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Identification of *ara*C Protein on Two-dimensional Gels, its *in vivo* Instability and Normal Level

*ara*C protein was identified on two-dimensional O'Farrell gels (O'Farrell, 1975) as a protein electrophoresing as two spots, both of molecular weight 30,500 and pI near 7·1, but differing by about 0·1 pH unit. The two spots were seen in crude extracts from cells overproducing C protein as specified by a plasmid, by a phage, and were also seen in C protein purified to about 20% purity on the basis of biological activity. A label-chase experiment indicated that both species of *ara*C are unstable *in vivo* and possess half-lives of about 60 minutes. The normal intracellular level of C protein is about 40 monomers per cell.

The regulatory protein of the L-arabinose operon in *Escherichia coli* is both an inducer and repressor of the *ara*BAD operon and apparently acts similarly on the two arabinose transport operons (Englesberg *et al.*, 1969; Kolodrubetz & Schleif, 1981). Additionally, *ara*C protein can regulate its own synthesis (Casadaban, 1976). Here we report the identification of *ara*C protein on two-dimensional O'Farrell gels (O'Farrell, 1975), where the protein migrates as two spots with slightly differing charge. We then use this information to show that *ara*C protein is unstable *in vivo*, with an apparent half-life of 60 minutes, and that the normal *in vivo* level of *ara*C protein is about 40 monomers per cell.

*ara*C protein was identified on the gels in three ways, all leading to the same conclusion. Shown in Figure 1 are autoradiograms of a pair of gels of proteins from cells containing a plasmid that overproduces *ara*C protein by 40-fold, and from cells containing a plasmid that overproduces *ara*C protein by a factor of three (Steffen & Schleif, 1977*b*).

Paradoxically, two protein spots vary in intensity in the expected fashion. To corroborate this finding, the experiment was repeated using cells infected with a pair of λ-p*ara*C bacteriophage as the source of *ara*C. The phage were isogenic except that one contained a nonsense mutation in the *ara*C gene. The results (Fig. 2) lead to the identification of the same two protein spots as the products of the *ara*C gene.

To confirm the above identification and to determine if only one of the spots might actually possess biological activity, purified *ara*C protein was electrophoresed on the same gels. This sample, which had been purified on the basis of inducing activity of *ara*C in the Zubay assay, was approximately 20% pure (Steffen & Schleif, 1977*a*). It, too, yielded the same two protein spots upon electrophoresis (data not presented). The identification of these spots required the addition of crude extract to the purified protein to provide "landmarks." The crude extract was added after protein denaturation to prevent any possible proteolytic processing of the purified *ara*C protein. This experiment does not resolve the question of whether one or both of the protein species are biologically active.

