二價汞及五價砷工業污染物誘導人類銅，鋅超氧化歧化酵素之結構改變

The structural changes of Human Cu,Zn-SOD molecule induced by the industrial contaminants mercury (II) and arsenate (V)

中文摘要

銅鋅超氧化物岐化酵素 (Cu,Zn-SOD) 屬同源雙體分子 (homodimer) 之抗氧化酵素。Cu,Zn-SOD 酵素活性降低是造成遺傳性肌肉萎縮性側索硬化症 (Familial Amyotrophic Lateral Sclerosis, FALS)的原因之一。在以往的相關報告中，在 E.coli SOD overexpression system 中加入重金屬以干擾 SOD 金屬結合位的實驗發現，環境中重金屬的污染，可能因上述機制而改變生物體內 Cu,Zn-SOD 的蛋白質結構，進而影響其酵素活性。因此，本研究中選擇以汞及砷酸鹽這兩種毒性最強，並且廣泛環境污染之重金屬元素，來觀察重金屬污染對細胞內 Cu,Zn-SOD 的影響。

利用大腸桿菌在 0.5 mM 的銅和 0.5 mM 鋅濃度下，過量表現之 SOD 蛋白具最高酵素活性。實驗顯示 E.coli 在 30 nM 的汞或 800 nM 的砷酸鹽仍可生存，但是當汞或砷酸鹽的濃度再提高時，E.coli 的生長則受到抑制。在 SDS-PAGE 分析中，發現 E.coli 之 Cu,Zn-SOD 蛋白質表現量，並不受各種濃度的汞或砷酸鹽的影響。SOD 酵素活性測試則顯示，在較高濃度的汞及砷酸鹽下所培養之 E.coli 表現的 SOD 蛋白，其酵素活性因汞及砷濃度之增加相對減少。以感應耦合電漿原子放射光譜儀 (ICP-AES) 偵測 SOD 蛋白內各金屬元素含量的實驗中發現，在 E.coli 培養液中，當汞高於 30 nM 或砷酸鹽高於 800 nM 的濃度時，SOD 蛋白中的含銅量減少；而其含鋅量在汞高於 10 nM，或砷高於 200 nM 時就增加約 35 % 及 100 %；SOD 蛋白內汞及砷的元素含量因外加劑量之增加而呈直線增加的趨勢。

利用圓振二向色性分析儀 (Circular Dichroism，CD)分析 Cu,Zn-SOD 來偵測 SOD 二級結構的實驗中發現，與不含重金屬的 SOD 蛋白比較，含有重金屬的 SOD 蛋白中，α-helix 和 β-sheet 的結構相對地減少。但若與 Cu,Zn-SOD 相比，則 SOD 是否具汞或砷皆不改變 Cu,Zn-SOD 之結構。

當含有四種重金屬的 SOD 蛋白與 apo-SOD 或 Cu,Zn-SOD 比較酵素活性時，含有四種重金屬之 SOD 的酵素活性明顯地比其他兩種 SOD 還低。另外在偵測這四種重金屬於 SOD 樣品中的含量時發現，當 SOD 含汞及砷時，蛋白中銅含量些微減少，但對蛋白中的鋅含量並沒有明顯影響。在四種金屬同時存在下，大腸桿菌所合成之 SOD 蛋白內除了銅和鋅外，又含有大量之汞及砷，可能因而影響了酵素活性。

外加的重金屬並不影響 E.coli 內 Cu,Zn-SOD 的表現量，但是經由替換銅的金屬

結合位會降低 SOD 蛋白的酵素活性。因此，重金屬的污染雖不對 Cu,Zn-SOD 之蛋白結構造成太大影響，卻使 SOD 酵素的蛋白與銅，鋅之結合不正確，進而降低 SOD 酵素活性，此極可能爲造成 ALS 致病原因之一。這些研究結果或能爲 ALS 的致病機轉提供一些新的研究方向。

英文摘要

Copper,Zinc-superoxide dismutase (Cu,Zn-SOD) is a homodimer antioxidant enzyme. The reduce in enzyme activity of Cu,Zn-SOD (SOD1) is one of the reasons that caused the familial amyotrophic lateral sclerosis (FALS). The pollution of heavy metal in the general environment may interfere metal binding site of the SOD protein, change its conformation, and lead to a reduced activity of Cu,Zn-SOD in the living being. Among the heavy metals, mercury and arsenate are two of the most toxic and widely polluted. Therefore, they have been selected to interact with Cu,Zn-SOD in this study.

The actitvities of purified Cu,Zn-SOD from E.coli overexpression system in LB broths containing various concentrations of copper and zinc were compared. The highest enzyme activity of Cu,Zn-SOD was purified from the system cultured in the 0.5 mM Copper and 0.5 mM Zinc . In the presence of 0.5 mM copper and zinc, E.coli can still grow in 30 nM of mercury or in 800 nM of arsenate. Once the concentration of mercury or arsenate got higher, the growth rate of E.coli declined rapidly. The Cu,Zn-SOD protein expression was not affected by the presence of different concentrations of mercury or arsenate in LB broth as judged by SDS-PAGE and Western blot. In addition, the activities of purified SOD proteins, from E.coli cultured in higher concentrations of mercury or arsenate, decreased dose-dependently. When the ICP-AES was used to detect the metal contents of SOD protein, it was found that less copper content was detected in the SOD protein when higher than 30 nM of mercury or 800 nM of arsenate was included in the LB broth. On the other hand, it appears that significant greater contents of zinc were found in the SOD proteins when more than 10 nM of mercury or 200 nM of arsenate were include in the LB broth. Furthermore, the secondary structures of Cu,Zn-SOD proteins from E.coli cultured in LB broth with various exogenous metals were analyzed with circular dichroism (CD). The data show that the SOD protein containing metals reduce the α-helix, β-sheet structures and turns as compared to the apo-SOD protein. However, there were not many changes among those containing various metals.

While comparing the enzyme activity of SOD protein from E.coli cultured in the presence of 4 metals (copper, zinc, mercury and arsenate) at the same concentration (15 nM each) in the LB broth, with that of apo-SOD protein and Cu,Zn-SOD, it was found that the former’s enzyme activity was least. Finally, the content of these 4

heavy metals in SOD protein was examed and found that the existence of mercury and arsenate caused a slight but not significant decrease of copper ion, however, the content of zinc ion was not affected by their presence.

In conclusion, either the presence of mercury or arsenate has affected the expression of Cu,Zn-SOD in E.coli, but they decreased the enzyme activity probably by replacing the binding of copper or zinc to the metal binding sites. Therefore, the presence of heavy metals in the culture broth might cause incorrect binding of metal ions to the SOD protein, and result in decreased the enzyme activity of SOD, which is a pathogenic reason of ALS. The obtained information may offer some directions to the studies on pathogenic mechanism of ALS.