嘉南藥理科技大學專題研究計畫成果報告

整合型計畫：硫氫化合物生物活性之研究
子計畫一：硫氫化合物之抗致突變性

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一、摘要

本研究探討硫氫化合物之抗致突變性，結果顯示四種硫氫化合物(0.0 - 5.0 mg) 對沙門氏菌變異株 TA98 與 TA100 均不會造成致突變性。在抗致突變之測試中，四種硫氫化合物對於 direct mutagen 的 MNNG 具有抗致突變活性。另外對於 indirect mutagen 的 IQ 亦具有抗致變活性，證實硫氫化合物同時具有抑制鹼基配對及支架移位突變之效應。

關鍵詞: 硫氫化合物、致突變性、抗致突變性

二、前言

硫氫化合物(thiols)是一種含有硫氫基之 mercaptan(硫醇)化合物。此種具有生理性功能之 thiols 被認為是防止細胞氧化之重要物質。彼等譬如 glutathione (GSH), captopril (CAP), cysteine (CYS), 及 homocysteine (HCYS)等廣為被研究，其已知之生理功計有 GSH 與 CYS 之 antitumor agent , anti-radiation,HCYS 之 atherosclerosis 之抑制性，及 CAP 之自由基捕捉性等功能(Demirkol et al., 2004)。再者，彼等亦被廣泛且深入探討其對其它功能障礙性之防止作用。邇來，有關基因毒性之探討頗為盛行，上述這些硫氫化合物是否具有此特性，有關這方面文獻甚為闕如。本研究乃針對上述硫氫化合物探討其對 IQ 與 MNNG 之抗致突變，以供消費者與產官學之參考。

三、結果與討論

硫氫化合物之致突變性

表一與表二為四種硫氫化合物對沙門氏菌變異株 TA98 與 TA100 在 S9 混合物存在下之致突變性試驗。由表中可看出添加 S9 混合物，0.5~5.0mg 之四種硫氫化合物所造成之回復突變數(revertants)與自突變數(spontaneous revertants)均很相近(顯示硫氫化合物不會造成致突變性)。另外在表三與表四亦可看出四種硫氫化合物對沙門氏菌變異株 TA98 與 TA100 在不添加 S9 混合物存在下，所造成之回復突變數與自突變數亦很相近。由表一至表四可得知四種硫氫化合物本身並不具備誘發致突變之特性。

硫氫化合物之抗致突變性

在測試抗致突變性之前，宜先釐清硫氫化合物是否具有毒性，同時宜測試不具毒性之劑量，以避免干擾實驗結果之判斷。本研究在添加或不添加 S9 混合物時，所使用 0.5-5.0mg 之硫氫化合物並不會對 TA98 與 TA100 造成毒性效應(data not shown) ，因此以 0.5-5.0mg 之硫氫化合物應用於 TA98 與 TA100 之抗致突變性試驗。表五為四種硫氫化合物對 IQ 誘發 TA98 致突變性之抑制效應。由四種硫氫化合物 0.5-5.0 mg 其抑制 IQ 誘發致變性為 0~30.1%，0~33.9%，0~37.5%與 0~20.3%，顯示四種硫氫化合物均表現出隨劑量增加有增加對 IQ 之抗致突變性。表六為四種硫氫化合物對 IQ 誘發 TA100 致突變性之抑制效應。四種 0.5~5.0mg 之硫氫化合物其抑制 IQ 誘發致突變性為 0~12.2%，0~32.6%，0~38.1%與 0~25.5%，與表五之結果有相同之趨勢。由表五與表六之結果可

看出硫氫化合物對IQ具有抗IQ之誘發突變之活性。表七為四種硫氫化合物對MNNG 誘發 TA98 致突變性之抑制效應，四種 0.5~5.0mg 硫氫化合物其抑制MNNG 誘發 TA100 致突變性約為 0~18.3% ， 0~27.4% ， 0~29.2% 與 0~13.7%。表八為四種硫氫化合物對MNNG 誘發 TA100 致突變性之抑制效應。四種 0.5~5.0mg 硫氫化合物抑制MNNG 誘發致突變性約為 0~31.6%，0~32.8%，0~36.8%與 0~28.6%，且隨劑量增加而增加。

綜合上述可知硫氫化合物對沙門氏菌變異株TA98與TA100均不會造成致突變性，而對 IQ 與 MNNG 則具有抗致突變性，至於其抗致突變性的有關機制則尚待進一步去探討。

四、參考文獻

Demirkol, O., Adams, C., and Ercal, N. 2004. Biologically important thiols in various vegetables and fruits. J. Agric. Food Chem. 52: 8151-8154.