To explore the possibility that the two *ara*C protein species might bear a

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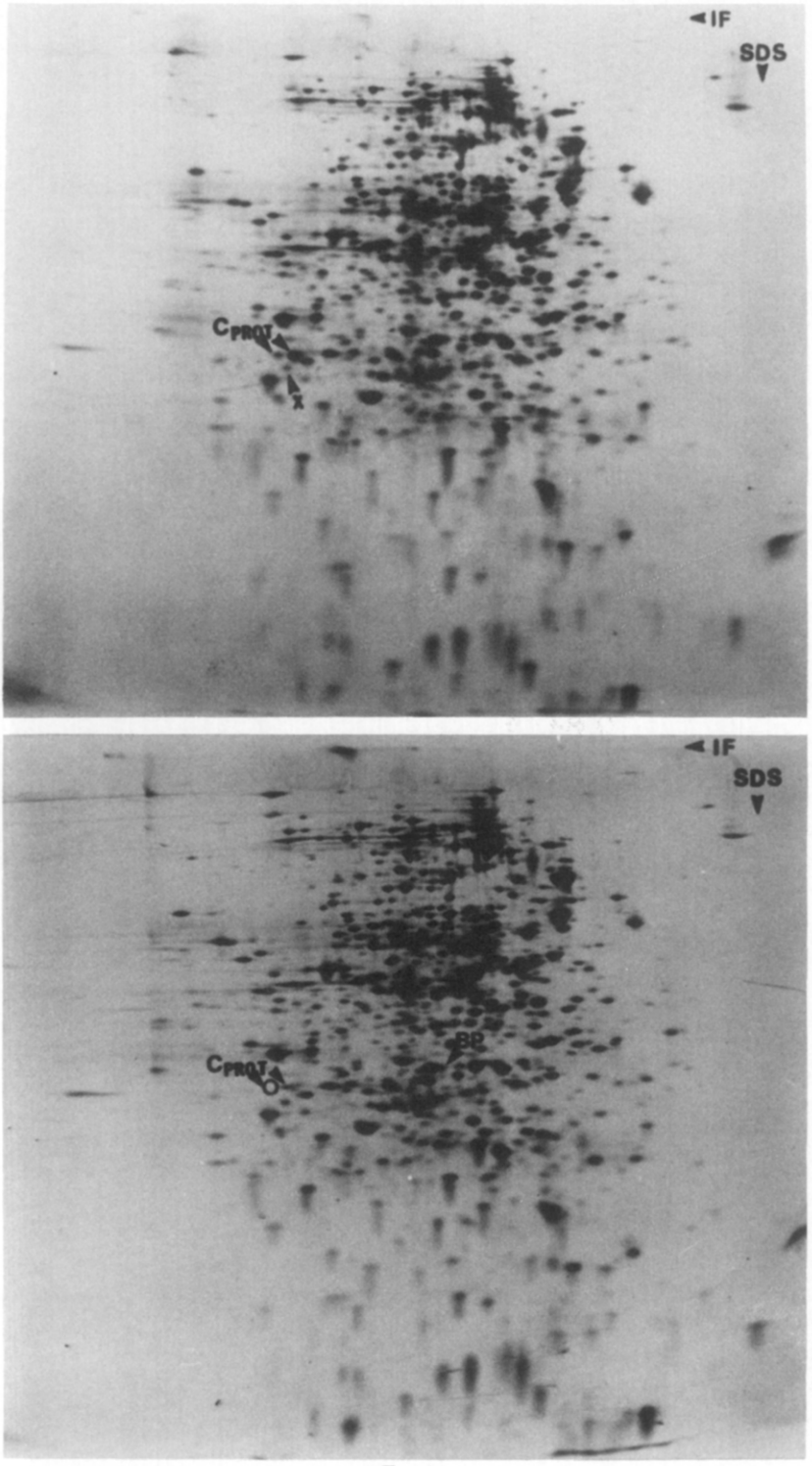

FIG. 1.

precursor-product relationship to one another, a label-chase experiment was performed. Figure 3 shows the gel of an extract prepared from cells grown six generations in the presence of label and a gel of an extract prepared from the same cells following an additional 135 minutes of cold chase. It is apparent from inspection of the gels that the relative intensities of the two *ara*C spots decreased in parallel by about a factor of six relative to the other spots on the gel. This leads to the conclusions that neither spot is a precursor of the other and that the *ara*C protein is unstable *in vivo* and possesses a half-life of about 60 minutes.

It is possible to estimate the levels of *ara*C protein in cells from these experiments. In Figure 1, the more basic of the *ara*C protein spots is about twice as intense as the spot labeled X just below it. In a gel not shown, spot X had the same intensity as the arabinose-binding protein spot. The level of the binding protein measured by equilibrium dialysis on similarly grown cells was determined to be 150 molecules per cell. Taking the sulfur content, since ^{35}S was used to label cells, to be similar in arabinose-binding protein and *ara*C protein, we calculate that the total amount of *ara*C protein in these cells is about 1500 monomers per cell. As these cells synthesize *ara*C protein at about 40 times the level found in wild-type cells, we conclude that the level of *ara*C protein normally present in a cell is about 40 monomers.

The molecular weight, 30,600, and pI, about 7·1, of the two spots identified as C protein are in good agreement with those found by Wilcox & Meuris (1976) and Steffen & Schleif (1977*a*) for purified C protein. Steffen & Schleif would not have observed the two C proteins since they only separated proteins by molecular weight and both C proteins have the same molecular weight. Wilcox & Meuris, however, did run C protein on an isoelectric focusing column. Examination of the procedures and data of Steffen & Schleif indicates that their system did not have sufficient resolution to see two proteins differing by only 0·1 pH unit if one species were present at 10% the level of the other.

Selective artifactual modification of a single charged amino acid, such as deamidation of glutamine or asparagine or oxidation of cysteine, could explain the existence of the two C protein spots. However, this would seem unlikely in view of the fact that C protein prepared by two different methods, the protein isolation procedure of Steffen & Schleif (1977*a*) and that of Ames & Nikaido (1976), gave the same two spots. The only step that the two procedures share is the final

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FIG. 1. The identification of C protein on 2-dimensional gels using C-overproducing plasmids.

