The Effect of Cyanogen Iodide and Mercuric Chloride on the Permeability of Cells of *Pseudomonas aeruginosa* and the Antagonistic Action of Sulfhydryl Compounds (35916)

FREDERICK BERNHEIM

Department of Physiology and Pharmacology, Duke University Medical Center, Durham, North Carolina 27710

In a previous paper (1) the effect of mercurials and sulfhydryl compounds on the rate of swelling of cells of *Pseudomonas aeruginosa* after addition of sodium and potassium phosphate was described. Under certain conditions, sulfhydryl compounds decreased the swelling rate and reversed the accelerating effect of mercurials and oxidizing agents. The cation determined the rate and extent of the reactions. In the present study, the effect of the anion was studied. Mercurials increased swelling rates in sodium chloride to a much greater extent than in sodium sulfate with the effect in sodium phosphate intermediate. The reversible action of the mercurials was compared with the irreversible action of cyanogen iodide (ICN). This compound reacts as rapidly as mercury salts with sulfhydryl groups. It was thus possible to compare the rate of reaction of these compounds with the sulfhydryl groups of the bacterial cell by adding sulfhydryl antagonists to the cells at different times after addition of mercury or ICN. The results show that 30–50% of the reactive sulfhydryl groups in the cell react with both compounds within 10 sec at 23° and that 80–90% have reacted after 9 min. The rapidly reacting sulfhydryl groups are presumably on the surface of the cell or on easily available sites. The more slowly reacting groups may be in the membrane or prevented from reacting rapidly by steric hindrance. Since the effect of mercury is reversible, one would predict that the antagonist would be equally effective regardless of the time of its addition if the antagonist has access to all the sites of interaction. This is true for mercaptoethylamine, cysteine hydantoin, and dithiothreitol, but not for cysteine. This compound cannot apparently penetrate the membrane very far because it becomes increasingly less effective in reversing the effect of mercury as the latter is allowed to react for longer periods of time with the cells.

Methods. A pigmentless strain of *Pseudomonas aeruginosa* maintained in this laboratory was grown for 24 hr in 100 ml of Difco nutrient broth. The cells were centrifuged twice with water and then suspended in 28 ml of water. When 1.5 ml of this suspension was added to 8.5 ml of water the optical density at 500 μm (OD $\times$ 1000) was 150–160. The experiments were performed as follows. ICN or $HgCl_2$ in equimolar amounts each in 0.1 ml water were put into 50 ml Erlenmeyer flasks. The controls were cells without additions, with the antagonist in 0.1 ml and with ICN or $HgCl_2$ without antagonist. The final concentration in 1.5 ml of cells for ICN or $HgCl_2$ was usually 1.0×10^{-4} *M*, and for the antagonists 2.0×10^{-3} *M*. The antagonist was added to ICN or $HgCl_2$ 10 sec before the cells or 10 sec or more after the cells had been added to ICN or $HgCl_2$. The volume in each flask was then made up to 8.0 ml with water and allowed to stand 40 min before the addition of 2.0 ml of 1.0 *M* salt solution. The OD was read immediately and after 10, 20, and 30 min incubation in a shaker. All the procedures were carried out at 23°. ΔOD values are the differences between the mean values of the three readings of the experimental and the appropriate controls.

ICN was supplied by the Nutritional Biochemicals Corporation. The cyanogen halides according to Aldridge (2) react with cysteine to produce the stable addition product:

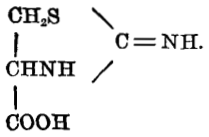

TABLE I. The Effect of Different Concentrations of ICN and $HgCl_2$ and One Concentration of *p*-Hydroxymercuribenzoate on the Swelling of Cells in Sodium Chloride, Sulfate, and Phosphate (pH 7.0) (0.2 *M* final concentration).

The compounds were added to 1.5 ml of cells, which were then diluted to 8.0 ml and incubated for 40 min before the addition of the salts. The ΔOD values are obtained by subtracting the mean of the readings at 10, 20, and 30 min incubation of the control from that of experimental.