表一、homosysteine (HCYS), cysteine (CYS), glutathione (GSH)與captopril(CAP)對 *Salmonella typhimurium* TA98 之致突變試驗

Table 1. Mutagenicity of homosysteine (HCYS), cysteine (CYS), glutathione (GSH) and captopril (CAP)toward *Salmonella typhimurium* TA98 with S9 mix

	No. of bacteria/plate*			
Sample (mg/plate)	HCYS	CYS	GSH	CAP
Spontaneous revertants	49 ± 2 (1.00) [ab**]	49 ± 2 (1.00) [a]	49 ± 2 (1.00) [a]	49 ± 2 (1.00) [a]
0.5	49 ± 4 (1.00) [ab]	50 ± 2 (1.02) [a]	48 ± 6 (0.98) [a]	49 ± 4 (1.00) [ab]
1.0	46 ± 1 (0.94) [b]	39 ± 1 (0.80) [b]	43 ± 4 (0.88) [a]	50 ± 1 (1.02) [ab]
2.5	52 ± 4 (1.06) [ab]	48 ± 5 (0.98) [a]	44 ± 3 (0.90) [a]	53 ± 1 (1.08) [ab]
5.0	55 ± 4 (1.12) [a]	49 ± 4 (1.00) [a]	45 ± 0 (0.92) [a]	55 ± 0 (1.12) [a]

* The no. of spontaneous revertants was determined without sample. Data are means ± SD of three plates. Mutagenicity ratio = induced revertants per plate/spontaneous revertants per plate.

** Data bearing same superscript letters within a column are not significantly different ($p < 0.05$).

表二、homosysteine (HCYS), cysteine (CYS), glutathione (GSH) 與captopril(CAP)對 *Salmonella typhimurium* TA100 之致突變試驗

Table 2. Mutagenicity of Homosysteine, cysteine, glutathione and captopril toward *Salmonella typhimurium* TA100 with S9 mix

	No. of bacteria/plate*			
Sample (mg/plate)	HCYS	CYS	GSH	CAP
Spontaneous revertants	142 ± 5 (1.00) [a**]	142 ± 5 (1.00) [a]	142 ± 5 (1.00) [a]	142 ± 5 (1.00) [b]
0.5	148 ± 6 (0.96) [a]	143 ± 8 (0.87) [a]	148 ± 8 (1.00) [a]	157 ± 7 (0.93) [a]
1.0	144 ± 5 (0.91) [a]	141 ± 4 (1.02) [a]	150 ± 1 (0.96) [a]	153 ± 5 (0.89) [ab]
2.5	150 ± 1 (1.09) [a]	149 ± 3 (1.02) [a]	144 ± 3 (1.17) [a]	154 ± 2 (1.28) [ab]
5.0	149 ± 1 (1.02) [a]	150 ± 0 (1.00) [a]	152 ± 5 (1.09) [a]	159 ± 0 (1.24) [a]

* The no. of spontaneous revertants was determined without sample. Data are means ± SD of three plates. Mutagenicity ratio = induced revertants per plate/spontaneous revertants per plate.

** Data bearing same superscript letters within a column are not significantly different ($p < 0.05$).

表 三、homocysteine (HCYS), cysteine (CYS), glutathione (GSH)與 captopril (CAP)對 *Salmonella typhimurium* TA98 之致突變試驗

Table 3. Mutagenicity of homocysteine (HCYS), cysteine (CYS), glutathione (GSH) 與 captopril (CAP) toward *Salmonella typhimurium* TA98 without S9 mix

	His^+ revertants/plate*			
Sample (mg/plate)	HCYS	CYS	GSH	CAP
Spontaneous revertants	46 ± 4 (1.00) ^{a**}	46 ± 4 (1.00) ^{a}	46 ± 4 (1.00) ^{ab}	46 ± 4 (1.00) ^{bc}
0.5	44 ± 3 (1.04) ^{a}	40 ± 2 (1.01) ^{b}	46 ± 1 (1.04) ^{ab}	43 ± 8 (1.11) ^{c}
1.0	42 ± 3 (1.01) ^{a}	47 ± 1 (0.99) ^{a}	44 ± 6 (1.06) ^{b}	41 ± 1 (1.08) ^{c}
2.5	50 ± 1 (1.06) ^{a}	47 ± 1 (1.05) ^{a}	54 ± 3 (1.01) ^{a}	59 ± 5 (1.08) ^{a}
5.0	47 ± 5 (1.05) ^{a}	46 ± 1 (1.06) ^{a}	50 ± 2 (1.07) ^{ab}	57 ± 1 (1.12) ^{ab}

* The no. of spontaneous revertants was determined without sample. Data are means ± SD of three plates. Mutagenicity ratio = induced revertants per plate/spontaneous revertants per plate.

