The Effect of Sulfhydryl Compounds and Mercurials on the Swelling Rate of Cells of *Pseudomonas aeruginosa* in Sodium and Potassium Buffers (34576)

FREDERICK BERNHEIM

Department of Physiology and Pharmacology, Duke University Medical Center, Durham, North Carolina 27706

Several investigations have shown that sulfhydryl groups near or on cell surfaces are important in the regulation of cell permeability. Oxidizing agents and mercury compounds cause either loss of intracellular ions or the more rapid entry of extracellular ones. It is probable that these effects are the results of conformational changes in the membrane protein which occur when the sulfhydryl groups react with oxidizing agents or heavy metals. Schaechter and Santomassino (1) showed that *E. coli* and certain other gram-negative organisms lysed when mercury salts and oxidizing agents were added to them. Passow and Rothstein (2) and Passow *et al.* (3) found that yeast cells leaked potassium ions in the presence of mercury compounds and oxidizing agents and that this was an all-or-none effect. Van Steveninck *et al.* (4) have studied the effect of mercury compounds on the red cell membrane. Mercuric chloride binds all the sulfhydryl groups in the ghost preparations and when added to intact cells increases sodium permeability. *p*-Chloromercuribenzene sulfonic acid binds only about 25 % because it does not penetrate the membrane, but it inhibits glucose transport and ATPase activity, and causes potassium loss from the cell. By measuring the swelling rate of a pseudomonad in sodium and potassium solutions, Bernheim (5) showed that mercuric chloride increased the rate to a greater extent in sodium than in potassium and that cystamine inhibited swelling to a greater extent in sodium than in potassium solutions at pH 7.7. It was, therefore, of interest to investigate the effect of other mercurials, sulfhydryl compounds, and oxidizing agents on the swelling of cells in different salt solutions.

Methods. A pigmentless strain of *Pseudomonas aeruginosa* maintained in this laboratory for 18 years was grown for 24 hr in Difco nutrient broth. The cells were centrifuged, washed twice with water, and then suspended in water. When 1.5 ml of this suspension was added to 8.5 ml of water the optical density (OD $\times$ 1000) was 140–150. The compounds tested were added to 1.5 ml of the undiluted suspensions and allowed to remain at room temperature for 30 min. Then potassium or sodium phosphate buffer, 0.1 *M*, pH 6.2 or 7.7, was added to a final volume of 10 ml and the OD read immediately. The addition of the buffer increased the OD to about 200 as a result of the osmotic shrinkage of the cells, and this shrinkage was 10–15 units greater at pH 6.2 than at pH 7.7. The suspensions were then placed in 50-ml Erlenmeyer flasks and incubated at 34° in a Dubnoff shaker. Aliquots were taken after 40, 80, 120, and 160 min incubation for OD readings in a Coleman Junior Spectrophotometer at 500 mμ. Upon incubation the OD decreases as the cells swell because of the uptake of ions and their water of hydration. (No swelling occurs if isosmolar sucrose is used.) The rate of swelling, in both sodium and potassium solutions is always greater at pH 7.7 than at pH 6.2, but after 160 min incubation the extent is approximately the same although always greater in potassium than in sodium. The mean values of 8–10 experiments, obtained by subtracting the final from the initial OD were 77 and 79 for potassium at pH 6.2 and 7.7, respectively, and 55 and 52 for sodium. The results with the compounds are expressed as the mean differences in the readings between the control and experimental. A+ signifies a de-

TABLE I. The Effect of 5×10^{-3} *M* Cysteine Hydantoin, Cysteine, and Mercaptoethylamine on the Swelling Rate of Cells Suspended in 0.1 *M* K or Na Phosphate, pH 6.2 or 7.7, and Incubated at 34°. The Figures Are the Means of the Differences of Four Readings at 40, 80, 120, and 160 min Incubation Between the Experimental and Control OD. A + Signifies Inhibition; a — Acceleration of Swelling Rate. Figures in Brackets Were Obtained when 5×10^{-3} *M* Dithiothreitol Was Added.

Buffer	Cysteine hydantoin	Cysteine	Mercapto-ethylamine
K 6.2	+47 (+40)	+29 (+25)	+25 (+21)
K 7.7	+5 (+3)	+4 (+7)	+3 (+4)
Na 6.2	+14 (+17)	—6 (—6)	+12 (+14)
Na 7.7	+5 (—9)	+3 (—3)	+26 (+6)

crease in swelling rate, a— an increase.

