The Effect of Formaldehyde and Acetaldehyde on the Swelling of Cells of *Pseudomonas aeruginosa* in Salt Solutions and Their Interactions with Amines and Carboxylic Acids (37092)

FREDERICK BERNHEIM

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

Gram-negative bacteria washed and suspended in water shrink when hypertonic solutions are added. If the membrane is permeable to the hypertonic agent, the cells subsequently swell and the rate and extent of this swelling is correlated with uptake of the agent. This has been shown quantitatively for potassium phosphate (1). Addition of compounds to the cells before the hypertonic agent affect the swelling rate either inhibiting or accelerating it depending on their reaction with the membrane. If certain compounds, *e.g.*, $HgCl_2$ react with specific groups, it is possible to infer the function of membrane sulfhydryl groups in regulating the permeability to anions and cations (2). With less specific compounds, *e.g.*, sulfonyl chlorides, their reaction with membrane amino groups can be inferred by the fact that carboxylic acids antagonize their effects (3). Formaldehyde reacts with amino groups and it like the sulfonyl chlorides increases the swelling rate. The present study was of the effects of formaldehyde, acetaldehyde and certain other compounds on the swelling rate and their interactions with carboxylic acids and amines.

Methods. A pigmentless strain of *Pseudomonas aeruginosa* maintained in this laboratory was grown for 24 hr at 34° in Difco nutrient broth. The cells were centrifuged and washed twice with water and then suspended in water so that 1.5 ml of this suspension diluted to 10 ml gave an optical density (OD × 1000) of 150–160 in a spectrophotometer at 500 nm. Compounds were added to 1.5 ml of the undiluted suspension and after 5 min water was added to 8 ml. Antagonists were usually added 5 min before the compounds. After approximately 60 min at 23°, 2 ml of *M* NaCl or Na phosphate (pH 6.8) were added to a final volume of 10 ml and the OD was read immediately and after 10, 20, and 30 min incubation with gentle shaking at 23°. As the cells swell the OD decreases. The values were substracted from the initial OD and the ΔOD was expressed as the mean of the three readings.

Results. Table I shows the effect of formaldehyde and acetaldehyde on the swelling of NaCl and the influence of previously added compounds. Acetaldehyde is approximately five times more effective in increasing the rate than is formaldehyde. Ethanolamine almost completely inhibits the effect of both aldehydes, but ethylenediamine more than doubles the effect of formaldehyde and partially inhibits that of acetaldehyde. Spermidine also increases the effect of formaldehyde but to a lesser extent and is a better inhibitor of acetaldehyde. Not shown in Table I are ethylamine which in 10 times the concentration has no effect on either aldehyde and spermine which almost completely inhibits both. Formate and acetate are approximately equally effective as inhibitors but antagonize acetaldehyde more than formaldehyde but propionate is less effective as is butyrate (not shown). Calcium chloride antagonizes both aldehydes but this is not specific since magnesium and barium chlorides do so also.

In order to study the effect of ethylene diamine in more detail the following procedures were used. Ethylenediamine dihydrochloride (400 mg) was dissolved in 1.0 ml water and 2, 1, 0.5 or 0.25 moles of formaldehyde were added and the mixture was diluted

TABLE I. The Effect of Various Compounds (Na Salts or Hydrochlorides) on the Swelling Rate of Cells in 0.2 M NaCl.[a]

	NaCl	Ethanolamine, 6.6×10^{-5} M	Ethylenediamine, 6.6×10^{-5} M	Spermidine, 6.6×10^{-5} M	Formate, 1.2×10^{-5} M	Acetate, 1.3×10^{-5} M	Propionate, 1.3×10^{-5} M	$CaCl_2$, 6.8×10^{-5} M
Formaldehyde, 5.0×10^{-3} M	33	4	80	62	27	24	30	2
Acetaldehyde, 2.2×10^{-3} M	77	0	62	30	32	34	61	36

[a] Formaldehyde or acetaldehyde were added to 1.5 ml cells 5 min after the compounds. The mixtures were diluted to 8.0 ml with water and 2.0 ml of M NaCl were added after 60 min. The concentrations are calculated on the basis of the final volume, 10 ml. The ΔOD are the mean of 3 readings at 10, 20, 30 min incubation. The control ΔOD has been subtracted.

to 10.0 ml with water. These mixtures were stable in the ice box for at least 1 mo. Table II shows the effect of these mixtures on the swelling rate of the cells. The mixture with 2 moles of formaldehyde was most effective, that with 0.25 mole was essentially ineffective in the concentrations used. However, 1600 μg of the 0.5 mole mixture and 800 μg of the 1.0 mole mixture have the same effect as 400 μg of the 2 mole mixture. This shows that any ethylenediamine which may not have reacted with formaldehyde does not affect the activity of the mixture.

