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PREIRRADIATION ACID-SOLUBLE SULFHYDRYL LEVEL-CHANGES IN THE CULTURES OF *ESCHERICHIA COLI* STRAIN 15T⁻ IN THE PRESENCE OF CYSTEINE

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Abstract—The addition of L-cysteine-HCl to growing cultures of *E. coli* 15T⁻ log. phase cells, resulted in a decrease of their sensitivity to X-ray radiation, even when cysteine was removed from the cell-suspensions prior to irradiation. Cysteine proved to be effective as a radiosensitivity-modifying agent at concentrations of 1–10 mM.

Simultaneously cysteine increased the cellular AS-SH* level of the bacteria. This rise was found to depend on the doses of cysteine and the length of the incubation period.

Extracellular cysteine at concentrations of 0·05 mM and upwards caused a detectable SH surplus intracellularly, which increased up to 5 mM. Higher concentrations of cysteine failed to induce a further rise of the bacterial SH content.

At a given concentration the AS-SH level increasing effect of cysteine set in 30 sec after adding it to the cultures, the maximum level being reached around the 15th min.

No direct correlation was found to exist between the changes in the radiosensitivity of the cells and their enhanced endogenous SH level.

In the field of radiation-pharmacology the opinion has been gaining the upper hand lately that it is not merely the physico-chemical effect of certain radioprotector compounds which is responsible for the radioprotective action of the latter, but that incorporation of the protector into the cells induces metabolic changes in them, thereby causing a decrease in their radiosensitivity.[1, 2] In mammalian cells the decrease is expressed in the rise of the SH level.[3]

In the case of microorganisms too, the metabolic or biochemical radioprotective effect is probably to be traced to an elevated SH content. This state of affairs, however, is complicated by the fact that the bacterial cell-wall has proved to be impermeable to a number of radioprotectors or compounds known as radiosensitors.[4, 5] These compounds exert their effect without leaving any trace intracellularly.

Experiments carried out earlier on *E. coli* B and *E. coli* 15T⁻ bacteria have shown that one of the components of the radioprotective action of the SH-containing protective agent cysteine acts on a cell-biochemical level.[5, 7, 8]

The present report refers to experiments with *E. coli* 15T⁻ cells in which the above-mentioned biochemical radioprotective effect of cysteine, the changes in the level of endogenous SH, as well as the presumable correlation between these two phenomena have been studied.

* Abbreviations used in this paper are: SH, sulfhydryl; AS-SH, acid-soluble sulfhydryl; SS, disulfide; PCA, perchloric acid, DTNB, 5,5′dithiobis-2-nitro-benzoic acid.

MATERIALS AND METHODS

L-Cysteine-HCl was added to *E. coli* 15T⁻ cells in a logarithmic phase of growth, the latter having been cultured in a glucose-salts medium[9] in a way to ensure that the final concentration of cysteine should be between 0·01 and 10·0 mM.

Thereupon samples were taken from time to time to determine the radiosensitivity and acid-soluble SH content of the bacteria.

Assay of survival

The samples were centrifuged, washed with phosphate-buffer (pH 7·0) and diluted therein until the number of bacteria reached 3×10^6 cells/ml. The bacterial suspensions in this buffer solution were irradiated by means of an X-ray apparatus under aerob conditions at room temperature. The dose rate was 1·6 KR/min. After irradiation the bacteria were plated on the same medium with 2% agar added. Survival was determined by the number of macrocolonies after 48 hr incubation at 37°.

Determination of acid-soluble thiols

The cells were sampled, centrifuged and washed as above, whereupon using the method of Ellman[10] we determined the AS-SH (endogenous SH) content of the 0·25 N PCA cold acid soluble supernatant of the bacterial suspension. SH content was calculated according to a given formula.[10]

RESULTS

Figure 1 indicates the changes in radiosensitivity of *E. coli* 15T⁻ cells to a given X-ray dose, as plotted against concentrations of cysteine.