The first experiment tested the effect of three sulfhydryl compounds, cysteine hydantoin, in which the amino and carboxyl groups are masked, cysteine, and mercaptoethylamine (MEA), all as hydrochlorides. They were added to the cells (the cysteine was neutralized with either sodium or potassium hydroxide) in a concentration of 5×10^{-3} *M* and incubated at room temperature 23° for 30 min before the addition of the buffer. The results are shown in Table I. Cysteine inhibits swelling only in potassium pH 6.2. Cysteine hydantoin shows the same effect but it causes a small but significant decrease in swelling in sodium pH 6.2. Dithiothreitol in equimolar amounts, which by itself has no effect on swelling rates, does not change the inhibition, which shows that the reduced form of these two compounds is the active one (figures in brackets in Table I). The behavior of MEA is anomalous. It causes significant inhibition except in potassium pH 7.7. Dithiothreitol does not affect the inhibition except in sodium pH 7.7. As previously shown, only the oxidized form is active under these conditions. It may, therefore, be acting as a diamine. It is well known that spermidine stabilizes membranes (6).

Under certain conditions mercury compounds and *N*-ethylmaleimide increase the swelling rate. One and a half milliliters of cells were incubated for 30 min at 23° with 5×10^{-5} *M* $HgCl_2$, 3.3×10^{-4} *M* sodium

TABLE II. The Effect of 5×10^{-5} *M* $HgCl_2$, 3.3×10^{-4} *M* PCMB and PCMBS, and 5×10^{-3} *M* NEM on the Swelling Rate of Cells Suspended in 0.1 *M* Potassium or Sodium Phosphate, pH 6.2 or 7.7, and the Effect of 5×10^{-3} *M* Cysteine Hydantoin (CH), Cysteine (C), and Mercaptoethylamine (MEA) Added 30 min after the Mercurials and NEM. The Figures and Symbols as in Table I.

Buffer	$HgCl_2$	+CH	+C	+MEA	PCMB	+CH	+C	+MEA	PCMBS	+CH	+C	+MEA	NEM	+CH	+C	+MEA
K 6.2	—40	+8	—1	+7	0				—30	—5	—11	—4	0			
K 7.7	—31	0	—13	—16	—15	—1	0	0	—28	—3	—3	—9	—8			
Na 6.2	—53	0	—26	—34	—31	—12	—7	—1	—33	—13	—6	—9	—34	—9	—16	—32
Na 7.7	—60	—4	—36	—35	—40	—7	—3	—7	—35	—11	—3	—5	—52	—33	—46	—48

TABLE III. The Effect of 1.25, 2.5, and 5.0 $\times$ 10^{-3} *M* Cysteine Hydantoin Added 30 min after 0.1 ml Iodine (Water Solution Saturated at 8°), 5 $\times$ 10^{-5} *M* $HgCl_2$, and 3.3 $\times$ 10^{-4} *M* PCMB on the Swelling Rate of Cells Suspended in 0.1 *M* Sodium Phosphate, pH 6.2. The Figures and Symbols as in Table I.

Compound		+1.25 $\times$ 10^{-3} *M*	+2.5 $\times$ 10^{-3} *M*	+5.0 $\times$ 10^{-3} *M*
I_2	−54	−46	−35	−19
$HgCl_2$	−56	−28	−8	+8
PCMB	−31	−9	−8	−2

p-chloromercuribenzoate (PCMB), 3.3 $\times$ 10^{-4} *M* sodium *p*-chloromercuribenzene sulfonate (PCMBS), or 5 $\times$ 10^{-3} *M* *N*-ethylmaleimide (NEM) before the addition of buffer. The results are shown in Table II. $HgCl_2$ increases the swelling rate under all four conditions but more so in sodium than in potassium. PCMB and NEM have little or no effect on potassium swelling but increase sodium swelling. PCMBS increases the swelling rate about equally under the four conditions. The effects of PCMB and NEM increase slowly with time of incubation, those of $HgCl_2$ and PCMBS remain constant. $HgCl_2$ apparently diffuses rapidly to its site of action, and PCMBS reacts only with surface sufhydryl groups. The organic mercurials and NEM increase the swelling rate in sodium to approximately the same extent and less than $HgCl_2$, probably because the inorganic compound has access to more sulfhydryl groups. It is, however, difficult to explain why PCMBS causes almost as much swelling in potassium as $HgCl_2$, whereas PCMB and NEM are essentally ineffective.

Table II also shows the effect of sufhydryl compounds on the increased swelling rate caused by the mercurials and NEM. The figures are the differences between the effects of the sulfhydryl compounds alone and in the presence of mercurials and NEM. All three compounds effectively prevent the increased swelling rate caused by PCMB and PCMBS. But only cysteine hydantoin, the least ionized compound, prevents the increase in swelling rate caused by $HgCl_2$ and NEM in sodium. Cysteine and MEA are as effective as cysteine hydantoin against $HgCl_2$ in potassium, pH 6.2, but only partially effective under the other conditions.

