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Environmental Control of the *In vivo* Oligomerization of Nucleoid Protein H-NS

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The nucleoid-associated transcriptional repressor H-NS forms both dimers and tetramers *in vivo*. Two types of two-hybrid systems, one capable of detecting protein dimerization and the other protein tetramerization, have been used to determine whether environmental changes could affect the oligomerization capacity of this protein in the cell. Increasing the temperature from 37 °C to 48 °C and changing the pH between 4.0 and 9.0 did not influence either dimerization or tetramerization, whereas lowering the temperature below 25 °C and increasing osmolarity were found to reduce the formation of H-NS tetramers, which are the active form of this protein, without affecting dimerization. These findings provide a rationale to explain the induction of H-NS expression during cold-shock, suggest a mechanism contributing to derepressing osmotic-shock genes transcriptionally regulated by H-NS and indicate that changes of the oligomerization properties of H-NS do not play a role in the H-NS and temperature-dependent control of virulence gene expression.

Keywords: two-hybrid system; protein dimerization and tetramerization; environmental control; transcriptional repression

The DNA-binding protein H-NS is an important component of the bacterial nucleoid as well as a modulator of gene expression.[1–6] It is well established that H-NS oligomerization is critical for determining the biological activity of this protein.[7–10]Recently, two types of two-hybrid systems were used to analyze and map the protein sites responsible for *Escherichia coli* H-NS oligomerization. This study demonstrated that *in vivo* this protein forms both dimers and tetramers with the latter being its active form and led to the suggestion that the DNA-binding domain (C-domain) of the protein contributes to tetramerization and that the tetramer contains a bundle of four α-helices, each contributed by an H-NS monomer.[11] Since a large proportion of the numerous genes directly or indirectly controlled by H-NS is involved in bacterial adaptation to changes of environmental conditions,[12,13] it was also hypothesized that changes in the nature and/or efficiency of oligomerization, triggered by changes of the external environment, could modulate the function of H-NS, both as an architectural component of the nucleoid and as a transcriptional repressor.[11] Since *in vitro* the oligomerization equilibria of H-NS were found to be affected by variations of temperature, ionic composition and ionic strength,[14,15] we decided to extend the studies with the two-hybrid systems to include an analysis of the *in vivo* effect of environmental parameters such as temperature, pH and osmolarity on H-NS dimerization and tetramerization.

Effect of temperature on H-NS oligomerization

A temperature of approximately 20 °C represents the lower threshold for balanced growth of *E. coli* and below this limit the cells react with the cold-shock response.[16,17] H-NS has been identified among the approximately 25 gene products whose level is increased after cold stress in *E. coli*[18] and its gene *hns* is essential for cell survival at low temperature[19] (also unpublished observations by

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Abbreviation used: AR, acid resistance.

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M. Giangrossi, Camerino). Indeed, already at a growth temperature of approximately 25 °C, the intracellular level of H-NS begins to increase above that found at 37 °C and continues to increase progressively with further lowering of the temperature so that at 10 °C it is four-to fivefold higher than the basal level.[16,18] On the other hand, above 38 °C the cell undergoes a heat-shock which, like cold shock, entails a transient reprogramming of gene expression.[20]

The transcriptional repression by H-NS of several virulence genes is strictly controlled within a very narrow temperature range. Although at least in the case of *Shigella virF* the temperature-sensitive switch has been identified in a temperature-sensitive DNA bend flanked by extended H-NS binding sites,[21,22] the hypothesis that the temperature-dependence of *virF* repression by H-NS might be related to variations of the aggregation state of this protein[23] has never been challenged.

Preliminary experiments, carried out *in vitro* by large zone gel permeation chromatography, demonstrated that the amount of H-NS tetramers in solution diminishes not only, as expected, with decreasing protein concentration, but also, somewhat surprisingly, upon lowering of the temperature. In fact, as seen in Figure 1(a), when the temperature is lowered from 25 °C to 15 °C at a protein concentration of 0.5 μM, the tetramers abruptly decrease from ≥70% of total H-NS to ≤10%; a similar behavior was observed also at higher protein concentrations, 10 μM and 50 μM (Figure 1(a)). Thus, in light of the potential importance of this finding as a clue for a physiologically significant protein behavior, we sought *in vivo* confirmation of this effect of temperature on H-NS tetramerization. Therefore, H-NS oligomerization was studied as a function of temperature using two established types of two-hybrid systems.[11] In excellent agreement with the results obtained *in vitro* (Figure 1(a)), the *in vivo* results shown in Figure 1(b) demonstrate that the extent of tetramerization starts to decrease in a strong temperature-dependent manner below 28 °C and continues to decrease until 18 °C. The same trend continues at even lower temperatures but we do not present these data because of the excessive fluctuation of the levels of β-galactosidase expressed at these suboptimal temperatures when translation of this protein becomes inefficient.[24] Unlike tetramerization, H-NS dimerization remained completely unaffected by the same temperature changes.

