Asian Journal of Biological and Life Sciences | Original Research

Polyamine Binding Studies with Silk gland and Testicular DNA of *Bombyx mori.* L (Lepidoptera: Bombycidae)

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Submitted : 20.08.2016 Accepted : 19.10.2016 Published : 30.12.2016

Abstract

Silkworms synthesize silk from the beginning of larval stages but, predominant production takes place during 5th instar larval stage. Silk is produced by a specialized organ of silkworms called as silk gland. Silk production and fecundity play a very important role in sericulture industry. Polyamines are low molecular weight polycations present in all living cells and proved to be regulators for growth and cell survival. Many studies have shown binding of polyamines to DNA. In the present study we investigated DNA binding efficiency of spermidine and spermine with silk gland DNA and testicular DNA taking calf thymus DNA as control. Results showed that both the polyamines caused hypochromic shift to the silk gland and testicular DNA similar to calf thymus DNA. The hypochromic effect increased with increase in the concentration of the polyamine. Moreover, spermine was better than spermidine in binding to DNA. In the presence of spermidine and spermine, melting temperatures of silk gland and testicular DNA increased more than calf thymus DNA. Thus the study shows binding of polyamines to silk gland and testicular DNA is similar to their binding to calf thymus DNA and cause DNA condensation.

Key words : Silkworm; silk gland; testis; Spermidine; Spermine; DNA binding.

INTRODUCTION

Silk produced by silk glands [SG] of mulberry silkworm, *Bombyx mori* (*B. mori*) is of very high economic importance. Silk is gaining lot of use in biomedical studies. Quality and quantity of the silk produced depends on various factors like healthiness of the larvae, growth, nutrition, temperature, humidity and other factors [1]. In India, the productive strains of *B. mori* have been developed by Central Sericulture Research and Training Institute (CSRTI), Mysore. The hybrid CSR2 × CSR4 is well known for its superior qualities. Approximately 1000 meters of filament length is produced from a single cocoon of this strain [2].

Polyamines play a very important role in cell growth, differentiation and regulate gene expression [3-6]. Polyamines bind to nucleic acids within the cells [7]. Apart from their transcriptional role they are also involved in regulating the structure of RNA and in translation [8]. Under physiological conditions spermine (Spm) and spermidine (Spd) are better than Putrescine (Put) in binding to nucleic acids [9]. Earlier reports observed that, positive charges on polyamines stabilize their DNA interaction [10]. Put, Spd and Spm increase the melting temperature (T_M) of DNA as much as 40˚C in a concentration dependent manner [10-11].

The present investigation was undertaken to check the effect of polyamine binding to SG and Testicular (TS) DNA of hybrid strain, CSR2 × CSR4. Results of the present study show that the polyamines, Spd and Spm bind to both SG and TS DNA. Spm showed better binding than Spd to SG DNA, TS DNA and calf thymus DNA (CT DNA). DNA melting studies showed increase in Tm values after polyamine binding. Moreover, the binding of Spd and Spm to SG and TS DNA is similar to their binding effect to CT DNA and caused DNA condensation.

MATERIALS AND METHOD

STANDARD POLYAMINES

Spermidine free base (RM 5438), Spermine (RM 7506), Calf thymus DNA (RM 362) were purchased from Hi media chemicals.

ISOLATION DNA FROM SG AND TS

A pair of SG and TS was isolated from *B. mori* and flash frozen in liquid nitrogen. Tissue were homogenized in lysis buffer (Tris 50mM, EDTA- 10mM, NaCl 100mM, SDS 2%) and treated with Proteinase K (200µg/ ml), incubated overnight at 37˚C. DNA was extracted by adding phenol: chloroform: isoamylalcohol followed by chloroform: isoamylalcohol (24:1 ratio). DNA was precipitated by adding 3M sodium acetate (pH 4.8) and ethanol followed by incubation at -86˚C for 2 hours. DNA was pelleted down and pellet was washed with 70% ethanol, air dried and was dissolved in autoclaved Millipore water. RNA contamination was removed by adding RNase A (100mg / ml) .The isolated SG and TS DNA were quantified at 260 nm and used for DNA binding studies.

DNA BINDING ASSAY

Different concentrations of Spd and Spm (50 µM and 100 µM) were prepared. CT-DNA (100 µg), TS DNA (100 µg) and SG DNA (100 µg) were taken, different concentrations of polyamines were added and the final volume was made to 1ml with Millipore water. The mixture was incubated at room temperature for 15 minutes. Absorbance was measured at different wavelengths ranging from 220 nm - 340 nm and graphs were plotted.

MELTING TEMPERATURE STUDIES

To SG, TS and CT DNA, polyamines were added and incubated at different temperatures (40°, 60°, 80° and 100° C) for 10 minutes. After incubation samples were cooled and absorbance was measured at 260 nm.

Table 1: DNA melting temperature of SG, TS and CT DNA

Melting temperature calculations of SG, TS and CT DNA after different polyamine treatments.