The upper gel shows the cytoplasmic proteins from a strain that overproduces C protein 40-fold from a plasmid, pDS1/DLS25. The lower gel shows the cytoplasmic proteins from a strain that overproduces C protein 3-fold, pDS5/DLS25. The arrows point to both electrophoretic species of C protein, the arabinose-binding protein (B.P.) and an unidentified protein, X, used in quantitating the level of C protein in the cell. The cells were labeled for 6 generations with $^{35}SO_4^{-2}$ while growing in M10 medium (Haggerty *et al.*, 1978), glycerol and tetracycline. The cells were harvested and disrupted by sonication. The proteins were prepared for gels as described by Ames & Nikaido (1976) using the 41,000 *g* supernatant as the cytoplasmic fraction and included a DNAase/RNAase treatment and the addition of solid urea to 9·5 M prior to dilution with sample dilution buffer. The protein samples were then electrophoresed on wide range (pH 4·5 to 8) isoelectric focusing gels, equilibrated and then run on a 12% polyacrylamide/sodium dodecyl sulfate gel, all as described by O'Farrell (1975). The gels were dried, exposed to Kodak XR5 X-ray film and then developed according to standard procedures. IF, isoelectric focusing; SDS, sodium dodecyl sulfate.

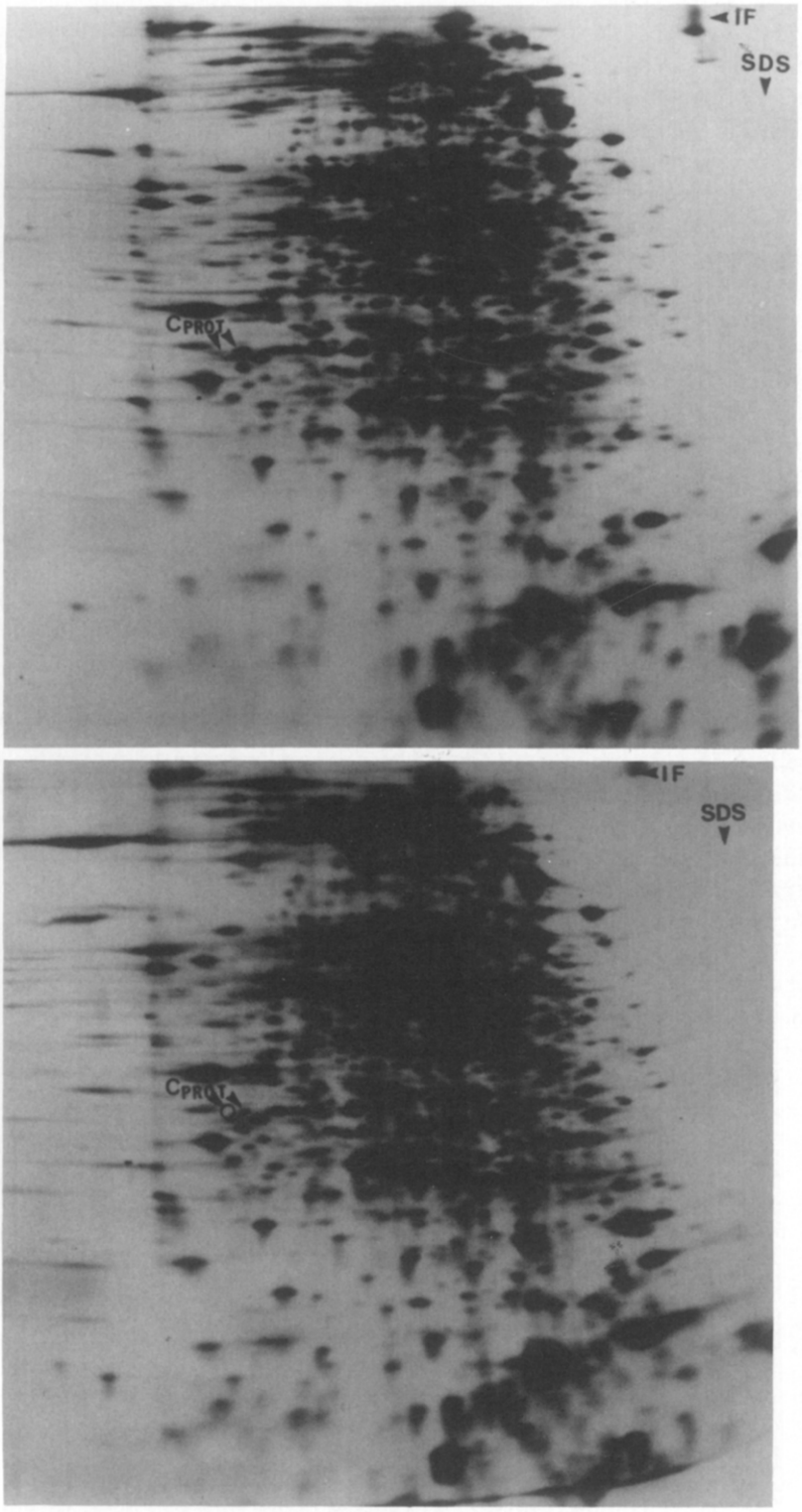

FIG. 2.

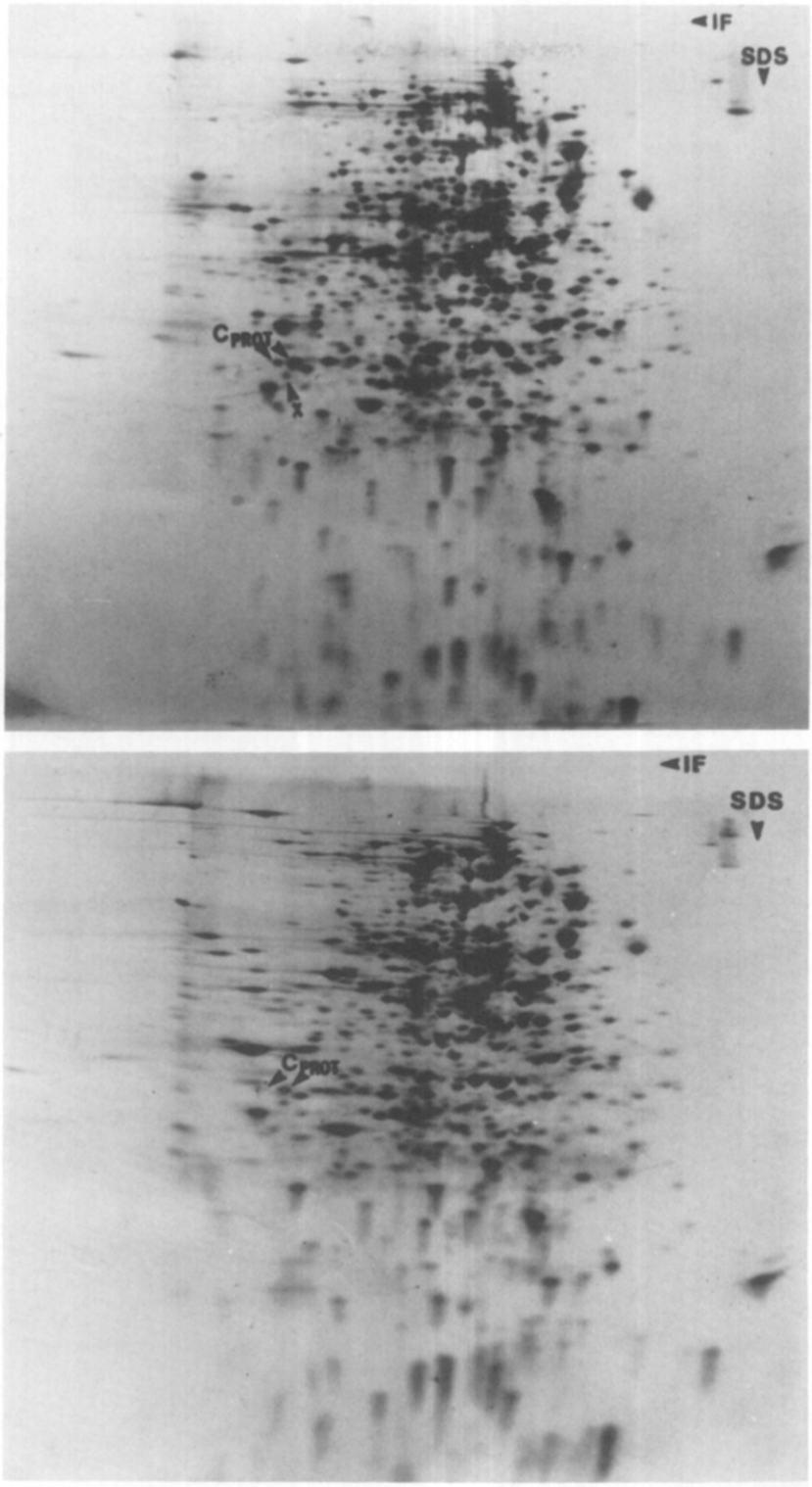

FIG. 3.