M conc × 10^{-4}	Sodium chloride		Sodium sulfate		Sodium phosphate	
	ICN	$HgCl_2$	ICN	$HgCl_2$	ICN	$HgCl_2$
1	67	52	17	39	45	49
2	81	75	38	49	61	52
4	82	93	38	50	62	69
6	83	94	34	34	62	50
8	83	93	42	28	66	—
Mean	79	81	34	40	59	55
			p-Hydroxymercuribenzoate			
4	24		1		8	

The reaction is quantitative 1 mole/mole. When they react with gluthathione, acethylcysteine, or hemoglobin, an unstable intermediate is formed, which breaks down to HCN on the further addition of sulfhydryl compounds. When 1.0×10^{-4} *M* cyanide was added to bacterial cells there was no detectable effect on the swelling. At a concentration of 1.0×10^{-3} *M*, there was an average 20% inhibition because the swelling is primarily the result of diffusion aided only minimally by metabolic activity. The effect of ICN, which greatly increases swelling, could not be due to cyanide release. ICN will also react with methionine. This reaction is much slower than its reaction with sulfhydryl compounds. Inglis and Edman (3) showed that when BrCN reacted with methionine containing proteins and peptides the reaction with few exceptions took hours to reach completion. It was run in formic acid, presumably under optimal conditions. Preincubation of ICN at 23° with a large excess of methionine for 10 min before the addition of the cells did not show any loss of ICN as measured by the subsequent swelling of the cells. This does not definitely exclude the reaction of ICN with methionine in the membrane protein but it makes it unlikely.

Table I shows the effect of different concentrations of ICN and $HgCl_2$ on the swelling of cells in sodium salts. The differential between chloride, sulfate, and phosphate is real, since in concentrations above 1.0×10^{-4} *M*, the ΔOD values are maximal which means that 2.0×10^{-4} *M* saturates all available sulfhydryl groups in the cell. Presumably any conformational change in the cell caused by the reaction of the sulfhydryl groups is more critical for chloride than for the other two anions. Moreover, the reasonable agreement between the effects of ICN and $HgCl_2$ suggests that ICN is reacting only with sulfhydryl groups. The effect of *p*-hydroxymercuribenzoate is also shown in Table I. In its highest effective concentration it increases swelling in chloride, slightly in phosphate, and not significantly in sulfate. Since it reacts primarily with peripheral sulfhydryl groups these groups seem to be critical for the chloride ion. The ΔOD in the presence of the mercuribenzoate is 30% of that in the presence of ICN or $HgCl_2$. As shown below the ΔOD after 10 sec exposure to ICN or $HgCl_2$ is about 40% of the final. Presumably these rapidly reacting sulfhydryl groups are also peripheral. If the osmotic agents are chlorides or sulfates of lithium, potassium, rubidium, or cesium instead of sodium the same relative relationships hold and in no instance does the mercuribenzoate have any effect on the swelling in sulfate. Thus the mean ratios in the chlorides of these cations, ΔOD experimental/ΔOD con-

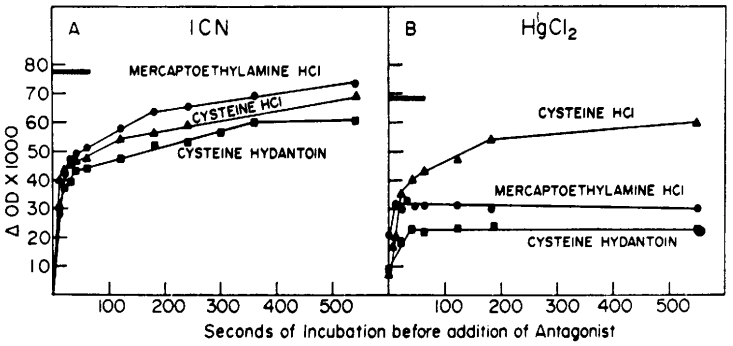

FIG. 1. The effect of different incubation times of cells with either 1×10^{-4} *M* ICN or $HgCl_2$ before the addition of 2×10^{-3} *M* cysteine HCL (neutralized), mercaptoethylamine HCL, or cysteine hydantoin on the rate of swelling in 0.2 *M* NaCl at 23°. The ΔOD is the OD of the experimental minus that of the appropriate control. The horizontal bar is the ΔOD in the absence of sulfhydryl compounds.

trol, was 2.85 for 1.0×10^{-4} *M* ICN and 2.44 for 0.5×10^{-4} *M* $HgCl_2$. In sulfate, the respective values were 1.90 and 1.69. For 4×10^{-4} *M* *p*-hydroxymercuribenzoate, they were 1.95 for chloride 0.95 for sulfate. The phosphates of lithium, rubidium, and cesium were too insoluble or not available. ICN and $HgCl_2$ have the same effect in sodium bromide and iodide as they do in chloride and BrCN behaves like ICN.