Table II also shows that the spermidine formaldehyde mixture is approximately half as effective mole for mole as the ethylenediamine mixture and that the spermine mixture is ineffective. Putresine formaldehyde mixtures are also without effect. The ethylenediamine mixture has a definite threshold; 100 μg is completely inactive. Table III shows that this amount acts synergistically with formaldehyde, butyl alcohol and to a lesser extent chloroform but antagonizes acetaldehyde. The spermidine mixture is synergistic only with formaldehyde, and inhibits or has no effect on the other three compounds. The spermine mixture inhibits all of them and also inhibits the effect of 400 μg of the ethylenediamine mixture when it is used alone. The synergism between formaldehyde and the ethylenediamine mixture is the same with 1.0 or 0.5 mg of formaldehyde but is absent with 0.1 mg of formaldehyde. Like the aldehydes themselves carboxylic acids and ethanolamine antagonize the effect of the ethylenediamine mixture. This is shown in Table IV. There are however some differences. With the aldehydes formate and acetate are essentially equally effective antagonists and propionate less effective. With the mixture, formate is less effective than acetate and propionate and butyrate are almost as good as acetate. With other compounds which may react with amino groups of the membrane, such as sulfamoyl chloride and 1,2,3, 4-cyclobutane tetracarboxylic acid, acetate is also more effective. Thus formate, acetate, propionate, and butyrate inhibit the effect of 50 μg of the cyclobutane compound 68, 88, 25 and 22%, respectively.

TABLE II. The Effect of Various Mixtures of Ethylenediamine 2·HCl and Formaldehyde and of Spermidine 3·HCl and Spermine PO_4 and Formaldehyde on the Swelling of Cells in 0.2 *M* NaCl.[a]

Ethylenediamine	μg	ΔOD	Spermidine	μg	ΔOD	Spermidine	μg	ΔOD
2 moles	100	0	2 moles	400	16	2 moles	940	0
	200	30		600	34			
	400	80		800	49			
1 mole	200	11						
	400	61						
0.5 mole	200	0						
	400	22						
0.25 mole	400	0						

[a] Conditions as in Table I. Moles signify the formaldehyde concentration in terms of the amine concentration. The control ΔOD has been subtracted.

On the assumption that carboxyl groups protect membrane groups against attack by the ethylenediamine mixture, succinate should be twice as effective as acetate as an antagonist. That this is so is also shown in Table IV. The carboxyl groups presumably react with cationic membrane groups. If so, the carboxylic acids should not protect against compounds which react with sulfhydryl groups such as cyanogen iodide and azobenzene sulfonyl bromide which reacts specifically with sulfhydryl groups (4). The carboxylic acids do protect against these compounds but are only half as effective. Thus the ΔOD with 1,2,3,4-cyclobutane tetracarboxylic acid was 87 and acetate inhibited 92%. The ΔOD with cyanogen iodide was 62 and acetate inhibited 45%. The carboxylic acids thus appear to "stabilize" the membrane in a nonspecific manner as well as protecting amino groups against aldehydes and sulfanyl halides.

The carboxylic acids are probably acting on the membrane. Cells suspended in water do not oxidize them and if they are preincubated at 23° for 60 min before the addition of the ethylenediamine mixture or other compounds they are just as effective as antagonists as they are if the preincubation is 5 min. Moreover, if 30 min after the addition of, for example, succinate, the cells are washed once with water, the protection against the ethylenediamine mixture is completely lost. This argues for a reversible probably electrostatic bond in or near the surface. The order of addition of the agonists and antagonists is important. Acetate added 5 min before the mixture inhibited 65%. If the two were added simultaneously the inhibition was 49% and acetate added after the mixture inhibited only 24%.

