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Antibacterial Activity of Matrix-Bound Ovotransferrin

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Ovotransferrin immobilized by covalent linkage to Sepharose 4B showed a bacteriostatic effect towards *Escherichia coli* similar to that of free ovotransferrin. The growth of the bacteria, after exposure to the gel-bound ovotransferrin and its removal, depended on the length of exposure. The results suggest that the antibacterial activity of transferrin is not due simply to the removal of iron from the medium.

Transferrins are iron-binding proteins which, in their free form, exhibit antibacterial activity against a variety of organisms. These proteins are present in blood serum (serotransferrin), in milk (lactoferrin), and in egg white (ovotransferrin) and are all glycoproteins, with molecular weights of about 80,000, containing two iron-binding sites per molecule (4).

In previous studies the bacteriostatic activity of ovotransferrin was investigated in detail (1, 6, 7). This note reports the results obtained with ovotransferrin covalently linked to a solid matrix. It will be shown that ovotransferrin immobilized on Sepharose 4B maintains its iron-binding capacity and its bacteriostatic activity; hence, the system might be useful to investigate the mechanism of the antimicrobial effect.

The ovotransferrin was obtained as a freeze-dried preparation (5). By starch gel electrophoresis and ultracentrifugation, the preparation showed a single protein component. Spectrophotometric titrations at 470 nm with iron citrate (pH 9) in the presence of 0.1 M $NaHCO_3$ gave endpoints corresponding to an iron-binding capacity of 1 ± 0.05 microequivalents for 40 mg of protein. This value corresponds closely to the theoretical binding capacity of two iron atoms per protein molecule (molecular weight, 80,000).

The preparations of immobilized ovotransferrin were obtained by coupling the protein at 4°C to BrCN-activated Sepharose 4B (3). The amount of protein immobilized on the gel was determined spectrophotometrically after correction for turbidity of the gel at 280 nm on the basis of $E^{1\%}_{1\,cm} = 11.2$. The sterility of the samples of bound ovotransferrin produced by the immobilization procedure was maintained by repeated washings with sterile solutions of 50 mM $NaHCO_3$. The antibacterial activity of both free and bound ovotransferrin towards *Escherichia coli* strain W1485 was tested in brain heart infusion with added $NaHCO_3$. The iron content of the culture medium was 2 μg/ml, as determined by an atomic absorption spectrophotometer (Perkin-Elmer model 360). The bacterial growth was measured in colony-forming units (CFU). In all of the experiments carried out with bound ovotransferrin, controls were performed with Sepharose 4B and with serum albumin bound to Sepharose 4B.

The results of the antibacterial activity of free and bound ovotransferrin are reported in Fig. 1. The antibacterial activity increased with the concentrations of free or bound ovotransferrin and was comparatively higher in the latter preparation. The higher bacteriostatic activity of matrix-bound ovotransferrin which was consistently found in all of the experiments and with all of the preparations has no clear explanation. At least in part, it might be ascribed to the higher stability of matrix-bound protein, as is commonly observed with other immobilized proteins (2).

Exposure of matrix-bound ovotransferrin to different concentrations of Fe^{3+} caused a loss of activity which is proportional to the degree of saturation with iron. This behavior is identical to that of free ovotransferrin. Also, the effects of citrate and bicarbonate are the same, using free (1) or immobilized ovotransferrin.

In a series of experiments, a culture medium containing 5×10^5 CFU/ml was mixed with Sepharose-bound ovotransferrin. After different times of contact, the matrix-bound protein was removed, and the supernatants were incubated at 37°C for 18 h. The bacterial growth was compared with controls in which the culture medium without *E. coli* was kept in contact for the same length of time with Sepharose-bound ovotransferrin. After the contact, the immobilized protein was removed, and the supernatants

† Dr. Rossi Fanelli died 6 March 1981.

