# Restoration of the calmodulin/adenylate cyclase interaction by the peptide methionine sulfoxide reductases system

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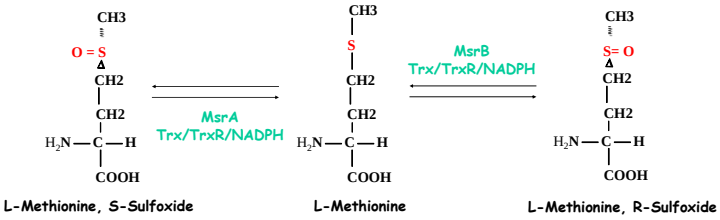
## INTRODUCTION

Proteins are sensitive to reactive oxygen species, and the accumulation of oxidized proteins has been implicated in the aging process and in other age-related pathologies. To prevent their accumulation in the cell, oxidized proteins can be either degraded through the proteasome pathway or repair by specific enzymatic repair systems. Methionyl residues are especially sensitive to oxidation leading to S- and R-methionyl sulfoxide diastereoisomers, the reversion of which is achieved by the peptide methionine sulfoxide reductases MsrA and MsrB respectively. In addition to its role in repair, the MsrA enzyme is part of the reactive oxygen species scavenging systems that are important in cellular antioxidant defence. Oxidation of methionine is generally associated with a loss of biological activity.

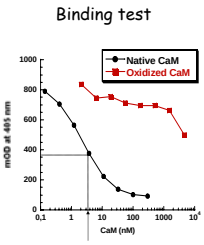
Methionine oxidation can also regulate protein function. In this study we investigated the interaction between Calmodulin (CaM) with Adenylate Cyclase (AC) from *Bordetella pertussis*, pathogenic agent of whooping cough. We focused on the potential role of methionyl residues and the effect of their redox state in this interaction.



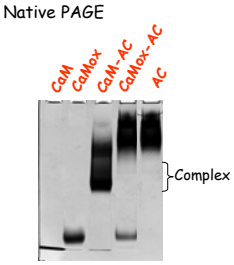

## RESULTS

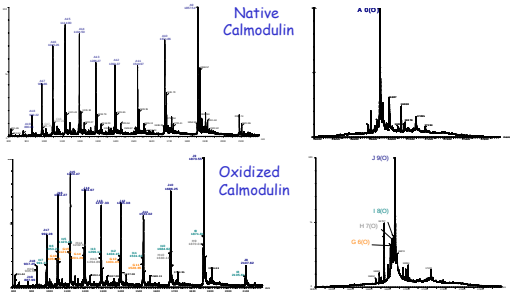
### 1- Oxidation of calmodulin methionyl residues abolishes the complex formation with adenylate cyclase.

*in vitro* calmodulin methionine are specifically oxidized in methionine sulfoxides with $H_2O_2$ 50 mM during 23h. SDS-PAGE and mass spectrometry (ESI-Q/TOF) analyses confirmed the oxidation of all nine methionines calmodul
A binding test experiment and native gel analysis showed that oxidized CaM did not interact with AC. ➡ The specific oxidation of methionine is critical for the complex formation.




$K_{0.5}$=3.5 nM




| Oxidized methionine | Symbol | Mesured Mass |
|---|---|---|
| 0 | A | 16708.95 ± 0.29 |
| 1 | B | 16724.79 ± 0.52 |
| 2 | C | 16740.62 ± 0.37 |

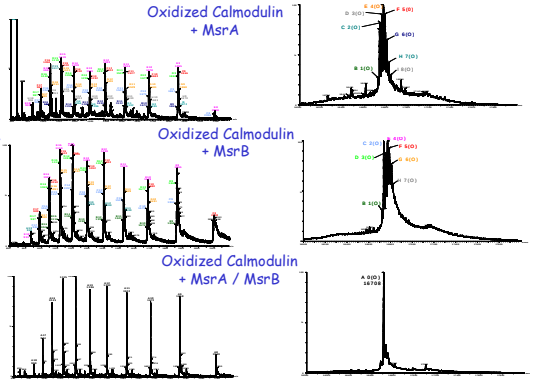
| Oxidized methionine | Symbol | Mesured Mass |
|---|---|---|
| 6 | G | 16804.84 ± 0.37 |
| 7 | H | 16821.87 ± 0.52 |
| 8 | I | 16836.64 ± 0.46 |
| 9 | J | 16852.69 ± 0.39 |

