# RNA Synthesis Stimulating Activity of Ascorbate in *Escherichia coli*

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In studies of the cell stimulating activity of a factor, called F V, from red kidney beans,[1] in plasmolyzed *E. coli* cells [2] it was observed that this factor was activated by reduction with 2-mercaptoethanol. In exploratory studies of the effects of other reductants it was found that ascorbic acid alone had a conspicuous cell stimulating activity in the bacterium system used. The present paper will report on this action.

*Material and methods.* Uridine-5-$^3$H and L-phenylalanine (ring-4-$^3$H) were obtained from Radiochemical Centre, Amersham; rifampicin from CIBA; inorganic chemicals from Merck, Darmstadt; and other chemicals and biochemicals were purchased from Sigma Chemicals Co. Cells of *E. coli* Q 13 (kindly provided by Dr. J. T. August, Albert Einstein College, Bronx) were plasmolyzed in hypertonic sucrose according to Fedorcsák *et al.*[2] The assay for stimulating activity was performed by incubating $6\times10^6$ cells per ml at 37° for 150 min, together with labeled precursor and at different ascorbate concentrations, in a suitable reaction mixture (RM) as described earlier.[2]

*Results and discussion.* Under the conditions of the test in the presence of 5 mM mercaptoethanol [1,2] a 5 – 10 times higher incorporation of uridine was obtained after addition of 1 mM ascorbate (Table 1). The maximum (saturation) stimulation of the system was reached at about 0.6 mM ascorbate. In similarity with F V from *Phaseolus vulgaris* seeds,[1,2] ascorbate is able not only to counteract the RNA synthesis depressing action of mercaptoethanol, but stimulates the cells to the same maximum level in the presence as well as in the absence of this thiol (Table 1).

Later studies have demonstrated that the action of ascorbate is not restricted to plasmolyzed cells. Under certain nutritional conditions a strong stimulation of *E. coli* cells is obtained in isotonic media as well.

*Table 1.* Influence of mercaptoethanol and ascorbic acid on the RNA synthesis in *E. coli* cells. Incorporation of uridine, in pmol/$10^6$ cells, was measured.

| Concentration of ascorbate (mM) | Concentration of mercaptoethanol (mM) 0 | 5 |
|---|---|---|
| 0 | 278 | 115 |
| 0.1 | – | 231 |
| 1 | 570 | 605 |
| 10 | – | 621 |

In general the action pattern of ascorbate in the *E. coli* cells is very similar to that of the bean factor, F V. Thus the time course of RNA, DNA, and protein synthesis in the presence of 1.4 mM ascorbate was identical with the effect of F V at 20 μg N/ml (*cf.* Fig. 1 of Fedorcsák *et al.*[2]).

F V, the molecular weight of which is about 10 000, contains about 20 mol % cystein.[2] Comparing ascorbate and F V in terms of the amount of stimulator which, per ml test suspension, provokes half maximum stimulation – *i.e.* the criterion used to define one (Bacterial) Unit of stimulator activity [2] – ascorbate may be said to be slightly less efficient (1 Unit $\approx$ 0.2 μmol) than F V (1 Unit corresponding to about 0.03 μmol cystein). The bean factor F V was so far not found to contain ascorbic acid.

The nature of the stimulation by ascorbate was studied preliminarily by measurements of the inhibitory effects of rifampicin and chloramphenicol (Table 2). Under conditions where 0.8 – 6 mM ascorbate provokes a 10-fold increase of RNA synthesis, a low concentration of rifampicin which provokes a decrease to 15 % in the unstimulated control is nearly completely counteracted by 6 mM ascorbate, about 80 % of the incorporation without inhibitor being reached (Expt. 1). In a second experiment a higher concentration of rifampicin was used, but a conspicuous tendency to competition with ascorbate is noticed. Chloramphenicol gives, in contrast, all signs of a non-competitive inhibition of the stimulation by ascorbate, the same relative reduction

*Table 2.* Influence of inhibitors on the stimulation by ascorbate of RNA and protein synthesis in *E. coli*. The incorporation of uridine or phenylalanine was measured.

| Experiment | Inhibitor | pmol/$10^6$ cells (in parenthesis % of control series) 0 mM ascorbate | 0.8 mM ascorbate | 6 mM ascorbate |
|---|---|---|---|---|
| 1 | None (control) | 47 (100) | 465(100) | 465(100) |
| (uridine | Rifampicin (4.5 μg/ml) | 7.1(15) | 279(60) | 382(82) |
| incorporation) | Chloramphenicol (4 μg/ml) | 20.3(43) | 166(36) | 182(39) |
| 2a | None (control) | 57 (100) | 275(100) | |
| (uridine | Rifampicin (9 μg/ml) | 1.7(3) | 48(17) | |
| incorporation) | Chloramphenicol (4 μg/ml) | 12 (21) | 70(25) | |
| 2b | None (control) | 10.5(100) | 46(100) | |
| (phenylalanine | Rifampicin (9 μg/ml) | 0.6(6) | 5.4(12) | |
| incorporation) | Chloramphenicol (4 μg/ml) | 1.7(16) | 2.2(5) | |

of uridine incorporation being observed in stimulated and non-stimulated cells. Measuring protein synthesis by phenylalanine incorporation it is seen that a tendency to competition of rifampicin and ascorbate is at hand. As far as protein synthesis depends on RNA available, this is expected in view of the stimulated uridine incorporation. Protein synthesis is hardly stimulable in the presence of 4 μg/ml chloramphenicol.

The mechanism of action of ascorbate in the observed effect cannot yet be explained. Kinetic studies in intact cells were unable to demonstrate unambiguously a competitive inhibition by rifampicin (which specifically inhibits DNA dependent RNA polymerase [4,5]) of the RNA synthesis stimulating action of ascorbate; and *in vitro* studies of RNA polymerase failed to show an antagonism of ascorbate and rifampicin. Ascorbate may therefore be supposed to interfere with the regulation of RNA synthesis either indirectly or by a mechanism not involving polymerase activity. In the cell systems studied ascorbate (like F V) no doubt interferes with the energy generating system. Continued investigations aim at a clarification of the possible role of this effect in the action on RNA synthesis.

In preliminary experiments ascorbate was found to play a role in the stimulation of lymphocytes.[3] It is of interest to note that the range of active concentrations of ascorbate, 0.1 – 1 mM, found in the present study corresponds to normally occurring tissue concentrations.

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