Iodine, 0.1 ml of a water solution saturated at 8°, and H_2O_2, 0.1 ml of a 3% solution, increase the swelling rate more in sodium than in potassium. Only cysteine hydantoin inhibits the increased swelling rate, but the inhibition occurs only in potassium at pH 7.7 and in sodium at pH 6.2. The other two compounds do not inhibit under any of the conditions. The oxidizing agents, the mercurials, and NEM increase the swelling rate in cells treated with 2.2 $\times$ 10^{-2} *M* formaldehyde. The percentage increase is approximately the same in control and treated cells. The increased swelling rate is not, therefore, the result of inhibition of cell metabolism but the result of changes in the cell membrane.

The sulfhydryl compounds must diffuse into the cell membrane, and this may be why high concentrations are needed and why the least ionized molecule is the most effective. It also follows that drugs which react nearest the surface should be affected by the lowest concentration of the antagonist. This is shown in Table III for cysteine hydantoin. The lowest concentration completely inhibits the effect of PCMB, partially inhibits the effect of $HgCl_2$, and produces little inhibition of iodine swelling. The next highest concentration inhibits $HgCl_2$ completely and iodine partially, and the highest concentration inhibits iodine, but not completely. In all cases the iodine was incubated with the cells for 30 min before the addition of cysteine hydantoin.

Discussion. With the exception of MEA in sodium at pH 7.7, the sulfhydryl compounds inhibit the rate of swelling when they are present in the reduced form. Their main effect is on the entry of potassium at pH 6.2. They may form mixed disulfides with sulfhydryl groups of protein or they may reduce disulfide linkages. In any case, they must

produce conformational changes which result in the prevention of the entry of potassium but only at pH 6.2. Anionic groups may be involved in the entry of cations into the cell because the rate of swelling is slower at pH 6.2 than at pH 7.7. Therefore, it appears that the sulfhydryl compounds are effective only when the ionization of protein anionic groups is depressed. The protein sulfhydryl groups which react with these compounds must be at a different locus from those which react with mercurials and NEM because these compounds increase the swelling rate more in sodium than in potassium and their effect is less dependent on pH. The greater effectiveness of $HgCl_2$ may be due not only to its ability to reach more sulfhydryl groups but to react with two of them, whereas the access of the organic mercurials and NEM may be limited by steric hindrance and their effectiveness in changing protein conformation may be less because one molecule reacts with only one sulfhydryl group.

All three sulfhydryl compounds reverse the effect of PCMB and PCMBS regardless of pH or cation. This is in agreement with the fact that these two compounds do not penetrate far into the cell membrane and, therefore, the charge on the sulfhydryl compound is not critical. But for $HgCl_2$, only the relatively uncharged cysteine hydantoin causes complete reversal regardless of pH and cation. Cysteine and MEA are completely effective only in potassium at pH 6.2, where they, when added alone, inhibit potassium entry. Cysteine hydantoin can also reverse NEM but not completely possibly because of the nature of the bond between NEM and sulfhydryl groups.

The results suggest that when sodium and potassium enter the cell under a diffusion gradient they go through different channels. The potassium channels are blocked by sulfhydryl compounds in the reduced form, the sodium channels are opened by mercurials and NEM.

Summary. Cysteine hydantoin, cysteine, and mercaptoethylamine inhibit the swelling rate of washed cells of a strain of *Pseudomonas aeruginosa* suspended in 0.1 *M* potassium phosphate, pH 6.2, but do not inhibit at pH 7.7. They are much less effective in sodium phosphate at both pH values.

Dithiothreitol, which itself has no effect on swelling rates, has no effect on the action of the sulfhydryl compounds with the exception of mercaptoethylamine in sodium phosphate at pH 7.7. In this condition, mercaptoethylamine is active only in the oxidized form, *i.e.*, as a diamine.

Mercurials and NEM increase the swelling rate in sodium phosphate at both pH values but have much less effect in potassium phosphate.

Sulfhydryl compounds reverse the effect of the mercurials. Cysteine hydantoin is the most effective.

Iodine and hydrogen peroxide increase swelling rates in sodium phosphate and to a lesser extent in potassium phosphate. Cysteine hydantoin is the best antagonist for both.

The oxidizing agents and the mercurials have similar effects in cells treated with formaldehyde.

1. Schaechter, M., and Santomassino, K. A., J. Bacteriol. **84**, 318 (1962).

2. Passow, H., and Rothstein, A., J. Gen. Physiol. **43**, 621 (1960).

3. Passow, H., Rothstein, A., and Loewenstein, B., J. Gen. Physiol. **43**, 97 (1959).

4. Van Steveninck, J., Weed, R. J., and Rothstein, A., J. Gen. Physiol. **48**, 617 (1965).

5. Bernheim, F., Biochem. Pharmacol. **15**, 1105 (1966).

6. Tabor, C. W., J. Bacteriol. **33**, 1101 (1962).

Received Sept. 18, 1969. P.S.E.B.M., 1970, Vol. 133.