EFFECT OF EDTA UPON BACTERIAL PERMEABILITY TO BENZYLPENICILLIN

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Millimolar EDTA (trisodium ethylenediamine tetraacetate) considerably enhances the penicillinase activity of whole-cell suspensions of several strains of Klebsiella aerogenes (Hamilton-Miller, 1964). This paper reports further experiments performed to elucidate this phenomenon.

Whole-cell washed suspensions of bacteria were prepared, as described by Smith and Hamilton-Miller (1963), in 25 mM sodium phosphate buffer pH 7.4. The penicillinase activity of each suspension was determined before and after disruption by ultrasonic treatment (Hamilton-Miller, 1964), using the hydroxylamine assay (Knox and Smith, 1962) with benzylpenicillin as substrate. For each strain, the "permeability factor" (Smith, Hamilton-Miller and Knox, 1964) for benzylpenicillin (= $P_{benzylpenicillin}$) was calculated thus: $P_{benzylpenicillin}$ = penicillinase activity of disrupted suspension ÷ penicillinase activity of suspension prior to disruption. As penicillinase is a wholly intracellular enzyme in each of the strains studied here (Hamilton-Miller, 1964), the magnitude of $P_{benzylpenicillin}$ for any strain can be taken as a measure of the permeability of intact cells of that strain to benzylpenicillin.

Table I shows that the penicillinase activity of whole-cell washed suspensions of 14 strains was increased in the presence of EDTA. The combination of benzylpenicillin and EDTA in concentrations present during assays did not cause either lysis (checked by measuring the extinction of

suspensions at 700 mμ before and after assay), or release of penicillinase activity from the cells; EDTA did not have a stimulating effect upon the

TABLE I. Effect of different concentrations of EDTA on penicillinase activity of whole-cell preparations of various Gram-negative bacteria

Species	Strain	P*	EDTA concentration					
			10mM	mM	500μM	100μM	50μM	10μM
Klebsiella aerogenes	1	6.5	6.2	5.9	5.7	4.65	2.9	
	43	11	8.75	7.6	6.4	4.9	1.7	1.25
	83	1.5	1.23	1.16	1.12	1.0		
	366	11		2.43	2.43	1.67	1.29	1.19
	370	8	2.6	2.25	2.25	1.9	1.2	1.08
	373	9	1.33	1.26	1.21	1.15	1.07	
	402	3	1.44	1.36	1.27			
	407	3	1.45	1.37	1.32	1.27	1.04	
	414	2	1.81	1.69	1.54	1.42	1.19	
	415	4	1.36	1.31		1.0		
	418	4	1.6	1.54	1.48	1.47	1.19	1.03
K. ozaenae	61	2.5	1.41	1.26	1.11	1.04	1.0	
Escherichia coli	214T	10	4.41					
	419	4	3.52			1.75		

* P = permeability factor for benzylpenicillin.

For each strain, activity of control suspension (no EDTA present) taken as 1.0. Substrate benzylpenicillin.

penicillinase activity of cell-free preparations from any of the strains used here. It was hence concluded that EDTA enhances the penicillinase activity of whole-cell suspensions by increasing the permeability of the bacterial cells to benzylpenicillin. Further studies on the permeability-increasing effect of EDTA (hereafter called the "EDTA-effect") were carried out using K. aerogenes strains 1 and 43, which show the effect the most markedly (Table I). The influence of pH on the "EDTA-effect" was investigated thus:

cell suspensions of 1 and 43 were assayed against benzylpenicillin in the presence of 25 mM sodium phosphate buffers of pH values ranging from 6.0 to 7.8, with and without the addition of mM EDTA. Table II shows that the magnitude of the "EDTA-effect" was strongly pH-dependent, reaching a maximum at pH values 7.4 - 7.6. This pH effect is not due to changes in the activity of the enzyme, as previous experiments (Hamilton-Miller, 1964) established that the activity of cell-free penicillinases from these two strains is practically independent of pH within the range used in these experiments.

TABLE II. Effect of pH value on action of EDTA on permeability of K. aerogenes strains 1 and 43 to benzylpenicillin

pH value	6.0	6.2	6.4	6.6	6.8	7.0	7.2	7.4	7.6	7.8
K. aerogenes 1	2.4	2.7	3.4	3.9	4.8	5.1	5.0	6.2	7.3	5.6
K. aerogenes 43	2.1	2.9	3.5	4.8	6.3	7.1	8.4	10.1	8.6	9.5

Assays carried out in presence of mM EDTA in phosphate buffer M/40 of stated pH value using whole cell suspensions. Results expressed as ratio of penicillinase activity in presence of EDTA to activity in control.

The following compounds did not affect the permeability of K. aerogenes 1 and 43 to benzylpenicillin, when tested at 1 mM in phosphate buffer pH 7.4: (a) other chelating agents (Hallaway, 1959) - oxalic acid, salicylic acid, αα'dipyridyl, o-phenanthroline, 8-hydroxyquinoline, Na pyrophosphate, glycine; (b) compounds containing primary, secondary or tertiary amino groups - β-phenylethylamine, DL lysine, Na p-aminosalicylate, Na p-aminobenzoate, diethylamine, dimethylformamide, p-dimethylaminobenzaldehyde; (c) sulphydryl and related compounds - thioglycollic acid, L cysteine, L cystine, glutathione (oxidised and reduced), β-mercaptoethylamine; (the last four compounds were also tested against K.aerogenes 366, 370 and 373, and E.coli 419; they had no effect).

(d) sulphydryl-blocking reagents - N-ethylmaleimide, Na p-chlormercuribenzoate. All these substances were also tested against cell-free penicillinase preparations, and in no case was there any inhibition or stimulation of activity. It appears, then, that the ability to increase the permeability of these Gram-negative bacteria is, so far, a property unique to EDTA.

Repaske (1958) reported that EDTA rendered E.coli, but not K.aerogenes, susceptible to lysis by lysozyme; this has been interpreted (Martin, 1963; Salton, 1964) as meaning that EDTA removes or reorganizes the outer layers of the cell-wall-membrane complex in E.coli. The present paper shows that EDTA also acts upon the surface structures of K.aerogenes, causing damage to permeability barriers. Such damage could be explained by the removal of divalent metal ions, as it has been found (Strange, 1964) that Mg^{2+} ions are necessary for the maintenance of functional permeability barriers in K.aerogenes. Indeed, results of preliminary experiments indicate that the "EDTA-effect" can be to some extent reversed by incubation of EDTA-treated cells with Mg^{2+} or Ca^{2+}. Further experiments are being carried out to determine the precise nature of the action of EDTA on the surface structures of K.aerogenes.

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