THE ANTIALDOLASE ACTIVITY OF PLAGUE ANTISERUM AND DIFFERENTIATION BETWEEN ALDOLASE AND TOXIN OF *Pasteurella pestis*

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When studying the mechanism of action of toxins, the investigator often has to examine the unpurified toxic fractions of microorganisms, in which, besides the actual toxins, other biologically active substances may be present. In such cases it may be difficult to decide where the action of the toxin ends and the action of the "contaminants" begins. Among the biologically active substances frequently accompanying toxins, bacterial enzymes may be found. For example, the staphylococcal enterotoxin contains apyrase [11], O-streptolyzin – diphosphopyridine nucleotidase [8], and staphylocoagulase – lipase [9]. Since many (if not all) enzymes, like toxins, possess antigenic properties [2], immunological methods can be used to purify toxins from their corresponding enzymes.

It was recently shown [4] that preparations of "mouse" toxin of *Pasteurella pestis* (fraction II) possess marked aldolase activity, and it was concluded from indirect evidence that aldolase and toxin are different components of the cell.

The object of the present investigation was to establish the presence of antialdolase activity in plague antiserum. Having done this, the next step was to compare the serological properties of the toxin and aldolas of *P. pestis*, and thereby to prove that these two components are different.

EXPERIMENTAL METHOD

The test object consisted of two toxic fractions of *P. pestis*. One of these (fraction II) was prepared from strain No. 17 by Baker's [6] method, the other (fraction III) was obtained from strain EB by our modification of Walker's method [3]. The mean lethal dose of fraction II when injected intrapertioneally into mice was 14.1 μg, and that of fraction III – 2.4 μg. Only fraction II possessed alsolase activity [4]. As antiserum, a serum was used that was obtained from a horse hyperimmunized with *P. pestis* strain EB.*

The presence of antialdolase in the serum was judged by the reduction in the aldolase activity in the supernatant fluid after keeping the test serum and the solution of the test fraction, initially at 37° (from 2 h), and then in the refrigerator (for 17 h). To determine whether the reduction in aldolase activity in the supernatant fluid was due entirely to precipitation of aldolase or whether some part was played also by neutralization with corresponding serum antibodies [4], the aldolase activity of the precipitate was also determined. The precipitate was separated by centrifugation of the mixture of serum with toxin at 10^4 rpm, washed three times, and resuspended in 2 ml of physiological saline.

The aldolase activity was determined by a modification of the method of Shibley and Leninger [5] and expressed as the degree of extinction, multiplied by 100 (units of activity†). The protein in the precipitates was determined by Lowry's method [10]. The standard used for construction of the calibration curve was horse serum, the protein concentration of which was estimated from the nitrogen content, determined by the micro-Kjeldahl method.

* The serum was obtained from L. E. Khundanov, to whom to record our gratitude.

† Using the type FEK-56 photocolorimeter and a No. 9 light filter.

TABLE 1. Effect of Plague Antiserum on Aldolase Activity of Fraction II

Concentration of fraction II (in mg of sample)	Protein content of precipitate (in mg)	Aldolase activity in presence of serum		
		normal control	immune	
			in supernatant fluid	in precipitate
10.0	11.2	3.256	0	1.008
5.0	9.3	1.960	0	448
2.5	6.7	980	0	0
1.2	4.2	420	0	0
0.6	2.6	168	0	0
0.3	2.3	90	0	0

Note. Precipitates were formed only in tests with immune serum.

TABLE 2. Effect of Sera on Aldolase Activity of Fraction II

Concentration of fraction II (in mg sample)	Aldolase activity in the presence of serum			
	normal	exhausted with fraction II	exhausted with fraction II	
			in supernatant fluid	in precipitate
5.0	2100	1848	0	630
2.5	1001	1008	0	0
1.2	525	504	0	0
0.6	140	196	0	0

Note. In the samples with serum exhausted with fraction II there was so little precipitate that it could not be estimated quantitatively.

To exhaust the immune serum, to every milliliter was added 5 mg of one of the test fractions. The mixture was kept initially at 37° for 2 h, and then in the refrigerator for 17 h. After centrifugation at 10^4 rpm, the precipitate was discarded. Since it was possible for the toxin to be removed only incompletely from the sera by the exhausting process, the toxicity of the supernatant fluid was verified in albino mice. Before this was done, this fluid was diluted in the same proportion as the mixture of normal serum with toxin used as a control; 0.2 ml* of the mixture contained approximately 2 LD_{50} of the toxin (the initial content of the toxic fractions in the normal serum was the same as that in the immune serum).