** Data bearing same superscript letters within a column are not significantly different ($p < 0.05$).

表四、homocysteine (HCYS), cysteine (CYS), glutathione (GSH)與 captopril (CAP)對 *Salmonella typhimurium* TA100 之致突變試驗

Table 4. Mutagenicity of homocysteine (HCYS), cysteine (CYS), glutathione (GSH)與 captopril (CAP) toward *Salmonella typhimurium* TA100 without S9 mix

	His^+ revertants/plate*			
Sample (mg/plate)	HCYS	CYS	GSH	CAP
Spontaneous revertants	138 ± 5 (1.00) ^{a**}	138 ± 5 (1.00) ^{b}	138 ± 5 (1.00) ^{b}	138 ± 5 (1.00) ^{b}
0.5	144 ± 2 (1.04) ^{a}	144 ± 4 (1.04) ^{ab}	150 ± 6 (1.09) ^{a}	139 ± 1 (1.01) ^{b}
1.0	145 ± 7 (1.05) ^{a}	147 ± 4 (1.07) ^{ab}	148 ± 1 (1.07) ^{ab}	147 ± 6 (1.07) ^{b}
2.5	150 ± 8 (1.09) ^{a}	148 ± 1 (1.07) ^{a}	148 ± 4 (1.07) ^{ab}	158 ± 4 (1.14) ^{a}
5.0	147 ± 5 (1.07) ^{a}	143 ± 4 (1.04) ^{ab}	144 ± 3 (1.04) ^{ab}	158 ± 2 (1.14) ^{a}

* The no. of spontaneous revertants was determined without sample. Data are means ± SD of three plates. Mutagenicity ratio = induced revertants per plate/spontaneous revertants per plate.

** Data bearing same superscript letters within a column are not significantly different ($p < 0.05$).

表五、homocysteine (HCYS), cysteine (CYS), glutathione (GSH)與 captopril (CAP)於 *Salmonella typhimurium* TA98 系統對 IQ 之抗致突變試驗

Table 5. Antimutagenicity of homocysteine (HCYS), cysteine (CYS), glutathione (GSH)與 captopril (CAP) against the mutagenicity of IQ toward *Salmonella typhimurium* TA98 with S9 mix

	No. of bacteria/plate			
	IQ (5.0 g/plate) *			
Sample (mg/plate)	HCYS	CYS	GSH	CAP
0	1849 ± 34 (0) a**	1849 ± 34 (0) a	1849 ± 34 (0) a	1849 ± 34 (0) a
0.5	1788 ± 34 (3.4) ab	894 ± 47 (4.2) ab	1738 ± 21 (6.2) ab	1316 ± 21 (0.1) a
1.0	1706 ± 16 (8.0) b	853 ± 16 (6.7) b	1730 ± 27 (6.6) ac	1292 ± 17 (4.0) a
2.5	1572 ± 42 (15.4) c	787 ± 49 (16.4) c	1649 ± 88 (11.1) bc	1218 ± 42 (3.1) a
5.0	1307 ± 35 (30.1) d	656 ± 17 (33.9) d	1174 ± 40 (37.5) d	915 ± 44 (20.3) b
Spontaneous revertants	47 ± 2	47 ± 2	47 ± 2	47 ± 2

* The no. of spontaneous revertants was determined without mutagen. Control was with mutagen but without sample. Data are means ± SD of three plates. Inhibition (%) = [1-(sample revertants per plate-spontaneous revertants per plate/control revertants per plate - spontaneous revertants per plate)]×100.

** Values in parentheses are percentages relative to control value (100 %). Data bearing different superscript letters within a column are significantly different ($p < 0.05$). IQ: 2-amino-3-methylimidazo(4,5-f)quinoline.

表六、homocysteine (HCYS), cysteine (CYS), glutathione (GSH)與 captopril (CAP)於 *Salmonella typhimurium* TA100 系統對 IQ 之抗致突變試驗

Table 6. Antimutagenicity of homocysteine (HCYS), cysteine (CYS), glutathione (GSH)與 captopril (CAP) against the mutagenicity of IQ toward *Salmonella typhimurium* TA100 with S9 mix

	His^{+} revertants/plate*			
	IQ (5.0 g/plate)			
Sample (mg/plate)	HCYS	CYS	GSH	CAP
0	962 ± 18 (0) [ab**]	962 ± 18 (0) [a]	962 ± 18 (0) [a]	962 ± 18 (0) [a]
0.5	988 ± 65 (0) [a]	954 ± 10 (1.0) [a]	930 ± 6 (4) [a]	968 ± 42 (0) [a]
1.0	932 ± 30 (3.7) [ac]	937 ± 11 (3.1) [a]	919 ± 2 (5.3) [a]	962 ± 28 (0.1) [a]
2.5	854 ± 72 (13.2) [bcd]	832 ± 20 (15.9) [b]	740 ± 21 (27.2) [b]	838 ± 32 (15.2) [b]
5.0	863 ± 128 (12.2) [d]	696 ± 10 (32.6) [c]	651 ± 34 (38.1) [c]	754 ± 11 (25.5) [c]
Spontaneous revertants	145 ± 6	145 ± 6	145 ± 6	145 ± 6

* The no. of spontaneous revertants was determined without mutagen. Control was with mutagen but without sample. Data are means ± SD of three plates. Inhibition (%) = [1-(sample revertants per plate-spontaneous revertants per plate/control revertants per plate - spontaneous revertants per plate)]×100.