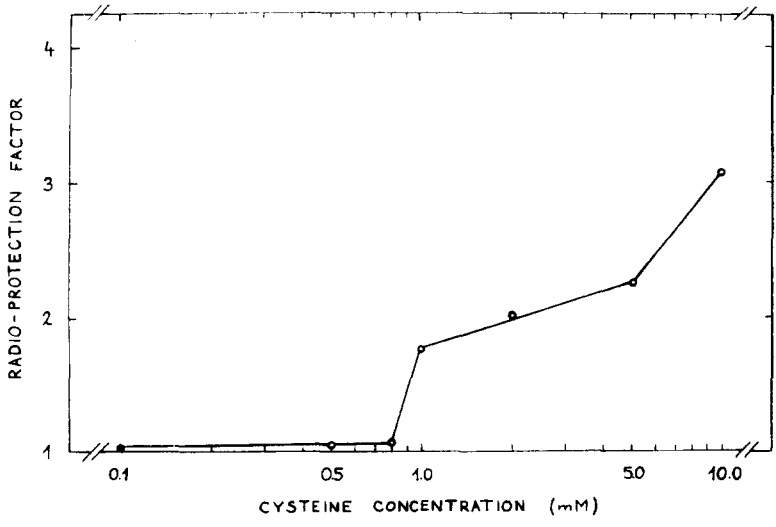

FIG. 1. Dependence of the radioprotection of *E. coli* 15T⁻ cells on cysteine concentration. Incubation time in the presence of cysteine prior to irradiation amounted to 30 min. The radio-protection factor was calculated from the quotien:

$$\frac{\text{percentage of surviving count cysteine treated cells}}{\text{percentage of surviving count untreated control cells}}$$

Each point represents the average of three independent experiments.

No changes in the radiosensitivity of the bacterial cells pretreated with cysteine could be observed in the course of an incubation period of 30 min provided that the concentration of cysteine did not reach 1 mM. A marked decrease of radiosensitivity set in when concentration of cysteine attained 1 mM and over. Analysis of the X-ray survival curves pertaining to the various concentrations of cysteine was made by us elsewhere. The fact is to be noted that incubation in the presence of cysteine for a period of 2 min had the same effect, asre gards decreasing the radiosensitivity, as a 30 min treatment.

Prior to studying the changes in the AS-SH level of the bacterial cells we deemed it necessary to determine the rate of oxydation of cysteine prevailing extracellularly in the culture medium during the incubation period, in view of the susceptibility of the SH group to oxydation.

TABLE 1. DISAPPEARANCE OF CYSTEINE-SH FROM THE AERATED BACTERIA-FREE CULTURE MEDIA*

Incubation time (min)	Measured SH concentration $\times 10^{-6}$ M/ml	Percentual value of SH groups as compared to point O
0†	47‡	100
15	46	98
30	45	95
60	37	78
120	33	70
300	19	40

* Concentration of supplemented SH amounted to 50×10^{-6} M/ml.

† O point is to be considered as relative; effectively 5–10 sec passed between the dissolving of cysteine and the adding of the DTNB reagent.

‡ The measured value of SH as compared to the introduced value is 94 per cent.

It can be stated that the effects of the possible oxidation products of cysteine i.e. —SS- and/or SO_3^{-2}, SO_4^{-2}—have not to be reckoned with unless the incubation period exceeds 60 min.

As plotted against cysteine at concentrations of 0·01 to 10 mM the AS-SH level of the *E. coli* 15T$^-$ cells shows the changes enumerated under Fig. 2.

Samples were taken from time to time after adding cysteine. As regards the level prevailing before treatment that of the AS-SH of *E. coli* 15T$^-$ cells showed an increase, in the presence of cysteine at a concentration as low as 0·05 mM. From then on the AS-SH content rose steeply up to 5 mM. Whereas, thereafter the level was found to mount at a lower rate thereby indicating that a certain degree of saturation had set in.

In view of the fact that a marked increase of the AS-SH content could be observed 2 min after adding cysteine, we considered it necessary to determine the rate of elevation of the AS-SH level the incubation period, given a certain concentration of cysteine.

This figure reveals that the AS-SH level rises within 30 sec, after adding cysteine. The peak is reached around the 15th min, and is then followed by a slow drop probably due to a process of elimination.