resuspension of the protein in the urea/NP40 gel loading buffer. It is possible that the two C protein spots are artifactually modified at this step. Perhaps C protein simply runs as two spots in this gel system, possibly due to preferential protein–solvent interactions.

The more intriguing possibility is that the two C protein spots reflect an *in vivo* modification of C protein. This may or may not then represent two different activities of C protein. The cells from which all of the C protein used in these experiments was isolated were grown in medium without arabinose. It would be interesting to determine the effect of growth on arabinose on the location, relative abundance and lability of the two C protein spots.

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Department of Biochemistry
Brandeis University
Waltham, Mass. 02254, U.S.A.

D. KOLODRUBETZ†
R. SCHLEIF

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† Present address: Molecular Biology Institute, University of California, 405 Hilgard Ave, Los Angeles, Calif. 90024, U.S.A.

FIG. 2. The identification of C protein on 2-dimensional gels using lambda phage with and without a functional *ara*C gene.

The upper gel shows the proteins that are made after infection by λ*para*C138 phage ($AraC^+$) of an Ara^+ λ-sensitive strain. The lower gel shows the proteins that are made after infection by λ*para*C163 ($AraC^-_{nonsense}$) (Steffen & Schleif, 1977*a*). Each gel also contains some labeled protein from pDS5/DLS25 as shown in Fig. 1 to localize the C protein produced by the phage. The small amount of C protein seen on the λ*para*C163 + pDS5/DLS25 gel is also present on a gel containing only the same amount of the pDS5/DLS25 labeled proteins. Cells were grown at 35°C to 2×10^8 cells/ml in 15 ml of M10 medium containing $MgSO_4$ adjusted to 10^{-4} M, also containing 0·2% (u/v) glycerol, 0·2% (w/v) maltose, and 10^{-3} M-$MgCl_2$. $^{35}SO_4^{-2}$ was added to 33 μCi/ml and 5 min later, phage, possessing both *c*I857 and *S*7 mutations was added at a multiplicity of infection of 7. After 20 min at 35°C, the culture was heated to 42°C for 15 min to ensure that all phage were induced. The culture was cooled to 35°C, grown with vigorous aeration for 5 h and harvested. Sample preparation and electrophoresis were as described in the legend to Fig. 1. IF, isoelectric focusing; SDS, sodium dodecyl sulfate.

FIG. 3. Turnover of *ara*C protein as seen on 2-dimensional gels.

The top gel shows the cytoplasmic proteins of the C-overproducing strain pDS1/DLS25 after being continuously labeled with $^{35}SO_4^{-2}$. The lower gel shows the cytoplasmic proteins from the same strain after being labeled with $^{35}SO_4^{-2}$ for 6 generations and then chased for 1 generation with unlabeled $MgSO_4$. These experiments were done on cells that contained a Tet^R plasmid, growing at 35°C in 50 ml of M10 medium containing 10^{-4} M-SO_4^{-2}, 0·2% (v/v) glycerol, 7 μg tetracycline/ml and 250 μCi $^{35}SO_4^{-2}$. At $2{\cdot}5 \times 10^8$ cells/ml, half of the cells were harvested while the other half were transferred into a new prewarmed flask and the $^{35}SO_4^{-2}$ was chased for 135 min by the addition of non-radioactive $MgSO_4$ to 10^{-2} M. These cells were then harvested at 5×10^8 cells/ml.

The cells from both preparations were treated as described in the legend to Fig. 1, and the cytoplasmic proteins extracted were electrophoresed and autoradiographed as described in the legend to Fig. 1.

The arrows point to the 2 C proteins as well as to an unidentified protein, X, used to determine by how much the level of C protein changed in the 2 gels. IF, isoelectric focusing; SDS, sodium dodecyl sulfate.

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