** Values in parentheses are percentages relative to control value (100 %). Data bearing different superscript letters within a column are significantly different ($p < 0.05$). IQ: 2-amino-3-methylimidazo(4,5-f)quinoline.

表七、homocysteine (HCYS), cysteine (CYS), glutathione (GSH)與 captopril (CAP)於 *Salmonella typhimurium* TA98 系統對 MNNG 之抗致突變試驗

Table 7. Antimutagenicity of homocysteine (HCYS), cysteine (CYS), glutathione (GSH)與 captopril (CAP) against the mutagenicity of MNNG toward *Salmonella typhimurium* TA98 without S9 mix

	No. of bacteria/plate*			
	MNNG (10.0 g/plate)			
Sample (mg/plate)	HCYS	CYS	GSH	CAP
0	629 ± 20 (0) ab**	629 ± 20 (0) a	629 ± 20 (0) b	629 ± 20 (0) ab
0.5	688 ± 34 (0) a	693 ± 20 (0) a	703 ± 28 (0) a	679 ± 52 (0) a
1.0	655 ± 56 (0) a	636 ± 49 (0) a	673 ± 45 (0) ab	650 ± 55 (0) ab
2.5	560 ± 25 (11.9) bc	543 ± 6 (14.9) b	527 ± 13 (17.6) c	631 ± 9 (0) ab
5.0	523 ± 15 (18.3) c	470 ± 18 (27.4) c	459 ± 10 (29.2) d	549 ± 34 (13.7) b
Spontaneous revertants	47 ± 2	47 ± 2	47 ± 2	47 ± 2

* The no. of spontaneous revertants was determined without mutagen. Control was with mutagen but without sample. Data are means ± SD of three plates. Inhibition (%) = [1-(sample revertants per plate-spontaneous revertants per plate/control revertants per plate - spontaneous revertants per plate)]×100.

** Values in parentheses are percentages relative to control value (100 %). Data bearing different superscript letters within a column are significantly different ($p < 0.05$).

MNNG: N-methyl-N'-nitrosoguanidine.

表八、homocysteine (HCYS), cysteine (CYS), glutathione (GSH)與 captopril (CAP)於 *Salmonella typhimurium* TA100 系統對 MNNG 之抗致突變試驗

Table 8. Antimutagenicity of homocysteine (HCYS), cysteine (CYS), glutathione (GSH)與 captopril (CAP) against the mutagenicity of MNNG toward *Salmonella typhimurium* TA100 without S9 mix

	His^+ revertants/plate*			
	MNNG (10.0 g/plate)			
Sample (mg/plate)	HCYS	CYS	GSH	CAP
0	1188 ± 86 (0) [a**]	1188 ± 86 (0) [a]	1188 ± 86 (0) [a]	1188 ± 86 (0) [a]
0.5	1092 ± 61 (9.2) [ab]	1121 ± 65 (6.4) [ab]	1178 ± 58 (1.0) [a]	1200 ± 11 (0) [a]
1.0	1026 ± 54 (15.6) [bc]	1013 ± 27 (16.8) [bc]	978 ± 57 (20.2) [b]	1160 ± 59 (2.7) [a]
2.5	935 ± 29 (24.3) [cd]	930 ± 18 (24.8) [c]	872 ± 11 (30.3) [bc]	977 ± 16 (20.3) [b]
5.0	859 ± 21 (31.6) [d]	846 ± 25 (32.8) [c]	804 ± 38 (36.8) [c]	890 ± 32 (28.6) [b]
Spontaneous revertants	145 ± 6	145 ± 6	145 ± 6	145 ± 6

[*] The no. of spontaneous revertants was determined without mutagen. Control was with mutagen but without sample. Data are means ± SD of three plates. Inhibition (%) = [1-(sample revertants per plate-spontaneous revertants per plate/control revertants per plate - spontaneous revertants per plate)]×100.

[**] Values in parentheses are percentages relative to control value (100 %). Data bearing different superscript letters within a column are significantly different ($p < 0.05$). MNNG: N-methyl-N'-nitrosoguanidine.