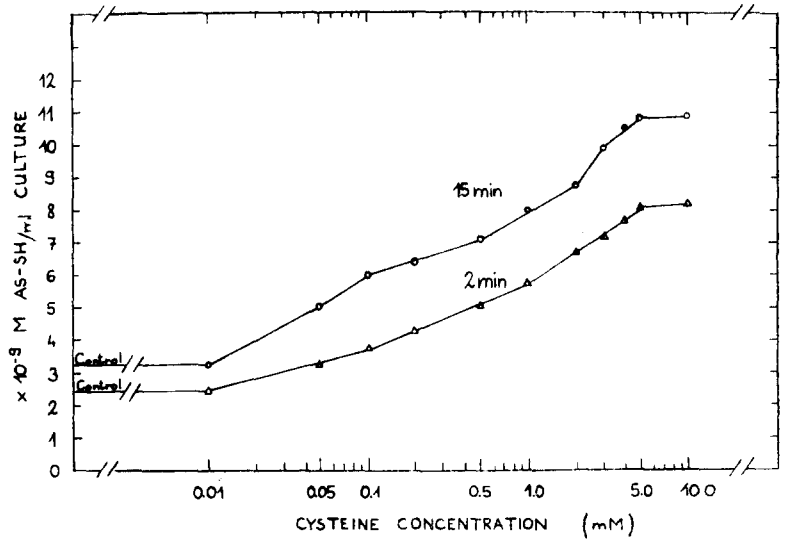

FIG. 2. Effect of different concentrations of cysteine on the AS-SH level of *E. coli* 15T⁻ cultures. Bacteria were supplemented with cysteine in the exponential phase of growth. Samples of bacterial cells were taken at the 2nd and 15th min after adding cysteine. Each point represents average of three independent experiments.

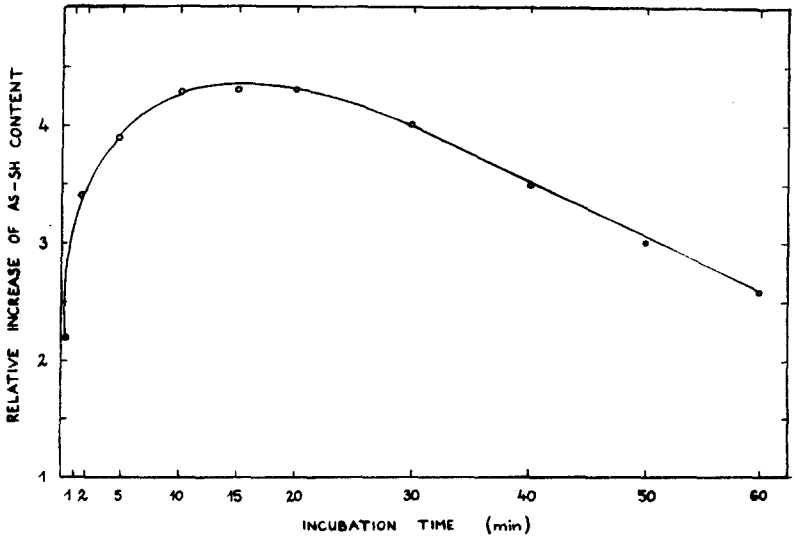

FIG. 3. The relative AS-SH level of *E. coli* 15T⁻ cells treated with 1 mM cysteine is plotted against incubation time, in relation to the level of the respective, untreated controls. Each point represents the average of three independent experiments.

DISCUSSION

We have ascertained from our experiments that the biochemical radioprotective effect of cysteine on *E. coli* 15T⁻ cells is only effective at a concentration of 1 mM and over. Radioprotection sets in very rapidly and this is also borne out by the finding that the survival counts carried out after incubation periods of 2 and 15 min respectively are practically identical.

