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Modification of external sulfhydryl groups of Ehrlich ascites tumor cells with 6,6′-dithiodinicotinic acid*

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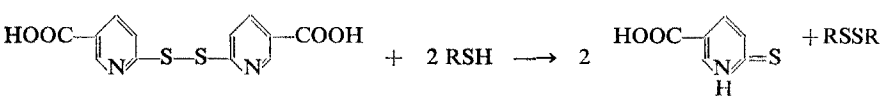
CERTAIN disulfides react with thiols in an irreversible manner, as exemplified for 6,6′-dithiodinicotinic acid (CPDS):[1]

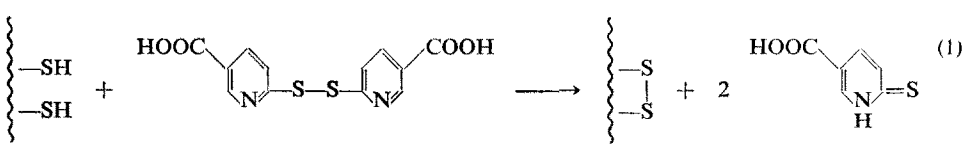


CPDS 6MNA

The fact that 6-mercaptonicotinic acid (6MNA) is virtually completely in the thione form prevents further interaction of this compound with disulfides. We have studied many other "thione-forming" disulfides, belonging to various heterocyclic systems, and found that they all react essentially irreversibly with thiols.[2] The shift in wavelength of the absorption maxima in the ultraviolet and visible range due to formation of the thione has been used as the basis of a method for the determination of thiols.[3]

Enzyme-catalyzed interactions occurring in living cells and leading to formation of 6MNA from CPDS have also been studied.[1] It has been shown that CPDS reacts with Ehrlich ascites (EA) cells to a lesser extent when these cells are intact than when they are broken.[1]

In the case of tissues, two possible reactions can be written between CPDS and cell thiols:



(Type A) (1)

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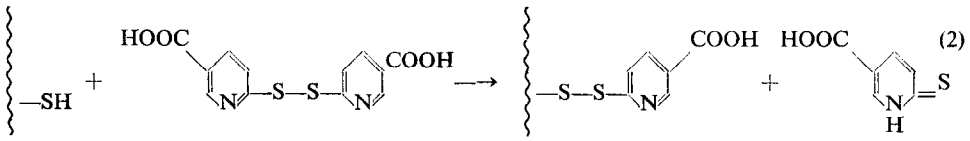

(Type B)

In both reactions the stoichiometry is consistent with the formation of 1 mole thione per mole of SH reacted. While it is difficult to assess the relative extent of these two possibilities, we present evidence that reaction 2 occurs to a significant extent with the peripheric SH groups of EA cells.

Treatment of washed EA cells with CPDS (10^{-3} M) (Table 1) results in the formation of 11×10^8 molecules of 6MNA per cell, in the supernatant. The total number of molecules of 6MNA obtained is a measure of the total amount of disulfides of types A and B (and of any disulfide bridges between

TABLE 1. INTERACTION OF 6,6′-DITHIODINICOTINIC ACID WITH EHRLICH ASCITES TUMOR CELLS*

Concn of 6,6′-dithiodinicotinic acid ($M \times 10^{-4}$)	Formation of 6-mercaptonicotinic acid (thione) [(Molecules/cell) $\times 10^8 \pm$ S. E.]			
	No.†	Supernatant S-1	No.†	Supernatant S-2
2	5	$7{\cdot}8 \pm 1{\cdot}2$	6	$0{\cdot}27 \pm 0{\cdot}05$
5	3	$9{\cdot}0 \pm 1{\cdot}7$	3	$0{\cdot}49 \pm 0{\cdot}11$
10	4	$11{\cdot}0 \pm 1{\cdot}9$	4	$1{\cdot}02 \pm 0{\cdot}25$