During the study of the antitoxic properties of the sera (original immune and exhausted), both fractions were added separately to each serum. The mixture was incubated for 2 h at 37° and then left overnight in the refrigerator; after removal of the precipitate the supernatant fluid was injected into albino mice. Before being injected, it was diluted in the same way as the supernatant fluid of the exhausted sera.

The results of the experiments on albino mice were read after 48 h. If necessary, they were subjected to statistical analysis [1].

EXPERIMENTAL RESULTS

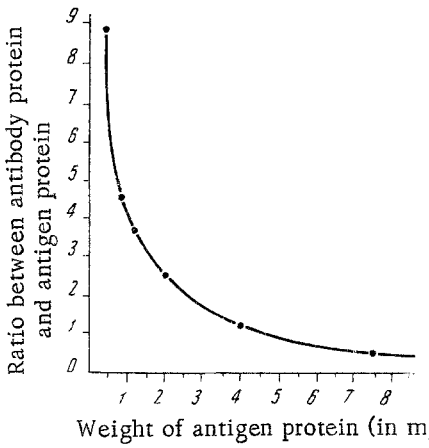
To study the effect of plague antiserum on the aldolase of fraction II, experiments were conducted by the method of the α-type flocculation reaction, i.e., increasing doses of antigen were added to a constant volume of

* The volume injected intraperitoneally into albino mice.

TABLE 3. Antitoxic Properties of Sera

Serum	Result of tests of sera in animals after addition of	
	fraction II	fraction III
Immune	0/25	0/25
Immune exhausted with fraction II	Experiment not carried out	23/2
Immune exhausted with fraction III	23/2	Experiment not carried out
Normal (control)	20/5	18/7

Note. 1. Numerator – number of animals dying, denominator – number surviving. 2. When the exhausted sera were tested 1 mg of the fractions was added each time, when the unexhausted and normal sera were tested – 5 mg was added.



Composition of precipitate from 1 ml antiserum and different doses of antigen – fraction II (composition of precipitate – ratio between antibody protein and antigen protein; protein content of antigen 75.4%; protein content of antibodies – difference between weight of precipitate and weight of antigen protein).

serum. In this way an attempt was made to attain a zone of equivalence at which the supernatant fluid possessed no aldolase activity, and to avoid "lag" zones [7]. We had to limit ourselves to a narrow range of antigen doses (from 0.3 to 10 mg of fraction II per sample). This was due, firstly, to the reduction in the solubility of fraction II in a dose higher than 10 mg per sample, and secondly, to the unreliability of the determination of aldolase if its concentration was less than 0.3 mg. The results of one of the experiments are given in Table 1.

The principal conclusion emerging from analysis of the results in Table 1 is that plague antiserum possesses antialdolase activity. This activity is specific, for it is not found in normal horse serum. Consequently, the aldolase of P. pestis is an antigen and leads to the formation of corresponding antibodies.

The second conclusion is that the absence of aldolase activity in the supernatant fluid is associated, not only when precipitation of aldolase, but also with neutralization of the enzyme by antialdolase. This is revealed by the decrease (by comparison with the controls) or complete absence of aldolase activity in the precipitates. One cause of the presence of aldolase in the precipitates during the interaction between the immune serum and large doses of antigen (10 and 5 mg of fraction II per sample), in our opinion, is to do with the composition of the precipitate. As the figure shows, with an increase in the dose of fraction II the content of antibodies in the precipitates falls rapidly. Evidently when the ratio between antialdolase protein and aldolase protein falls below a certain level (in our case below 2.6), the neutralization of aldolase becomes incomplete on account of a shortage of antibodies.

Experiments were next carried out with exhausted sera. Preliminary tests on albino mice showed that these sera did not contain the toxic fractions by which they had been exhausted.

A comparison of the results given in Tables 2 and 3 shows that the immune serum, when exhausted with fraction III (which does not possess aldolase activity), contains antialdolase but not antitoxin. At the same time, in the immune serum exhausted with the toxic fraction II, which possesses aldolase activity, neither antialdolase nor antitoxin is present.

Hence, judging by the results of the experiments with exhausted sera, the aldolase and toxin of P. pestis are different antigens.

SUMMARY

Antiplague serum possesses antialdolase activity. This activity is specific since it was not revealed in normal horse serum. Antialdolase of antiplague serum not only precipitates the aldolase contained in the preparations of P. pestis toxin (fraction II), but also neutralizes it.

By cross serological reactions and by experiments on animals it was revealed that P. pestis aldolase and toxin were different antigens.

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