OAKLEY, C. L. & WARRACK, G. H. (1958). *J. gen. Microbiol.* **18**, 9-12

The Cysteine-Inactivated Proteinase (δ -antigen) of *Clostridium histolyticum*

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SUMMARY: Evidence is provided in support of the view of MacLennan, Mandl & Howes (1958) that some strains of *Clostridium histolyticum* produce a cysteine-inactivated proteinase—the δ -antigen.

Three soluble antigens in *Clostridium histolyticum* filtrates have been differentiated by immunological means: the necrotizing and lethal toxin α , the collagenase β , and the cysteine-activated proteinase γ . Though the existence of a cysteine-inactivated proteinase had been demonstrated by van Heyningen (1940), Oakley & Warrack (1950) were unable to show its presence in the culture filtrates they had used to differentiate the α -, β - and γ -antigens. Since then Mandl, MacLennan & Howes (1953) and MacLennan, Mandl & Howes (1958) have found a cysteine-inactivated proteinase (δ -antigen) in some filtrates of *C. histolyticum* cultures examined by them, and we here provide some evidence to support their views.

METHODS

Culture filtrate preparations. Through the courtesy of Dr J. D. MacLennan we received six dried partly purified preparations from filtrates of *Clostridium histolyticum*: P199, P208, P235, P245, P278 and P279.

Antisera. We used the six *Clostridium histolyticum* antisera referred to by Oakley & Warrack (1950).

Diluents. A mixture of equal volumes of sodium chloride solution (1%, w/v) and broth (broth+saline) was used as a non-activating diluent. For activation with cysteine the culture filtrate preparations were dissolved in 0.1M-cysteine (neutralized) made by dissolving cysteine in a little distilled water, adjusting the pH value to 7.1-7.3 (checked electrometrically) and diluting the mixture to 0.1M-cysteine with distilled water.

Assay of antisera. The antisera were assayed by conventional methods against RR2001 (100 units/ml.) as standard, with azocoll as indicator (Oakley, Warrack & van Heyningen, 1946). The level of test was 0.25 unit, and the standard indicating effect was the release of a certain amount of 'colour' from the azocoll. A suitable end-point tube was chosen from the assay of the standard serum that was always included in any set of tests, and used to determine the end-point, by interpolation when necessary, in tests of other sera. In tests with cysteine-activated filtrates, the tubes should be filled nearly to the top to prevent oxidation of the cysteine. The values given in the Tables are the average of at least three determinations; the error of a single test appears to be about 20%.

RESULTS

Activity of the preparations

All the preparations, dissolved in broth + saline, rapidly dissolved azocoll, releasing 'colour'; they had little or no effect on collagen paper, and no necrotizing effect on intradermal injection into guinea-pig's skin. Solution in cysteine increased the effect on azocoll of all preparations except P 245, whose activity was decreased.

Effect of cysteine on the test dose of the filtrate preparations

As Table 1 shows, the test doses of these preparations were invariably altered by solution in cysteine, as compared with those of material dissolved in broth + saline. In all but P 245 the test dose was much decreased; in P 245 it was increased twofold. Oakley & Warrack (1950) have already shown that the gamma antigen of *Clostridium histolyticum* is activated by cysteine, so that solution in cysteine, if it affected the test dose at all, would be expected to decrease it, if gamma were the only antigen affected. The fact that the test dose of P 245 was increased by solution in cysteine suggests that a cysteine-inactivated antigen was present in the preparation.

Table 1. *Test doses of Clostridium histolyticum filtrate preparations dissolved in broth + saline or in neutralized 0.1 M-cysteine*

Filtrate preparation	Test dose (mg.) of preparation dissolved in		Ratio of test dose in broth saline test dose in cysteine
	Broth + saline	Neutralized 0.1 M-cysteine	
P 199	0.125	0.062	2
P 208	0.125	0.018	7
P 235	0.44	0.021	21
P 245	0.38	0.68	0.5
P 278	0.20	0.023	9
P 279	0.20	0.022	9

Table 2. *Values of antisera against Clostridium histolyticum filtrate preparations dissolved in broth + saline, with azocoll as indicator*

Serum	Values of sera against							
	α -antigen	β -antigen	γ -antigen	P 199	P 208	P 235	P 245	P 278 P 279
RR 2001*	100	100	100	100	100	100	100	100 100
RR 2005	240	350	160	90	90	80	75	70 80
RR 2008	27	380	360	165	170	40-160†	175	150 160
RR 2035	570	250	150	200	185	100-400	185	170 200
RR 8003	55	210	550	125	125	60-240	120	130 120
RR 8006	73	160	410	150	140	80-320	150	140 140

* Standard.

† Inadequate amounts of preparation for closer test.

Values of antisera against partly-purified filtrate preparations dissolved in broth + saline

Table 2 gives the values of the six antisera against the known *Clostridium histolyticum* antigens for comparison with their values against the purified preparations. It is clear that the latter figures bear no relationship to the

values against the α -, β - or γ -antigen; we may therefore postulate another antigen (δ) as that responsible for the end-points in these tests.

Effect on antiserum values of solution of the filtrate preparations in cysteine

As Table 3 shows, solution of the preparations in cysteine had a considerable effect on the antisera assayed against them. Most of the values obtained in these tests agree very well with the anti- γ values of the antisera, if one allows for the accuracy of the tests; the values for antiserum RR 2005, and that for antiserum RR 8003 against filtrate preparation P 245, are however, rather lower than the known anti- γ values, and may be due to some other antigenic proteinase. When these results are compared with those for the change in test dose produced by solution in cysteine, it is clear that delta antigen is inactivated by cysteine.

Table 3. *Values of antisera against Clostridium histolyticum filtrate preparations dissolved in neutralized 0.1 M-cysteine, with azocoll as indicator*

Serum	Values of sera against								
	α -anti- gen	β -anti- gen	γ -anti- gen	P 199	P 208	P 235	P 245	P 278	P 279
RR 2001*	100	100	100	100	100	100	100	100	100
RR 2005	240	350	160	110	115	115	100	125	120
RR 2008	27	380	360	415	475	475	310	530	510
RR 2035	570	250	150	230	220	230	180	275	245
RR 8003	55	210	550	530	630	710	380	750	650
RR 8006	73	160	410	350	390	470	300	450	400

* Standard.

DISCUSSION

It is evident that when a culture-filtrate preparation contains a cysteine-activated and a cysteine-inactivated proteinase, solution of the preparation in cysteine will increase its activity, leave it unaffected, or decrease it, depending on the relative amounts of the two components and on the concentration of activating substances present. If we take the test doses of the filtrates in broth + saline as inversely proportional to their δ -antigen content, and those in cysteine as inversely proportional to the γ -antigen content, it is clear that a very considerable degree of purification has been obtained in some of them. Thus, at one end of the scale filtrate preparation P 245 has a γ : δ ratio of about 0.5:1, whereas filtrate preparation P 235, at the other, has a γ : δ ratio of 21:1, a forty-fold increase in purity for γ -antigen. The other preparations show intermediate increases in purity. Obviously the presence of other reducing agents in the broth + saline solutions may decrease their δ activity slightly, and thus slightly alter these ratios, but judging from the amount of cysteine necessary for complete inactivation of δ -antigens, this effect is very small. The δ -antigen is not destroyed by cysteine, for when the cysteine is oxidized by shaking the mixture with air the δ values are obtained when the shaken preparation is being used as antigen in serum assays.

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(Received 20 February 1957)