S.No	Biomolecules	Concentration (µM)	Melting temperatures (T_M) °C
1	CT DNA	----	60
2	CT DNA	SPD 100	62
3	CT DNA	SPM 100	65
4	SG DNA	----	65
5	SG DNA	SPD 100	68
6	SG DNA	SPM 100	70
7	TS DNA	----	65
8	TS DNA	SPD 100	69
9	TS DNA	SPM 100	72

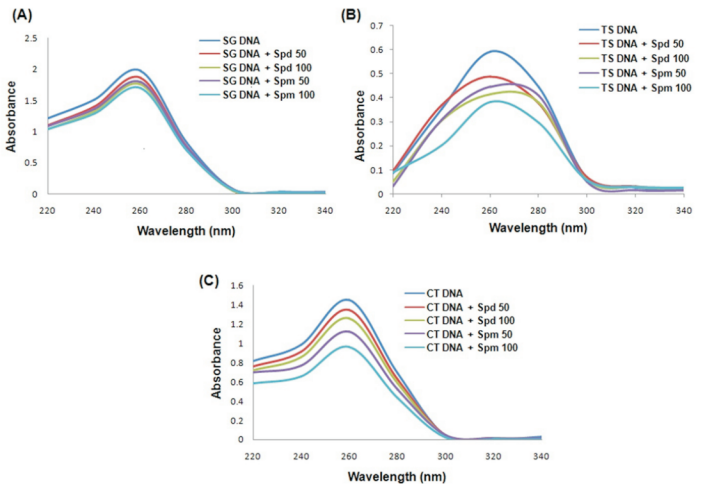

Fig 1: DNA binding study

Binding of Spd and Spm to SG, TS and CT DNA. (A) Represents binding to SG DNA (B) Represents binding to TS DNA and (C) Represents binding to control CT DNA. X-axis represents Wavelength in nm and Y- axis represents absorbance

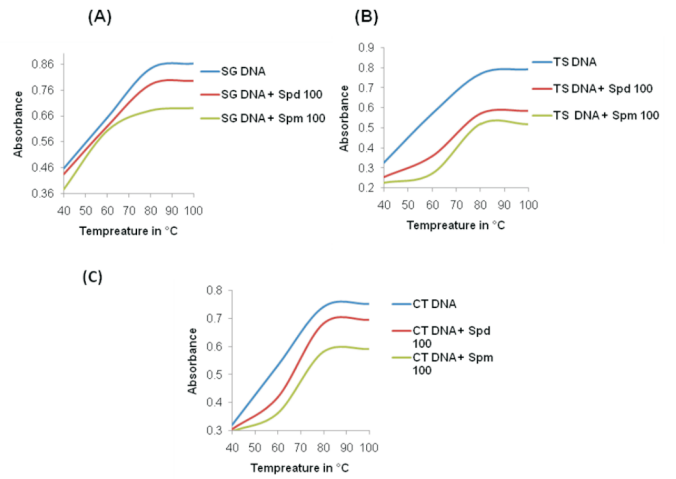

Fig 2: Thermal denaturation studies

Melting temperature studies of Spd and Spm with SG, TS and CT DNA. (A) Represents the melting studies of SG DNA (B) Represents the melting studies of TS DNA and (C) represents the melting studies of control CT DNA. X-axis represents Temperature in °C and Y- axis represents absorbance at 260 nm.

RESULTS

The present study was focused on the interaction of Spd and Spm with SG, TS and CT DNA by UV- visible spectroscopy. 50 µM and 100 µM concentrations of polyamines were checked for binding studies (Fig 1). Hypochromicity (decreased absorbance) was observed upon addition of Spd and Spm to SG, TS and CT DNA. SG and TS DNA showed greater hypochromic shift when compared to CT DNA. 100 µM showed more hypochromic effect compared to 50 µM in both SG and TS DNA. Of the two polyamines, Spm showed more hypochromic effect than Spd.

MELTING TEMPERATURES

Melting temperature was used to determine the denaturation capability of DNA by UV- spectroscopic method. The values of T_M in the presence and in the absence of polyamine (Spd and Spm) were determined at temperatures ranging from 40 °C to 80 °C for SG, TS and CT DNA. The melting curves were plotted (Fig 2) and the T_M values were calculated (Table 1). The T_M values increased upon addition of Spd and Spm to SG, TS and CT DNA. The T_M of CT DNA increased by 2 °C with Spd 100 µM and by 5 °C with Spm 100 µM. The T_M of SG DNA increased by 3 °C with Spd 100 µM and by 5 °C with Spm 100 µM. The T_M of TS DNA increased by 4 °C with Spd 100 µM and by 7 °C with Spm 100 µM. Change in the T_M was more with the TS DNA than SG and CT DNA upon addition of Spm 100 µM.

DISCUSSION

Gradual decrease in absorbance of SG and TS DNA with Spd and Spm indicated possible electrostatic interaction between Spd and Spm with DNA. Spectroscopy study also showed no shift in the wave length upon interaction with polyamines. The molecular mechanism of polyamine function in DNA condensation is presumed to involve neutralization of the negatively charged DNA backbone by positively charged amino group of polyamines [12-14]. Hypochromic effects are spectral features of DNA concerning its double helix structure [15]. Hypochromism results

from the contraction of DNA along the helix axis, as well as from the change in conformation on DNA [16]. Further studies are required to explore the molecular changes caused by polyamines after binding to SG and TS DNA.

Intercalation of small molecules with DNA can influence T_M. By binding, they stabilize the molecule structure and increase the T_M [17]. The interaction of Spm 100 µM with SG and CT ds-DNA showed increased T_M values nearly by 5°C and TS showed increased by 7°C. The ΔT_M suggest that Spm and Spd exhibit intercalation binding mode with ds-DNA and stabilise the DNA structure.

CONCLUSION

The present study is a first report of the DNA binding studies of polyamines with silkworm DNA. Studies from our lab have also shown that addition of Spd cause upregulation of fibroin [18] and Myc gene expression whereas expression of Atg 8 and Catalase were found to be unchanged after Spd addition [19]. Addition of Spd and Spm in micromolar concentrations cause hypochromic shift and increased the melting temperature of silkworm DNA thus resulting in conformational changes. More detailed studies may be required to find the local changes in the DNA conformation which results in different expression levels of DNA.

ACKNOWLEDGEMENTS

This work was supported by University Grants Commission (UGC) as UGC MRP grant (Grant Number: MRP-MAJOR-AGRI-2013-13665) to Anitha Mamillapalli.

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