INACTIVATION OF α-HEMOLYSIN IN CULTURES OF A HEMOLYTIC STRAIN OF Escherichia coli

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In the exponential phase of growth of a culture of Escherichia coli P678 Hly$^+$ the increase in hemolytic activity runs parallel to the increase in the number of cells. At the beginning of the stationary phase of growth the hemolytic activity starts to decrease and then falls sharply. Changing the culture fluid during this period for fresh medium leads to restoration of the hemolytic activity of the culture. The culture medium, freed from cells in the stationary phase of growth has an inhibitory effect on α-hemolysin activity. The factor inhibiting α-hemolysin activity withstands heating to a 100°C for 15 min.

KEY WORDS: α-hemolysin of Escherichia coli.

α-Hemolysin is one of the factors of pathogenicity of the hemolytic strain of Escherichia coli. It appears in the culture about 1 h after inoculation. During the exponential phase its titer rises to reach a maximum at the beginning of the stationary phase of growth, after which it falls rapidly [3]. The reason for this rapid and sudden change in the hemolytic activity of the culture is not yet clear. An attempt to shed light on this problem was made in the investigation described below.

EXPERIMENTAL METHOD

Strain E. coli P678 Hly$^+$, kindly provided by Professor D. G. Kudlai, was used. The hemolytic activity factor (Hly) was transmitted to this strain from the prototrophic strain E. coli 195 Hly, isolated from an affected child [1]. Alkaline extract broth [3] was used as the nutrient medium. The cells were grown at 37°C in tubes containing 10 ml broth. The original concentration of the bacteria was about 5×10^8 cells/ml. The number of cells in the culture was estimated from the density of the bacterial suspension as measured in a photoelectric colorimeter (FÉK-56, No. 4 filter). The hemolytic activity was monitored from the optical density of the hemoglobin (FÉK-56, No. 3 filter) liberated during lysis of a 1% suspension of human red cells. The optical density after complete hemolysis of a 1% red cell suspension was taken as 100% hemolysis. The unit of hemolytic activity was taken as the optical density of a hemoglobin solution corresponding to 10% hemolysis. The composition of the incubation mixture was: 0.5 ml hemolysin solution, 0.2 ml of 0.2 M $CaCl_2$, 0.3 ml of 0.15 M NaCl, and 1 ml of a 2% red cell suspension in 0.15 M NaCl. Incubation was carried out in an ultrathermostat at 37°C for 1 h. To stop hemolysis the samples were placed for 10 min in an ice bath, then centrifuged, and the optical density of the supernatant was determined. In the experiments to study the effect of the culture medium on hemolysin activity a preparation of hemolysin isolated by the writers from the culture fluid of E. coli P678 Hly$^+$ by the method of Zwadyk and Snyder [4], was used. The activity of the preparation was 134 units/mg protein.

EXPERIMENTAL RESULTS

The data given in Fig. 1 show that as the number of cells in the culture increased, the level of hemolytic activity rose to reach a maximum at the end of the logarithmic phase and beginning of the stationary

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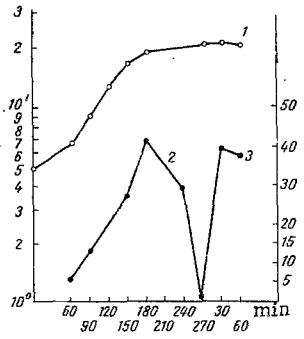

Fig. 1

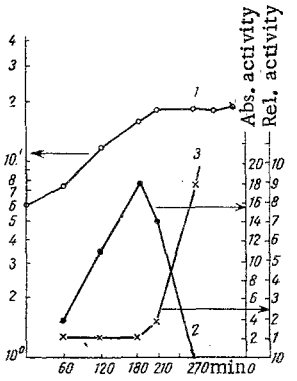

Fig. 2

Fig. 1. Growth of cells and hemolytic activity of a culture of E. coli: 1) growth of culture; 2) hemolytic activity of culture; 3) hemolytic activity of culture after change of medium. Abscissa, duration of culture (in min); ordinate, left) logarithm of number of cells, right) hemolytic activity (in units/ml).

Fig. 2. Changes in hemolytic activity of E. coli culture during change of medium: 1) growth of culture; 2) absolute hemolytic activity (in units/ml); 3) relative hemolytic activity (in conventional units). Remainder of legend as in Fig. 1.

