FUNCTIONAL PROPERTIES OF *Escherichia coli* SINGLE-STRANDED DNA BINDING PROTEINS IN VIVO

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Interest in chromosomal proteins of nonhistone nature has increased considerably in recent years because these proteins are considered to participate in the regulation of genome expression. Some chromosomal nonhistone proteins are universal, from the biological point of view, i.e., they are found in cells of organisms of all species, beginning with bacteria and ending with man. This group of proteins includes the single-stranded DNA binding proteins (SSB proteins). Irrespective of their origin these chromosomal binding proteins possess certain common properties and they play an important role in the regulation of DNA replication and repair [3].

SSB of *Escherichia coli* have received the closest study from the structural and functional points of view. However, although three functional domains have been identified in them [4, 8], the functional properties of *E. coli* SSB proteins have been characterized mainly in vitro. Yet the functions of these proteins in vivo and their participation in concrete biochemcial processes have not yet been fully explained. An elucidation of these problems could assist with the study of the biochemical "behavior" of mutants effective for SSB proteins.

Two conventionally lethal temperature-sensitive mutations in the ssb gene, determining the structure of the SSB proteins, are now known [4, 9]. One of these mutations (ssb-1) took place through substitution of tyrosine in position 55 for histidine in the first domain, where it interacts directly with single-stranded DNA. As a result of this mutation [6] the mutant SSB-1 proteins, at a nonpermissive temperature (42°C), can no longer bind firmly with single-stranded DNA, i.e., with the replication on forks. Loss of this fundamental property makes the antinuclease barrier, formed by SSB proteins on replication forks, imperfect, so that replication is halted, DNA degraded, and the viability and radioresistance of cells with the ssb-1 mutation are sharply reduced [1].

The second point mutation in the ssb gene, which has been called ssb-113, is located in the third domain and is associated with substitution of proline in position 171 for serine [4]. As a result of the second mutation the degree of affinity of the mutant protine SSB-113 for single-stranded DNA is increased (compared with the wild-type SSB protein). Consequently, mutant SSB-113 proteins form a stronger antinuclease barrier on the replication forks than wild-type proteins. Apparently neither degradation of DNA at the nonpermissive temperature nor a sharp decrease in radioresistance (compared with the wild type and ssb-1 mutants) should not take place in cells carrying the ssb-113 mutation. However, our experimental data, described below, are evidence to the contrary.

EXPERIMENTAL METHOD

Experiments were carried out on the following strains of *E. coli* K-12: JGC158 ssb^+ (wild type); JGC155 ssb-1 [5], PAM2611 ssb-113 [6]. Methods of determination of radioresistance and of the degree of DNA degradation in intact and irradiated wild-type *E. coli* cells, and carrying a temperature-sensitive mutation for binding protein ssb-1 and ssb-113, were described previously [1].

EXPERIMENTAL RESULTS

Data on the intensity of DNA degradation at temperatures of 30 and 42°C in intact and irradiated (with UV- or γ-rays) cells are given in Figs. 1 and 2.

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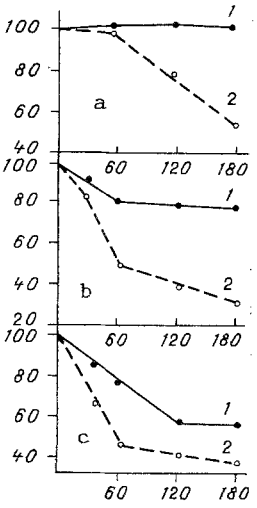

Fig. 1

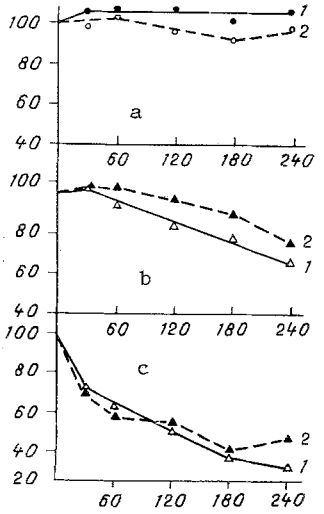

Fig. 2

Fig. 1. Spontaneous (a) and UV- (b) and γ-ray-induced (c) DNA degradation in cells of ssb-1 mutant of *E. coli*. a: 1) Intact cells, 30°C; 2) the same, 42°C; b: 1) UV-irradiation, 18.0 J/m², 30°C; 2) the same, 42°C; c: 1) γ-irradiation, 100 Gy, 30°C; 2) the same, 42°C. Here and in Fig. 2: abscissa, incubation time (in min); ordinate, radioactivity of acid-insoluble fraction (in %).

