ELIMINATION OF THE PENICILLINASE INDUCIBILITY GENE OF Staphylococcus aureus BY ACRIDINE ORANGE

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UDC 576.851.252.097.22: 615.33].095.57

The dynamics of penicillinase formation was compared in a penicillin-resistant (MIC > 1000 units/ml) inducible strain of Staphylococcus aureus 16/160 and its subcultures which had lost their capacity for inducible penicillinase synthesis through contact with acridine orange or spontaneously. The optimal dose of the inducer for the original culture was a penicillin concentration of 10 units/ml. Subcultures whose resistance to penicillin and salts of the heavy metals was reduced by acridine orange or spontaneously lost their capacity for induced penicillinase synthesis. Possible ways of origin of noninducible mutants are suggested.

A penicillin-resistant strain of Staphylococcus aureus is capable of inducible penicillinase (β-lactamase) synthesis, rising to a maximum 4 h after addition of the inducer (penicillin) to the medium [5-7]. The inducibility of strains of Staphylococcus aureus is controlled by a regulator gene (i^+) linked with the structural gene controlling penicillinase synthesis (P^+) [15]. The regulator gene i^+ lies side by side on the penicillinase plasmid with the structural penicillinase gene P^+ [13, 14] and determinants of resistance to salts of the heavy metals (mercury, cadmium, etc.).

The object of this investigation was to compare the dynamics of penicillinase formation induced by penicillin in a P^+i^+ strain of Staphylococcus aureus and its subcultures which had lost their capacity for inducible synthesis of β-lactamase through contact with acridine orange or spontaneously.

EXPERIMENTAL METHOD

Staphylococcus aureus strain 16/160, highly resistant to benzylpenicillin (minimal inhibitory concentration MIC > 1000 units/ml), and possessing episomal and chromosomal determinants of pencillinase activity [8-10] were used. The method of illumination was indistinguishable in principle from those used previously [2-4] and it was used in the modification [1]. To investigate inducibility, the original strain was used together with its subcultures, the first of which (AO) had acquired resistance to benzylpenicillin in a dose of 10 units/ml after contact for 30 min with acridine orange (100 μg/ml), whereas previously resistance was observed only with a dose of 1000 units/ml. Another subculture of strain 16/160 had acquired the same resistance through spontaneous elimination. The resistance of both subcultures to $HgCl_2$ was reduced from 50 to 10 μg/ml, and to $Cd(NO_3)_2$ from 100 to 50 μg/ml, indicating that the decrease in resistance to penicillin could take place through a disturbance of replication of genes located on the penicillinase plasmid [17, 18]. The 24-h cultures of the staphylococci were diluted to a density, checked with a photoelectric colorimeter, equivalent to 10 cell density units (CDU). Samples of 3 ml of the culture were added to 27 ml Hottinger's broth and grown on a shaker at 37°C for 4 h. The density of the bacterial suspension of the subculture and of the original strain was adjusted to 10 CDU, and the suspension was then distributed in equal volumes among 4 tubes: 1 control (with the antiboitic) and 3 experimental tubes, to which the inducer (benzylpenicillin) was added in final concentrations of 10, 100, and 1000 units/ml. Samples from the control

Laboratory of Biochemistry of Metabolism in Microorganisms, N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR, O. V. Baroyan.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 77, No. 5, pp. 107-109, May, 1974. Original article submitted October 16, 1972.

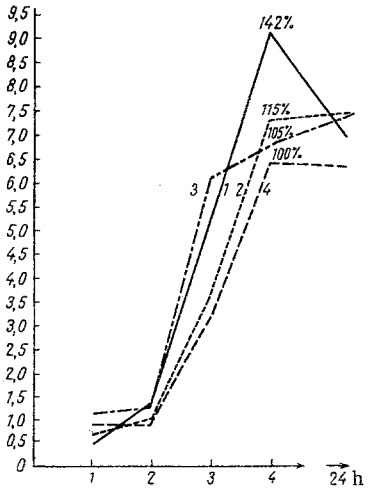

Fig. 1

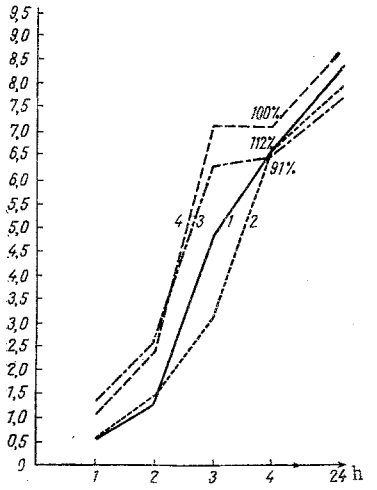

Fig. 2

Fig. 1. Dynamics of penicillinase activity of orginal strain Staph. aureus 16/160 during induction by benzylpenicillin. Abscissa, time of induction (in h); ordinate, penicillinase activity (in units/10 CDU): 1) induction by penicillin, 10 units/ml; 2) 100 units/ml; 3) 1000 units/ml; 4) control without penicillin.

Fig. 2. Dynamics of penicillinase activity in a subculture of strain 16/160 obtained by elimination with acridine orange. Legend as in Fig. 1.

and experimental suspensions were taken after 1, 2, 3, 4, and 24 h and the density of the suspensions was standardized at 10 CDU. After centrifuging, dilutions of the supernatant were tested. The penicillinase activity was determined by a microiodometric method [11] with a final substrate (benzylpenicillin) concentration of 1000 units/ml.

EXPERIMENTAL RESULTS

As Fig. 1 shows, the maximal yield of penicillinase from the original strain 16/160 was observed after 4 h. The optimal concentration of the penicillin inducer was 10 units/ml. If larger doses of inducer were used, the penicillinase yield was reduced but was still higher than in the control. The possibility cannot be ruled out that with the use of penicillin in doses of 100 and 1000 units/ml a combination of two processes – induction and inhibition of penicillinase synthesis – was observed, as the writers found when using ceporin [9]. Meanwhile, having lost its resistance to penicillin through exposure to acridine orange, the culture was unable to produce an increased amount of enzyme under the influence of penicillin induction (Fig. 2). Similar results were obtained in experiments with spontaneous elimination.

The results of these and previous experiments [13-16] suggest that eliminants that had lost the regular gene of penicillinase inducibility (i^+) had been obtained.

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