TABLE 1. Effect of Culture Fluid on α-Hemolysin Activity

Expt. No.	No. of units in sample			
	native culture fluid		heated culture fluid	
	control	expt.	control	expt.
1	9,0 3,0	4,4 0	— 3,0	— 0
2	1,8	0,5	1,8	0,6
3	3,6 5,6	0 2,3	— 5,6	— 2,4
4	3,2	1,7	6,4	3,4

*In the control α-hemolysin was preincubated with the original nutrient medium.

phase of growth (after culture for about 3 h). During the next 1.5 h the activity declined to a minimum, and often disappeared completely. The pattern of these results is basically the same as that obtained by Smith [3]. To determine the reason for the rapid decline in α-hemolysin activity in the culture on reaching the stationary phase of growth, two series of experiments were carried out. A 4.5-h culture, the hemolytic activity of which was minimal or zero, was centrifuged to remove the culture fluid. In the experiments of series I the cells were washed twice with a fresh portion of medium, heated to 37°C, suspended in the original volume of this medium, and incubated for a further 1 h. After only 30 min (Fig. 1) the hemolytic activity of the culture fluid had risen to its initial maximum. The number of cells in the culture, however, was unchanged. Consequently, E. coli cells in the stationary phase of growth, when transferred to fresh medium, were able to synthesize α-hemolysin at the original rate. The decrease in hemolytic activity of the culture in the stationary phase of growth could be due either to cessation of synthesis or to inactivation of α-hemolysin. The hypothesis of the cessation of hemolysin synthesis is unlikely to be true, for it does not explain the sudden change in hemolytic activity of the culture. Despite the fact that α-hemolysin is thermolabile [2, 4], according to the present writers' observations 1-1.5 h is too short a time for considerable destruction of it to take place at 37°C. Inactivation due to the gradual accumulation of a certain factor in the culture, affecting hemolysin activity or facilitating its rapid destruction, is a more likely explanation. To test this hypothesis the experiments of series II were carried out to study the effect of the culture fluid of a 4.5-h culture on the activity of α-hemolysin added from an outside source. For this purpose the hemolysin previously isolated was preincubated with culture fluid (1 h at 37°C), and the changes in the hemolytic activity of the mixture were then studied as described in the section "Experimental Method." The culture fluid of the 4.5-h culture was shown to inhibit the activity of the added extrinsic α-hemolysin (Table 1). The degree of inactivation varied from one experiment to another.

Serial dilution of the culture fluid led to a gradual increase in α-hemolysin activity. The hypothesis that inactivation of the hemolysin in the culture was due to the action of proteases or other enzymes found

no support. The results in Table 1 show that heating the culture fluid for 15 min at 100°C did not change its inhibitory effect on hemolysin. Consequently, a thermostable factor was responsible for the inhibition of α-hemolysin activity. To ascertain at what period of growth of the culture this factor appeared, a special experiment was carried out. Samples were taken after culture for 1, 2, 3, 3.5, and 4.5 h, and the number of bacterial cells and the hemolytic activity were determined in them. The cells from each sample were then transferred to fresh medium and, after incubation for 30 min, the number of cells and the hemolytic activity were again determined. The results showed that transferring the E. coli cells into fresh medium after 1, 2, and 3 h did not change the hemolytic activity of the culture. During this period the activity increased gradually (Fig. 2). After 3.5 and 4.5 h, during the period of commencing decline and complete loss of activity, respectively, changing the medium led to restoration of hemolytic activity to the characteristic level at the maximum. The change in the hemolytic activity of the culture when the medium was changed at each of these periods is shown in Fig. 2 (curve 3). The change is expressed in conventional units of relative activity, the ratio between activity before and after transferring the cells into fresh medium. The increase in the relative α-hemolysin activity with a decrease in its absolute activity indirectly indicates the time of appearance of the factor inhibiting α-hemolysin activity in the culture. The character of the curve describing the hemolytic activity of the culture (Figs. 1 and 2) evidently reflects by its rise an increase in the synthesis of α-hemolysin by the dividing cells up to the maximal value, and by its fall the appearance of a factor inhibiting hemolysin activity in the culture.

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