行政院國家科學委員會專題研究計畫成果報告

計畫編號: NSC 88-2313-B-019-048
計畫名稱: 單胞藻對 DNA 外接鑑結物之切割修補作用
執行期限: 民國 87 年 8 月 1 日至 88 年 9 月 30 日
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一, 中文摘要

以抗癌藥劑 cisplatin 處理過之質體 DNA 為體外 DNA 修補試驗之基質證實小球單胞藻抽取液含有核酸切割修補作用蛋白質。單胞藻抽取液可使受 cisplatin(16.8 pmole)處理之質體 DNA(1 μg)增加 9 倍之修補合成作用。當固化在 agarose 顆粒上之紫外線傷害 DNA 與單胞藻抽取液在 30° C 培養 20 分鐘後, 此抽取液幾乎喪失切割修補 cisplatin 所造成 DNA 損害之能力,然而與未照射固化 DNA 培養之抽取液則有非常明顯之修補活性。此一現象顯示固化後之紫外線傷害 DNA 可有效吸附小球單胞藻抽取液中之 DNA 修補蛋白質。SDS-PAGE 蛋白電泳分析吸附後之蛋白質鮮示有一分子量介於 70 至 72 KDa 者對紫外線傷害 DNA 有明顯親和力,表示此一蛋白質極有可能參與小球單胞藻之核酸切割修補作用。

Abstract

An in vitro DNA repair synthesis assay monitoring nucleotide excision repair(NER) was established in cell-free extracts of unicellular alga *Chlorella pyrenoidosa* using cisplatin-damaged plasmid as the repair substrate. The ratio of cisplatin(16.8 pmole)-induced to control dAMP incorporation was estimated to be 9.0 in several experiments. A biotin-labeled 27 mer(2 μg) was irradiated with UV(27 KJ/m^2) and immobilized on streptavidin-conjugated agarose beads, and this insolubilized DNA was incubated at 30° C for 20 min with algal extracts to pull down NER factors by affinity adsorption. The extracts post incubation with the beads carrying unirradiated or no DNA ligand showed a repair factor between 8 and 9 for cisplatin-damaged DNA, whereas the extracts incubated with the beads carrying UV-damaged DNA almost lost NER capacity, reflecting that some factors crucial to NER had been captured by UV-damaged DNA immobilized on the beads. SDS-PAGE analysis of the captured proteins clearly indicated the preferential binding of a polypeptide about 70 to 72 KDa to UV-irradiated DNA. The binding of polypeptide p72 to control DNA was barely detectable, and this polypeptide was therefore highly speculated to play a role in NER in *C.pyrenoidosa*.

二. 緣由與目的

生物體 DNA 因紫外線照射而引起 CPD 及 6-4PPs 雙嘧啶體之形成已有詳細研究報告[1]。此兩種類型之 DNA 傷害在一般生物體內是由具有廣效性修補作用之核酸切割修補途徑(簡稱 NER)所去除[2,3],而辨識受傷 DNA 位置已知為整個 NER 機制之速率決定步驟。植物因光合作用之需要必須長時間暴露於太陽光包括紫外線之照射下,因此必須具備良好之 DNA 修補機制方能維持遺傳物質之完整。已有報告指出但植物植物可進行 NER 以移除紫外線產生之雙嘧啶體[4-6],但植物 DNA 修補蛋白之特性除少數高等植物外其他仍未明瞭[7]。本實驗室之前利用體外 DNA 切割試驗已證實小求單胞藻 *Chlorella pyrenoidosa* 抽取液中含有 NER 活性[8],並且利用離子交換樹脂與肝醣親和性管柱層析法自單胞藻抽取液分離出一個可結合受紫外線及抗癌藥物 cisplatin 傷害 DNA 之辨識活性,而此活性係由三個分子量分別為 72,80,90 KDa 之蛋白質所構成[9]。為確定此三個蛋白質是否與 NER 有關, 本計畫將一段寡核甘酸照射紫外線後固定在醣類顆粒上,再藉此寡核甘酸與單胞藻抽取液進行短時間親和性吸附測試何種蛋白質會與帶有雙嘧啶體作用, 若吸附上之蛋白質與 NER 有關則吸附後之抽取液 NER 活性與對照組比較應明顯下降。本計畫主要從功能性角度探索 *Chlorella pyrenoidosa* 抽取液中之 NER 相關蛋白。

三.結果與討論

將抗癌藥物 cisplatin 傷害過之 pGEMIV 質體 DNA 或正常質體分別與單胞藻抽取液反應進行 DNA 修補合成試驗,發現單胞藻蛋白可產生與 cisplatin 劑量呈正相關之修補合成作用,此一修補合成作用與每一管中正常 pBR322 質體 DNA 之放射性核甘酸參入量做比較時更為明顯(圖一)。以紫外線照射過或未照射過之固化寡核甘酸進行修補蛋白之親和性吸附, 發現被紫外線照射過寡核甘酸所吸附過之單胞藻抽取液已無修補 cisplatin 傷害 DNA 之能力,但是被正常寡核甘酸所吸附過之抽取液仍有明顯修補能力且此能力與祇與醣類顆粒反應之抽取液修補能力甚為接近(圖二), 表示本實驗採用之親和性吸附法確可抓下單胞藻抽取液中之 NER 蛋白。SDS-PAGE 蛋白電泳分析吸附後之蛋白質鮮示照射過與未照射過紫外線之寡核甘酸均可吸附相當多之蛋白質,惟有一分子量介於 70 至 72 KDa 者對紫外線傷害 DNA 有明顯親和力(圖三),表示此一蛋白質極有可能參與小球單胞藻之核酸切割修補作用。