* Washed Ehrlich ascites cells[4] (800×10^6) were incubated for 5 min at 25° with CPDS (0–10 μmoles) in a total volume of 10 ml Krebs–Ringer phosphate (KRP) buffer, pH $7{\cdot}2 \pm 0{\cdot}2$. The gas phase was air. After the incubation, the cells were separated by centrifugation (5 min at 800 *g*). The supernatant fluid (S-1) was filtered through glass paper (Whatman GF/C) and the absorbance of the filtrate at 344 mμ determined against a blank containing the initial amount of CPDS in an equal volume of buffer. The cell pellet was washed several times with KRP buffer by repeated suspension and centrifugation. Four washings were usually sufficient to remove all the CPDS and 6MNA present. The washed cell pellet was resuspended in 5 ml of a solution of reduced glutathione (10^{-3} M in KRP buffer, pH $7{\cdot}2 \pm 0{\cdot}2$). After standing for 5 min at 25°, the suspension was centrifuged 5 min at 800 *g*. The supernatant fluid (S-2) was heated 5 min in boiling water. (This heat treatment does not affect 6MNA and permits the elimination of proteins which may decrease the accuracy of the determination. Such treatment is not possible when CPDS is present, as this compound is decomposed by heat.) After filtering through glass paper, the absorbance of the filtrate at 344 mμ was determined against the supernatant fluid of EA cells treated in the same manner, but in the absence of CPDS. Complete u.v. spectra in the range 220–400 mμ were taken of S-1 and S-2, in order to confirm the fact that the absorbance at 344 mμ was actually due to 6MNA.

† Number of determinations.

cells), in addition to 6MNA formed by enzymatic processes. The CPDS-treated cells can be separated and washed several times with buffer to remove all the 6MNA and CPDS in solution. If these washed cells are then treated with a thiol (glutathione, 10^{-3} M), an amount of 6MNA will be liberated, corresponding to about 1×10^8 molecules per cell. This figure represents the number of molecules of 6MNA which were attached to the cells as mixed disulfide (type B).

Enzymatic processes leading to formation of 6MNA occur at a considerable rate,[1] and could account for the difference between 11×10^8 and 1×10^8. By this method we are therefore not able to assess the formation of intracellular disulfides according to reaction 1, or of disulfide bridges between cells.

The amount of 6MNA formed per cell on treatment with CPDS increases with the concentration of CPDS (Table 1). This may be due in part to denaturation of the cell surface proteins by CPDS exposing further SH groups, and may indicate a slow penetration of CPDS into the cell. At the

concentrations of CPDS studied, less than 10 per cent of the 6MNA liberated is due to formation of mixed disulfides of type B.

Manometric experiments, carried out with the conventional Warburg technique,[5] showed that treatment of EA cells with 10^{-3} M CPDS, followed by washing, does not alter their rates of respiration and glycolysis. Respiration and glycolysis of EA cells in the presence of 10^{-3} M CPDS are also essentially unaffected. The results of preliminary experiments indicating that CPDS is not toxic are consistent with these findings.

The results reported in this paper provide a method for the determination of external (membrane-bound) and intercellular SH groups. The possibility is opened, through the reactions described here, of binding selectively the external SH groups of EA cells, thus preventing their normal reactions.

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Arequipa Foundation,
Russ Building,
San Francisco, Calif. 94104, U.S.A.

D. R. GRASSETTI
J. F. MURRAY, JR.

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Effect of 5-methylpyrazole-3-carboxylic acid on plasma free fatty acids and blood sugar in geese*

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THE ADIPOKINETIC effect of the catecholamines can be inhibited by a number of compounds,[1] but there is less information regarding the influence of pharmacological agents on the adipokinetic effect of glucagon. Because the lipolytic effects of catecholamines and glucagon are believed to be mediated by the same biochemical mechanism,[2] it is of interest to study whether the adipokinetic effect of glucagon is inhibited by substances known to inhibit the adipokinetic effect of the catecholamines.

The pyrazole derivative, 5-methylpyrazole-3-carboxylic acid (U-19425), has been shown to decrease the plasma free fatty acid (FFA) concentration of eviscerated rats and of intact rats receiving subcutaneous injection of glucose, and to inhibit the release of FFA from rat adipose tissue *in vitro*.[3] This compound lowers plasma FFA and blocks the adipokinetic effects of epinephrine and of human growth hormone in man.[3, 4]

The present report describes the results of experiments designed to test the effect of 5-methylpyrazole-3-carboxylic acid on the elevations of plasma FFA and blood sugar produced by glucagon in geese.[5]

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