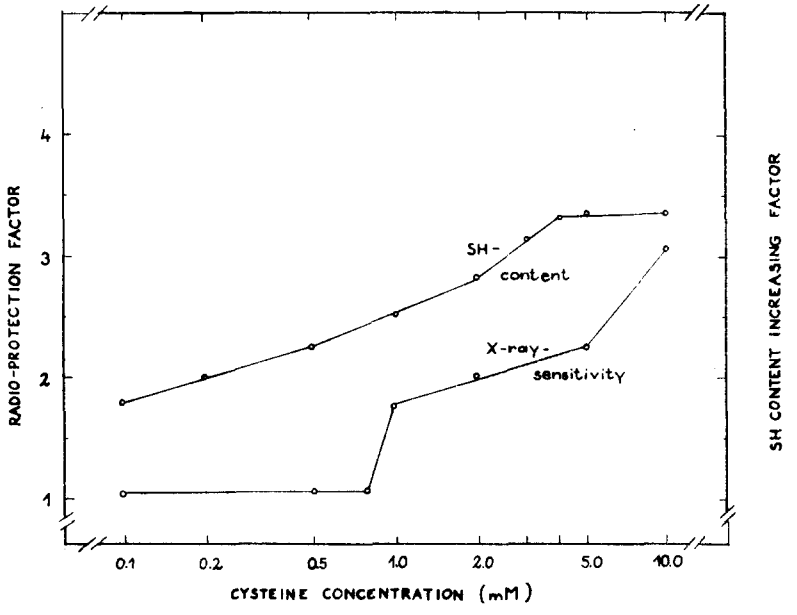

FIG. 4. Changes in the radiosensitivity to X-ray irradiation and the SH content of *E. coli* 15T⁻ cells as plotted against concentrations of cysteine. Both factors were calculated according to the quotient indicated in Fig. 1.

The curve representing the changes in the AS-SH level induced by cysteine may be divided into two parts (see Fig. 2). In the case of extracellular cysteine up to a concentration of 1 mM (first part of the curve) a practically direct ratio was found to exist between the rise of the intracellular AS-SH level and the increase of the concentration of extracellular cysteine. Thereafter the increase of cysteine in the culture medium failed to raise the AS-SH level to any marked degree (second part of the curve).

As could be seen the AS-SH-enhancing action of cysteine took effect very quickly. The AS-SH content showed an increase already during the first minutes of the incubation period, reaching its maximum in a very short time (see Fig. 3). It has naturally to be taken into account that in the course of the period under examination the cysteine-SH level in the environmental medium kept on decreasing (see Table 1) and thus the cells were exposed to the effect of extracellular SH-groups of increasingly fewer numbers.

Figure 4 reveals a correlation between the decrease of the radiosensitivity of the cells induced by cysteine and the rise of the endogenous SH level.

This figure shows the increase of the radioprotection as well as that of the SH level subsequent 15 min treatment as plotted against the concentrations of cysteine. It will be observed that a certain degree of radioprotection involves a given level of SH in the

bacteria. At the same time it seems likely that a certain threshold endogenous SH content is required for a decrease of radiosensitivity to set in.

The question now arises whether a direct or indirect correlation exists between the rise in radioresistance and that in the SH level; further, whether the enhanced SH content measured in the bacterial cells is to be ascribed directly to the cysteine-SH, or whether we are here dealing with an accumulation of SH groups of endogenous origin resulting from some kind of catalytic process induced by cysteine.

In our opinion the SH level expresses the metabolic state of the cells treated with cysteine. This inference is based on two facts:

(1) Changes take place in the SH level of the bacteria during the incubation period, whereas the changes in their radiosensitivity occur only at the initiation of this period. Saturation point is reached by the former around the 15th min, while the latter does not vary after the 1st minute.

(2) A decisive shift in radiosensitivity can be ascertained should the SH content remain constant.

We can not exclude the possibility either, that cysteine induces a so-called biochemical shock within the bacteria and this shifts the SH/SS balance in an indirect manner in favour of the SH groups.

In this connection it might be postulated that the aforesaid unfavourable state of the cells enhances their post-irradiation capacity for repair of initial lethal damage the increase in the number of surviving bacteria being thus chiefly due to this repairing process.

In addition to the aforesaid interpretations the possibility of cysteine getting bound to the cell surface (cell wall and/or cytoplasmic membrane) by means of its SH group might further be raised; this would then cause a disturbance of the membrane function. At the same time it is well known that the physiological metabolism of the cells (cromosome replication,[11] enzymatic activities[12] etc.) requires the cell membrane to be intact. The disturbed membrane function results in unbalanced growth of the bacteria thus being the cause of an enhanced radioresistance of the cells.[13] Hence the changes within the membrane might be considered secondary effect, the primary site of action being the cell surface.

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