Fig. 2. Spontaneous (a) and UV- (b) and γ-ray-induced (c) DNA degradation in cells of *E. coli* mutant for binding protein SSB-113. a: 1) Intact cells, 30°C; 2) the same, 42°C; b: 1) UV-irradiation, 9.3 J/m², 30°C; 2) the same, 42°C; c: 1) γ-irradiation, 100 Gy, 30°C; 2) the same, 42°C.

TABLE 1. Comparative Sensitivity of Strains ssb-1 and ssb-113 of *E. coli* to UV- and γ-Rays

Strain	D_{37}			
	UV-irradiation, J/m²		γ-Irradiation, Gy	
	30 °C	42 °C	30 °C	42 °C
JGC158 ssb+	10,0	10,0	50,0	50,0
JGC155 ssb-1	6,2	5,0	31,2	15,0
PAM2611 ssb-113	2,2	2,0	40,0	17,0

It can be concluded from these results that the presence of mutant protein SSB-113, with enhanced affinity for single-stranded forms of DNA, does not prevent the development of their degradation either when growth of replication forks ceases under nonpermissive temperature conditions (42°C) or in the case of irradiation of mutants ssb-113 cells by UV- and γ-rays.

The experimental data given in Table 1 show that the presence of mutant proteins in the cells — both SSB-1 and SSB-113 — makes them equally sensitive to UV- and γ-radiation.

It can be concluded from these results that the function of antinuclease protection of replication forks, performed by SSB-proteins, is neither the main, nor an essential, function for the viability of cells subjected to the action of agents with affinity for DNA (UV- and γ-rays). The question accordingly arises, through what molecular mechanisms do mutant proteins SSB-113 induce DNA degradation and an increase of sensitivity to UV- and γ-rays.

This question is answered by the view expressed previously and confirmed experimentally by the authors [1-3], namely that an excess of free chromosomal binding proteins, accumulating in the case of drastic inhibition or termination of DNA replication processes, triggers

a series of consecutive molecular events, overcoming the antinuclease barrier (protecting the replication forks) and leading to the development of DNA degradation, which is ultimately the cause of death of the cells.

This idea was based on one of the functional properties of binding proteins, linked with third domain. This property consists of the ability of SSB proteins to interact selectively with certain repair enzymes (DNA-polymerase II of bacteria) and to form complexes with them. The catalytic activity of the repair enzymes is modified when in the composition of these complexes and they are converted from reparative into destructive [7].

When the above-mentioned property of SSB-proteins is examined, it must be noted that the degree of affinity of the third domain for some enzymes is much lower than the degree of affinity of the first domain for replication forks. The formation of complexes between SSB-proteins and enzymes in vivo is therefore possible only when growth of replication forks has ceased whereas synthesis of SSB-proteins continues. These conditions are created in what are called unbalanced situations, when replicative DNA synthesis is drastically inhibited or completely stopped but synthesis of proteins, including SSB-proteins, still continues at the previous rate.

Unbalanced situations may arise under the influence of various causes: endogenous (for example, thymine starvation, strong and prolonged stress reactions, cell aging processes, and so on) and exogenous [for example, selective inhibitors of replicative DNA synthesis such as nalidixic and oxolinic acids in bacteria, araC — the arabinose derivative of cytosine (Cytosar) in mammals, ionizing and UV-radiation, and also other agents with affinity for DNA].

Thus in unbalanced situations one of the functions of SSB proteins, linked with their third domain, is manifested and realized. This function is responsible for strictly determined modification of the catalytic activities of some enzymes, and leads to their conversion from reparative into destructive.

Thus functional property of the SSB-proteins is responsible for their participation in the development of several pathological processes [3], such as malignant and aging of cells, disturbance of immune reactions (the abortive blast-transformation phenomenon), the cell depopulation syndrome during the development of radiation sickness, and so on.

In conclusion it must be emphasized that under normal conditions of cell metabolism, i.e., in the absence of unbalanced situations, vitally important functions of SSB-proteins, linked with their first and second domains, are manifested and realized. These functions include functions of unwinding of the double helix, stabilization of single-stranded matrices, antinuclease protection of replication forks, and so on. The functions listed above guarantee the realization of processes of replication, repair, and recombination of DNA, so vitally important for cells.

SSB-proteins, which are polyfunctional structural chromosomal proteins, can thus participate both in normal physiological processes and also in the formation of pathological processes in dividing cells.

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