Figure 1A shows the time course for the reversal of the effect of ICN by three sulfhydryl compounds. The zero time value of zero was obtained by mixing ICN with the compounds 10 sec before the addition of cells and shows that no ICN remained to react with the cells. The horizontal bar is the mean of the ΔOD values in the absence of any of the antogonists. The rest of the points represent the ΔOD values corresponding to the times of incubation of ICN with the cells before the addition of the antagonist. After 10 sec incubation, the swelling is 36–51% of the final value; at the end of 9 min it is 78–87% of the final value. These variations are not significant. Between 10 and 20 sec the rate changes abruptly. Since sulfhydryl compounds only react with free ICN, these results show that ICN reacts with an average of somewhat less than 50% of the available –SH groups on the cell in 10 sec and that it requires 9 min or more to react with the rest of them. This delay presumably is the result of a slow diffusion to the remaining groups.

The effect of the sulfhydryl compounds on $HgCl_2$ is somewhat different (Fig. 1B). In the first place the zero time values are not zero. This does not imply that $HgCl_2$ reacts slowly with the compounds because it occurs when the mixing time is prolonged before addition of cells. It suggests that there is a competition during the preincubation period between the sulfhydryl compound and the cell –SH groups for the $HgCl_2$. Secondly, only cysteine has an effect similar to the effect all the compounds have with ICN. This suggests that cysteine can only react with the $HgCl_2$ when it is still in the cell periphery. As the $HgCl_2$ penetrates further, cysteine no longer has access to it. But mercaptoethylamine and cysteine hydantoin can apparently penetrate to all the sites with which $HgCl_2$ has reacted so that, after an initial irreversible phase, reversal occurs regardless of time of preincubation. The initial irreversible phase, which amounts to 12–13 OD units or about 18% of the total reactive –SH groups in the cell, is difficult to explain but it may indicate that some cell –SH groups have a higher affinity for $HgCl_2$ than the sulfhydryl compounds. Dithiothreitol behaves like the other sulfhydryl compounds with ICN and like mercaptoethylamine and cysteine hydantoin with $HgCl_2$. These same relationships hold when sodium sulfate or phosphate are used instead of sodium chloride, but since the ΔOD values are smaller it is more difficult to get accurate readings.

2-Mercaptopropionic acid, deaminated cysteine, penetrates the membrane but more

slowly than mercaptoethylamine. When added up to 60 sec after $HgCl_2$, it can almost completely reverse the effect of $HgCl_2$. Added 9 min after $HgCl_2$, the reversal is not complete, as it is for mercaptoethylamine, which indicates that there has been a delay in its penetration. Thus it is the presence of both an amino and carboxyl group in cysteine that blocks its access to all the reactive sulfhydryl groups.

Discussion. The rapid and irreversible reaction of ICN with sulfhydryl groups makes it possible to estimate the relative accessibility of such groups in the bacterial cell. The rapid but reversible reaction of $HgCl_2$ with cell sulfhydryl groups makes it possible to determine the degree of penetration of various antagonists to the different sulfhydryl sites. Both compounds react with approximately 40% of all available sulfhydryl groups in 10 sec of incubation at 23°. These groups can be considered to be on or near the surface of the membrane and are the ones that probably react with the nonpenetrating *p*-hydroxymercuribenzoate.

ICN and $HgCl_2$ have a much greater effect on the swelling of these cells in sodium chloride than in sodium phosphate or sulfate. This indicates that the conformational change which results from their reaction with sulfhydryl groups facilitates chloride entry to a greater extent than phosphate or sulfate. Since *p*-hydroxymercuribenzoate is much more effective in chloride, it is probable that the peripheral sulfhydryl groups on the cell are involved in chloride diffusion.

Summary. ICN reacts irreversibly with sulfhydryl groups in cells of *Pseudomonas aeruginosa*. Addition of sulfhydryl compounds to cells treated with ICN for different times indicates that approximately 40% of the reactive –SH groups in the cell react in 10 sec at 23°. Equimolar $HgCl_2$ produces the same increase in swelling as ICN. The mercury effect is reversible by compounds which can penetrate the membrane but not by those that do not. Both ICN and $HgCl_2$ as well as *p*-hydroxymercuribenzoate increase swelling rate to a greater extent when chloride is the anion than when phosphates and sulfate are.

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