### 2- Reduction of oxidized CaM by MsrA/MsrB restores its ability to bind AC

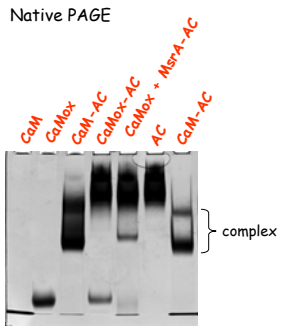
The reduction by MsrA or MsrB of oxidized CaM leads to a heterogeneous population containing one to seven methionine sulfoxides (see mass spectrum). The SDS-PAGE shows several bands corresponding to conformational differences of calcium fixation. From the binding test analysis, partially repaired CaM binds to AC less efficiently ($K_{0.5}$= 25 nM) than native CaM ($K_{0.5}$= 3.5 nM). Native gel reveals that whereas a fraction of repaired CaM can bind AC, it remains unbound CaM and AC.

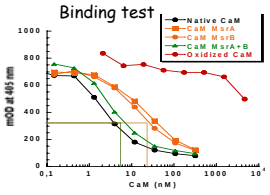
The incubation of oxidized CaM with MsrA and MsrB reduced totally the nine methionine sulfoxides. The repaired CaM migrates as the native protein on the SDS-PAGE. The mass spectrometry analysis showed a single major peak of 16708 Da corresponding to the molecular weight of native CaM. The binding test revealed a similar affinity for AC between repaired CaM ($K_{0.5}$= 6 nM) and native CaM ($K_{0.5}$= 3.5 nM). The migration as a complex on native gel confirmed this result.

➡ The oxidation of CaM is reversed by the peptide methionine sulfoxide reductases system and repaired CaM binds AC as efficiently as the native CaM.

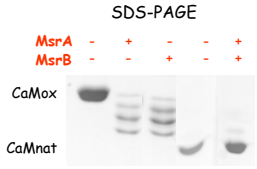



| Oxidized methionine | Symbol | Mesured Mass |
|---|---|---|
| 0 | A | 16709.00 ± 0.44 |
| 1 | B | 16725.59 ± 0.40 |
| 2 | C | 16741.19 ± 0.47 |
| 3 | D | 16757.03 ± 0.23 |
| 4 | E | 16773.39 ± 0.86 |
| 5 | F | 16789.88 ± 0.95 |
| 6 | G | 16804.84 ± 0.37 |
| 7 | H | 16821.87 ± 0.52 |
| 8 | I | 16836.64 ± 0.46 |
| 9 | J | 16852.69 ± 0.39 |



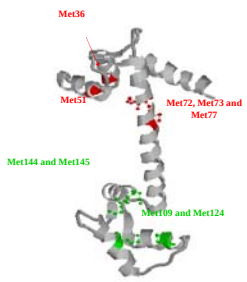

$K_{0.5}$ (CaM MsrA) = 25 nM
$K_{0.5}$ (CaM MsrA+B) = 6 nM




### 3- Identification of methioninyl residues implicated in the complex formation

The oxidized CaM partially reduced by MsrA is heterogeneous and contains one to seven methionine sulfoxides. This population is loaded on an AC-affinity chromatography column to isolate CaM forms which bind to AC. The unbound and bound fractions are collected and analysed by mass spectrometry (MALDI-TOF) after a total tryptic digestion. The methionyl residues located in the amino-terminus extremity, *i.e.* Met 36, 51, 72, 73 and 77, are oxidized in the peptides detected both in the unbound and bound fractions. Conversely, the C-terminus methionyl residues, *i.e.* Met109, 124, 144 and 145 are oxidized only in the unbound fraction.

➡ This result suggests that oxidation of the C-terminus methionyl residues abolished the CaM ability to bind to AC.




### Conclusion

The CaM binding to AC is prevented by its methionyl residues oxidation.The peptide methionine sulfoxide reductases system repairs the oxidized CaM in vitro and restores the interaction between CaM and AC. Among the nine CaM methionyl residues, the carboxy-terminus methionyl residues are implicated in the interaction with AC. Our data provide evidence that CaM function and the AC toxin action are regulated via methionyl residues oxidation/reduction by the peptide methionine sulfoxide reductases system.