行政院國家科學委員會專題研究計畫成果報告

結核桿菌之銅鋅超氧歧化酵素之調控及致病角色以及疫苗之研究

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一. 中英文摘要

Recent studies report that Mycobacterium tuberculosis CuZnSOD could protect specific membrane-associated targets from oxy-radical damage in bacterial intracellular survival. The predicted protein sequence contains 240 amino acids with a putative signal peptide at the N-terminus. We hypothesize that the signal peptide plays the roles in the export of this protein. We have constructed three forms of recombinant sodCs (L-sodC, M-sodC, and S-sodC) containing a poly-histidine tag at the very N-terminus and expressed these proteins in *E. coli*. L-sodC possessed the signal peptide whereas M-sodC and S-sodC are proteins without the signal peptide. Western blot with anti-sodC antiserum showed a smaller protein fragment of discrete size was present in the total bacterial extract of L-sodC, but not in those of either M-sodC or S-sodC. This smaller fragment was not detected with anti-His antibody specific for the N-terminal poly-histidine tag suggesting that the smaller protean was a product of L-sodC processed at the N-terminus. Functionality of the signal peptide was further supported by immunogold labeling electron microscopic experiment showing that L-sodC proteins were present in inclusion bodies as well as the periplasmic space. Surprisingly, recombinant M-sodC and S-sodC, which did not possess the signal peptide, were also found in the periplasmic space suggesting the presence of a parallel signal peptide-independent secretion mechanism.

最近的研究顯示 Mtb CuZnSOD 能保護 Mtb 免於氧化壓力的傷害而存活於吞噬細胞內。我們假設 Mtb CuZnSOD N-端的訊號蛋白質與 Mtb CuZnSOD 的分泌有關，利用分生方法我們合成三種蛋白質 L-sodC, M-sodC 及 S-sodC，其 N-端含有一 poly-histidine，這三種蛋白質皆能藉由大腸桿菌表現出來。L-sodC 含有 N-端的訊號蛋白質, M-sodC 及 S-sodC 則無；使用 sodC 抗體的西方墨氏反應發現有一小片段的蛋白質存在於 L-sodC 的全細菌萃取物，但 M-sodC 及 S-sodC 則無；改用 poly-histidine 抗體，L-sodC 一小片段的蛋白質則消失不見。此結果顯示 L-sodC 的 N-端的訊號蛋白質可在大腸桿菌被切除。利用電子顯微鏡切片染色顯示 L-sodC 存在於 inclusion body 及 periplasmic space。此研究結果顯示 Mtb CuZnSOD N-端的訊號蛋白質將 L-sodC 從 inclusion body 送至 periplasmic space 而成為分泌的 sodC.

二. 計畫緣由與目的

Cu,ZnSOD, encoded by the *sodC* gene and will be called *M. tuberculosis* SodC herein, of *M. tuberculosis* possessed a putative signal peptide at the N-terminal end and this protein was located at the periphery of the bacteria (42). In the report, we use biochemical fractionation as well as immunogold labeling electron microscopic examination to show that recombinant *M. tuberculosis* SodC expressed in *E. coli* can be processed at the N-terminal end and the processed forms are exported to the periplasmic space. We further show that *M. tuberculosis* SodC can also be transported to the periplasmic space in a signal peptide-independent manner. Significance of *M. tuberculosis* SodC being a secreted protein is discussed.

三. 結果與討論

***SodC* is secreted to the environment of *M. tuberculosis*.**

We have previously found that *M tuberculosis sodC* is present in the periphery of the bacterium (Wu, 1998). *sodC* was present in the ST-CF of 4 independent clinical isolates of *M. tuberculosis* indicating that sodC is indeed secreted to the environment.

M. tuberculosis sodC* protein is processed at the N-terminal end in *E. coli

We have constructed three different forms of fusion protein between a poly-histidine track and *M. tuberculosis sodC*, which can be purified to near homogeneity from the insoluble inclusion bodies. When the total extracts of *E. coli* expressing the recombinant proteins were subjected to a Western blot with the anti-*M. tuberculosis sodC* antibody, a novel band of smaller molecular weight was observed in the extract of L-sodC. This extra band is unlikely to be a non-specific proteolytic product of L-sodC since no equivalent product was found in M-sodC or S-sodC (FIG. 1). We reasoned that this product should be a specific proteolytic product of L-sodC and most likely to the result

of cleavage by *E. coli* signal peptidase recognizing the putative signal peptide of *M. tuberculosis sodC* protein. When the same blot was reprobed with antibody specific to the N-terminal poly-histidine track, the smaller band disappeared while L-sodC, M-sodC, and S-sodC remained stained.

M. tuberculosis sodC* protein is secreted to the periplasmic space in *E. coli.

Based on the previous results, it seems that the putative signal peptide of *M. tuberculosis sodC* can be recognized in the Gram-negative bacterium *E. coli*. If it were the case, then the protein should be transported to the periplasmic space and the transported protein should be in a processed form while the protein inside the bacterium should remain unprocessed. We tested this hypothesis by examining the subcellular localization of the processed *M. tuberculosis sodC* in *E. coli* by fractionation of the bacterial extract. Only the processed form of L-sodC was present in the periplasmic fraction while the full-length L-sodC was present only in the insoluble inclusion bodies. The shorter form of L-sodC is processed at the N terminal end as shown by probing the same blot with anti-His antibody. These results support the hypothesis that the putative signal peptide of *M. tuberculosis sodC* is functional in mediating secretion to the periplasmic space of *E. coli* and is cleaved upon transport. Surprisingly even though both M-sodC and S-sodC have the putative signal peptide removed in the constructs, these two proteins can also be transported to the periplasmic space.

Subcellular localization of *M. tuberculosis sodC* in *E. coli*

Subcellular localization of *M. tuberculosis sodC* in *E. coli* was further demonstrated by electron microscopic immunogold labeling experiment. The study shows sections of *E. coli* expressing *L-sodC* probed with anti-*M. tuberculosis sodC* antiserum. The gold particles were concentrated in the inclusion bodies, which represent the insoluble fraction. Heavy labeling was also found in the periplasmic space and in the areas alone the inner membrane. Bacteria expressing M-sodC (lacking the putative signal peptide) also showed labeling in both the inclusion bodies and the periplasmic space suggesting a non-signal peitide-mediated mechanism might also exists in the *M. tuberculosis sodC* protein.

計畫成果自評

In the report, we use biochemical fractionation as well as immunogold labeling electron microscopic examination to show that recombinant *M. tuberculosis* SodC expressed in *E. coli* can be processed at the N-terminal end and the processed forms are exported to the periplasmic space. We further show that *M. tuberculosis* SodC can also be transported to the periplasmic space in a signal peptide-independent manner.

We have done that the expression of *M. tuberculosis CuZnSOD* in a *E. coli* system could provide protection from exogenous ROS. Expression of L-CuZnSOD could enhance the concentration of both the periplasmic and intracellular SOD activities and therefore can provide protection against hydrogen peroxide and superoxide.

Because this study was only granted only for one-year, we could not study further the pathogenesis and vaccine development.

Refernece

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