The ethylenediamine mixture (2 moles) was evaporated at room temperature to minimize any volatilization of formaldehyde. Flat plates of different crystalline form from

TABLE III. The Effect of Equimolar Amine Formaldehyde Mixtures (2 Moles) on the Action of Formaldehyde, Acetaldehyde, Butyl Alcohol and Chloroform on Cell Swelling on 0.2 *M* NaCl.[a]

		Acetaldehyde		Butyl alcohol		Chloroform	
	ΔOD	(mg)	ΔOD	(mg)	ΔOD	(mg)	ΔOD
Formaldehyde, 1.5 mg	33	0.5	77	24	58	4.6	51
+100 μg ethylenediamine mixture	96		57		93		67
+200 μg spermidine mixture	65		35		58		40
+235 μg spermine mixture	3		0		13		0

[a] Conditions as in Table I.

TABLE IV. The Effect of Carboxylic Acids (Na Salts), Ethanolamine, Putrescine 2·HCl and Spermidine 3·HCl on the ΔOD caused by 400 μg Ethylenediamine Mixture (2 moles).[a]

Compound	Concn (*M*)	ΔOD	% Inhibition
—		87	
Formate	1.3×10^{-5}	46	47
Acetate	1.3×10^{-5}	26	70
Propionate	1.3×10^{-5}	34	61
Butyrate	1.3×10^{-5}	32	63
Succinate	0.65×10^{-5}	24	72
	1.3×10^{-5}	3	97
Ethanolamine	6.6×10^{-6}	4	95
Putrescine	6.6×10^{-6}	74	15
Spermidine	6.6×10^{-6}	65	25

[a] Conditions as in Table I.

ethylenediamine turned brown on slight warming. This indicated that a reaction had occurred between formaldehyde and ethylenediamine. The crystals were completely inactive. There are many possible reactions between the two compounds and whatever is formed as the solution is concentrated must be different from that present in dilute solution.

Discussion. The procedure used in these experiments measures the rate of cell swelling which depends on the rate ions and water enter the cells primarily by a diffusion process. The entry of water accompanies or immediately follows ion uptake because if sucrose is used as the osmotic agent the cells remain shrunk. Since regulation of ion entry is a function of the membrane, factors which affect entry must act on the membrane. A number of chemicals can change the configuration of the membrane and allow more rapid entry of ions and loss of osmotic regulations so that the cells swell and eventually may lyse. The data presented above show that certain compounds can protect the cell membrane from attack by the agents which cause swelling or lysis.

Ethylenediamine can slow the swelling rate of cells and partially counteract the effects of butyl alcohol and certain other agents which damage the membrane. Presumably it combines with membrane groups by electrostatic forces and acts as a cross-linking agent. In the presence of formaldehyde it damages the membrane and allows the cell to swell more rapidly. Formaldehyde must react with the amine groups and thus decrease their charge but since the configuration of the formaldehyde–ethylenediamine complex is unknown it is impossible to say what forces bind the complex to the membrane.

Ethanolamine antagonizes the effects of all the agents tested which increase swelling including those which react with sulfhydryl groups. Under certain conditions it is a better antagonist than spermine and spermidine. As an alcohol it could make a hydrogen bond with the membrane and as an amine bind electrostatically. Why this should make it such a good stabilizing agent is not clear. It is possible that it may enter the phospholipid layers and orient itself with the phosphatidyl ethanolamine in such a way as to block ion penetration through the membrane.

The carboxylic acids combine with the membrane and this is probably the required step for the induction of permeases. Under the conditions of these experiments with the cells suspended in water permeases are not induced. There is no indication that the acids enter the cell, because they can be easily removed by washing. They probably combine with amino groups because they antagonize agents which react with these groups. That they also have some protective action against agents which react with sulfhydryl groups indicates that they stabilize the membrane in some nonspecific way as well. Recently it has been shown that acetate and other short chain fatty acids inhibit the uptake of amino acids by *B. subtilis* and also inhibit growth and oxygen uptake (5, 6). These effects are also reversible.

Summary. A mixture of 1.0 mole of ethylenediamine and 2.0 moles of formaldehyde cause a rapid swelling of cell of *Pseudomonas aeruginosa* shrunk in 0.2 *M* Na chloride or phosphate. It is much more effective than formaldehyde alone. A similar mixture of spermidine and formaldehyde is less effective, and the spermine–formaldehyde mixture inhibits swelling. Low concentrations

of ethanolamine antagonize the effect of the ethylenediamine mixture as well as that of other agents which increase swelling rates. Ethylamine is inactive. Carboxylic acids also antagonize the effect of the ethylenediamine mixture as well as other agents. Low concentrations of the mixture which are by themselves without effect act synergistically with formaldehyde, butyl alcohol and chloroform.

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