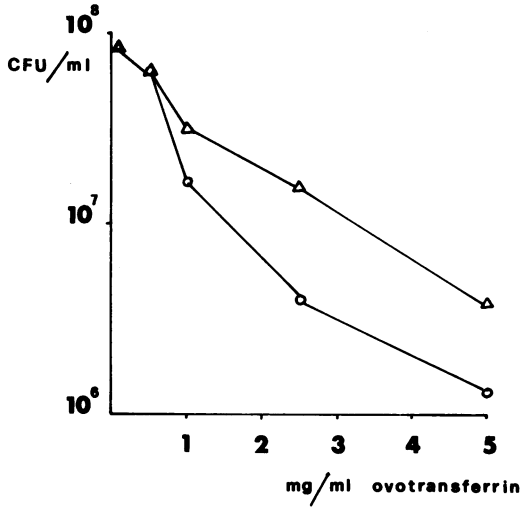

FIG. 1. Antibacterial activity towards *E. coli* strain W1485 (5×10^5 CFU/ml) of different concentrations of free (△) and matrix-bound (○) ovotransferrin with $NaHCO_3$ (50 mM). Ordinate, colony-forming units per milliliter; abscissa, concentrations of ovotransferrin.

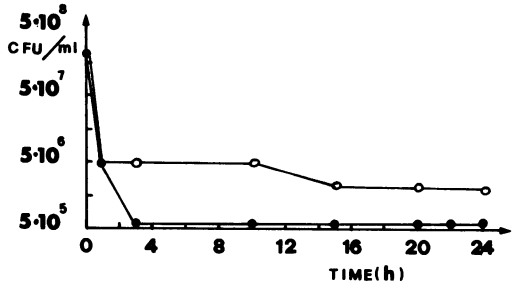

FIG. 2. (●) Growth of *E. coli* strain W1485 (5×10^5 CFU/ml) in the medium with Sepharose-bound ovotransferrin (5 mg/ml) and (○) in the medium previously kept in contact with Sepharose-bound ovotransferrin (5 mg/ml), at different times.

were inoculated with *E. coli* (5×10^5 CFU/ml) and incubated at 37°C for 18 h.

The results of these (Fig. 2) and similar experiments, in which the inoculum was varied from 5×10^5 to 1×10^7 CFU/ml (Fig. 3), always showed that, in the culture medium treated with bound ovotransferrin before adding the inoculum, the growth of *E. coli* was higher than in the culture growing in the presence of bound ovotransferrin.

These experiments showed that (i) on removal of the Sepharose-bound ovotransferrin, the microorganisms regain their full capacity to grow after the addition of iron to the medium, and (ii) the growth of bacteria in the absence of added iron depends on the length of time that the bacterial suspension is exposed to the matrix-bound ovotransferrin.

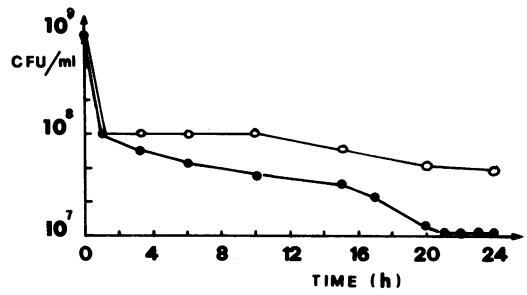

FIG. 3. (●) Growth of *E. coli* strain W1485 (10^7 CFU/ml) in the medium with Sepharose-bound ovotransferrin (5 mg/ml) and (○) in the medium previously kept in contact with Sepharose-bound ovotransferrin (5 mg/ml), at different times.

These results cannot be interpreted simply on the basis of removal from the medium of the iron available to the bacteria by ovotransferrin. It should be noted that the concentration of iron in the medium falls essentially to zero after even a brief (<1 h) exposure to the immobilized transferrin. However, the ability of the bacteria to grow in this medium (in the absence of newly added iron) decreases with the length of time of exposure to the transferrin. This indicates that in the presence of ovotransferrin, slow changes which progressively limit their ability to grow may occur in the microorganisms. It could be argued therefore that these changes reflect a direct interaction of the protein with the bacteria or a deprivation of an internal iron pool in slow equilibrium with the medium or both.

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