As aforementioned, above 38 °C the *E. coli* cells undergo a heat-shock reponse. However, as seen from the *in vivo* results of Figure 1(b), neither dimerization nor tetramerization of H-NS are influenced by temperatures increases up to 10 deg·C above the heat-shock threshold, indicating that changes of the quaternary structure of this protein do not play a role in the transcriptional control of heat shock genes.

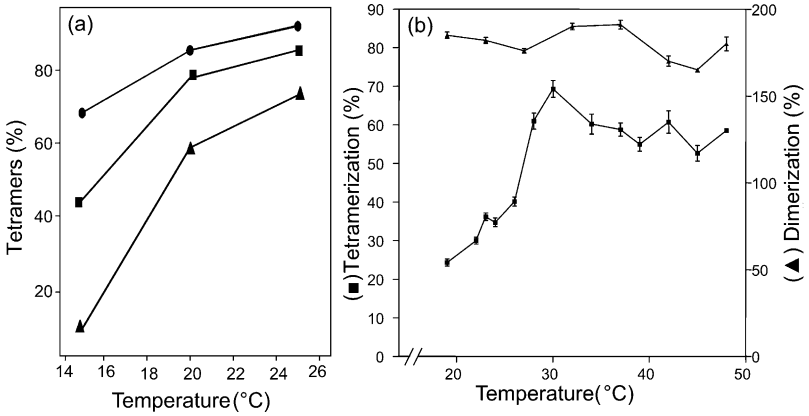

Figure 1. Effect of temperature on H-NS oligomerization. (a) Effect of temperature on H-NS tetramerization *in vitro*. Formation of tetramers was studied by large zone chromatography as described[15] at 0.5 μM (▲), 1 μM (■) and 50 μM (●) H-NS concentrations and at the temperatures indicated on the abscissa. The amount of tetramers is expressed in the ordinate as % of the total protein. (b) Effect of temperature on H-NS oligomerization *in vivo*. H-NS oligomerization was studied by two types of two-hybrid systems using the chimeric constructs and the bacterial hosts described in detail elsewhere.[11] These cells were grown at the temperatures indicated on the abscissa. In the dimerization test[32] the expression of the reporter gene *lacZ* depends upon the activity of a promoter overlapped by two adjacent bipartite hybrid operator sites, $Or2_{434}Or1_{P22}$ and $Or1_{434}Or1_{P22}$, both located within the promoter core. Each bipartite site is recognized by the DNA-binding domain of two lambdoid phage repressors (P22 and 434), which must undergo heterodimerization to yield a functional repressor. Thus, the extent of H-NS dimerization is measured by the capacity of chimaera consisting of H-NS fused to the DNA-binding domains of the 434cI and P22cI repressors to prevent the expression of the *lacZ* reporter gene. The tetramerization test[33] is based on the capacity of chimaera consisting of H-NS fused to the DNA-binding domains of phage λcI repressor to prevent RNA polymerase access to the −35 element of the *lacZ* promoter by specifically binding, as a result of tetramerization, to both the high-affinity site Or1 and to the low-affinity site Or2. In both dimerization and tetramerization tests *lacZ* expression is quantified by the β-galactosidase activity assayed as described[34] and expressed as % of the activity displayed by wt 434/P22 repressors (in the dimerization test) or λcI repressor (in the tetramerization test) after subtraction of the background represented by the activity of 434cI(Δ102-263)/P22(Δ102-263) or λcIN(ΔC), respectively.[11] The dimerization and tetramerization tests were performed three times taking triplicate experimental points in each case so that the error bars represent the deviation from the average of nine measurements.