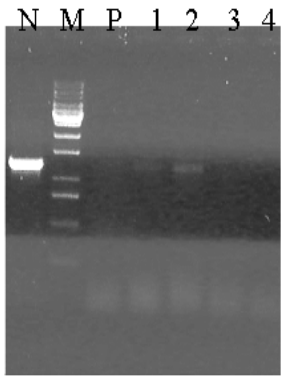

Figure 3

M: DNA marker, 1: 5 mM LiCl, 2: 10 mM LiCl, 3: 15 mM,4: 20 mM.

Figure 3. Amplification results showing effect of different concentrations of LiCl on different sizes of amplicons

Table. 1. ***Effect of different PCR enhancers on PCR inhibition by LiCl.***

Component	Concentration and status of amplification			
BSA (w/v)	0.2% (No)	0.4(No)	2% (No)	4% (No)
DMSO (v/v)	1%(No)	5%(No)	10%(No)	20% (No)
Tween 20 (v/v)	0.2%(No)	0.5%(No)	1%(No)	2%(No)

CONCLUSION

The PCR reaction depends the enzymes in which metal ions play important role. Lithium chloride shows inhibitory reaction on PCR functioning. It is known that lithium ions bind to nucleic acid bases, leading to an increased H-bond stability. In the present case it seems that mode of inhibition of the PCR is mainly due to the interference of LiCl with Mg^{2+} ions i.e. it inhibits availability of Mg^{2+} ions to bind with dNTPs, DNA and as cofactor for *Taq* DNA polymerase. In the absence of adequate free magnesium, *Taq* DNA polymerase is inactive. *Taq* DNA polymerase is more sensitive to Mg^{2+} concentration (http://www.promega.com/paguide/chap1.htm). Thus with the increased concentration of Mg^{2+} ions (in the form of $MgCl_2$) the inhibition of PCR by LiCl gets countered to some extent.

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