四.計畫成果自評

本計畫成果約 80%達到預期成果,未來將朝分析 NER 蛋白氨基酸序列方向發展。本計畫成果已投稿於 SCI 期刊 Plant Science[10],目前已經過初步審查修改後進入再審查狀態, 表示本計畫成果應具有學術價值。

五.參考文獻

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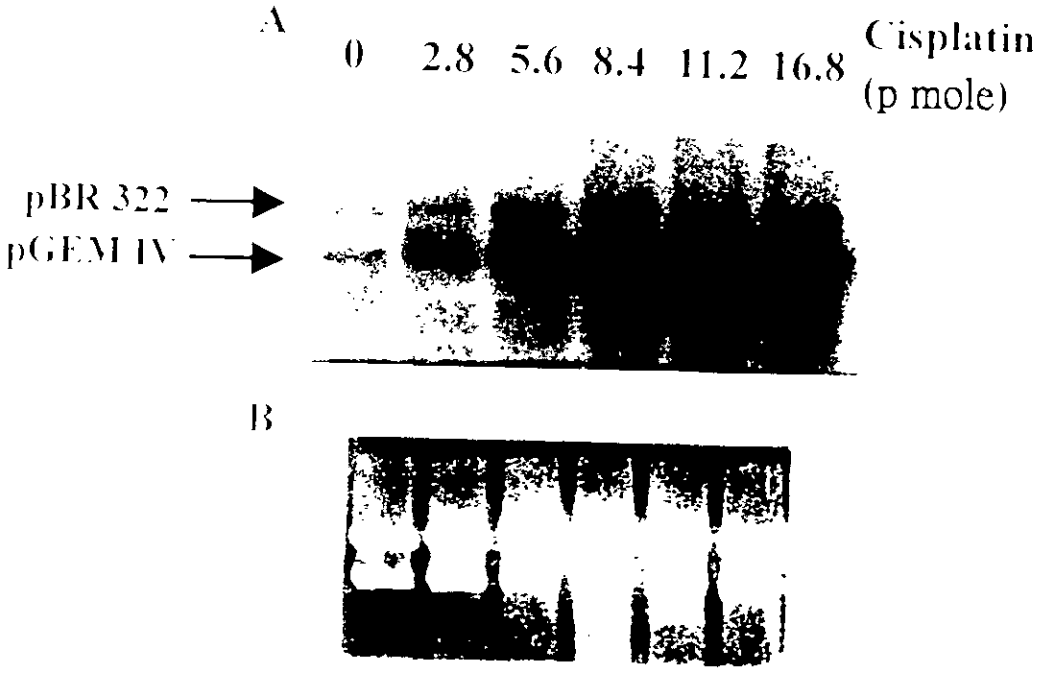

Fig.1 Excision repair of cisplatin-damaged plasmid DNA in cell-free extracts of unicellular alga *Chlorella pyrenoidosa*. Excision repair of cisplatin-treated pGEM IV plasmid was determined by DNA repair synthesis whose intensity was dependent on the amount of radioactive nucleotides incorporated. Plasmid pBR322 was included in each repair reaction as an internal control. (A) Autoradiography and (B) ethidium bromide staining of the gel are shown.

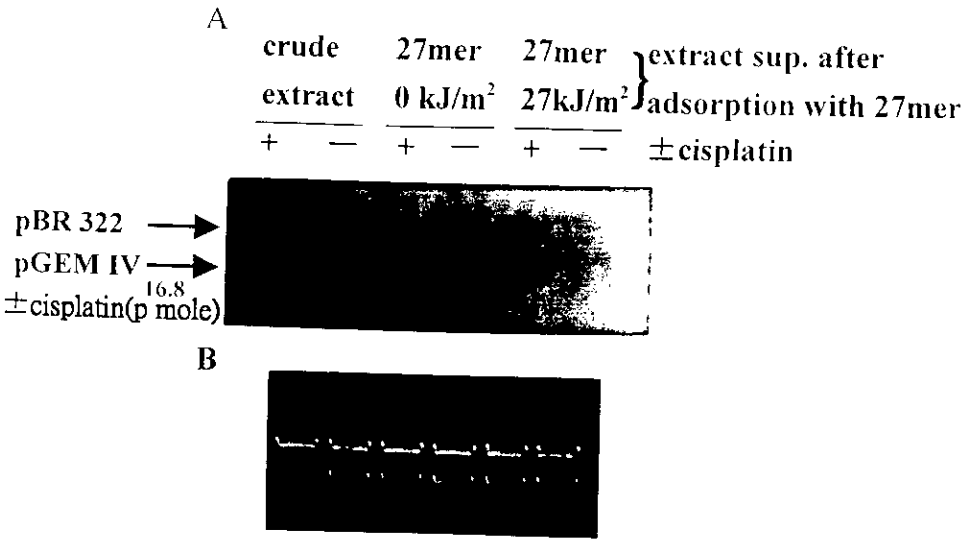

Fig.2 Affinity adsorption of repair proteins from cell-free extracts of *Chlorella pyrenoidosa*. Algal extracts were incubated with agarose beads carrying UV-irradiated, unirradiated or no DNA, and the extract supernatant was taken for DNA repair synthesis assay using cisplatin(16.8 pmole)-damaged DNA as the repair substrate. (A) Autoradiography and (B) ethidium bromide staining of the gel are shown.

Fig. 3 SDS-PAGE analysis of the proteins captured on the beads after affinity adsorption. Lanes 1-3 are nonspecific binding proteins washed away from the beads and lanes 4-6 are specific binding proteins captured by beads carrying no DNA, unirradiated DNA and UV-irradiated DNA, respectively.