

## Structure and thermodynamics of Leishmania protein Sirtuin interaction with resveratrol polyphenol.

Paola P. Bertuzzo (IC), Regina Adão (PQ), Carlos H. I. Ramos (PQ),

### Abstract

Resveratrol consumption has been linked to numerous health benefits, for its supposed interaction with proteins, such as Sirtuin - which have many functions in cellular health. It is known that resveratrol is a sirtuin activator; however the process of interaction is not entirely clear, this work aims elucidate the types and thermodynamics from the sirtuin and resveratrol interactions using a Leishmania protein as model.

*Key words: Protein Structure/Function, Protein/Ligand Interaction, Protein folding.*

### Introduction

Sirtuins (Silent Information Regulator-Sir) is a  $\text{NAD}^+$  dependent family of enzymes which is highly conserved, from *E. Coli* to humans. Its deacetylase activity is important to many biological purposes, such as gene suppression, DNA repair, aging and rDNA recombination. Some classes of small molecules, such as resveratrol are known to be excellent sirtuin activators due to its lowering of Michaelis constant for  $\text{NAD}^+$  and the acetylase substrate. To date, the nature of the interaction is not entirely clear, however elucidating the mechanism would be of great interest for medicinal therapy.

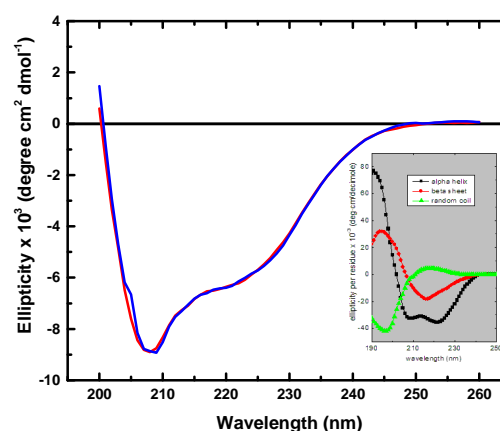
### Results and Discussion

In order to obtain the recombinant protein, a pET system was used, in combination with *E. Coli* cells BL21(DE3). The protein was obtained from cell lysates and was purified using two chromatography steps. Purity was assessed by SDS-PAGE gel electrophoresis.

Biophysical experiments were carried out to obtain information regarding sirtuin and its interaction with resveratrol. Differential scanning calorimetry was used to study the heat involved in the transition from the native state of sirtuin to its denatured state, obtaining its calorimetric behavior and transition temperature ( $T_m$ ). It was observed that the transition temperature of sirtuin is  $53^\circ\text{C}$ , and that the thermal unfolding was not reversible in the conditions tested (data not shown).

Circular dichroism is an experiment used to analyze the secondary structures of a protein in order to obtain information about the secondary structure, its dynamics and interactions. The experiment was done using PBS 50 mM, and TBS 50mM to resolve the sirtuin structure.

Figure 1. Molar ellipticity for sirtuin.



- Sirtuin in Phosphate buffer 50 mM, NaCl 50 mM, EDTA 2 mM, pH 7.4
- Sirtuin in Tris buffer 50 mM, NaCl 150 mM, EDTA 2 mM, pH 7.4

Insert figure: secondary structure pattern

From the spectrum it is possible to conclude that the protein was partially folded, having both  $\alpha$ -helical and random structures.

### Conclusions

As a result of these experiments, it's possible to conclude that sirtuin was produced pure and was partially folded.

The transition temperature observed for sirtuin was  $53^\circ\text{C}$ , and it is irreversible.

Experiments are underway to investigate the effect of sirtuin on these characteristics.

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