*ISOLATION OF A CHLORAMPHENICOL-RESISTANT PROTEIN FROM λ-INFECTED CELLS**

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Abstract.—During infection with bacteriophage φX174, a protein, made in the presence of 30 γ chloramphenicol/ml but not made in the presence of concentrations greater than 100 γ/ml, has been shown to be essential for viral DNA replication. A protein with similar chromatographic properties and "chloramphenicol resistance" has now been demonstrated in cells infected with λ bacteriophage.

Introduction.—Circular λ DNA replicates in the presence of 30 μg/ml of chloramphenicol, albeit more slowly (about 1/5 the rate) than in the absence of this drug. λ circular DNA is not replicated in the presence of 100 μg/ml.[1] A similar situation is found with circular φX174 replicative form (RF) which is synthesized in 30 μg/ml of chloramphenicol,[2] but not in 100 μg/ml.[3, 4] In φX-infected cells a viral-specific protein required for progeny replicative-form replication is synthesized in 30 μg/ml of the drug but not in 100 μg/ml. This protein (coded by cistron VI of φX174) has been isolated by chromatography on $CaHPO_4$ columns, with the aid of a double-label technique in the presence of 30 μg/ml of chloramphenicol.[5] The similarities between φX RF and circular λ DNA replication and their differential sensitivities to the drug concentrations suggested that a λ-directed protein might be detected by utilizing the same techniques successfully employed to isolate the φX-chloramphenicol-resistant protein.

Experimental.—To investigate this possibility, a 40-ml culture of *E. coli* CR34 (a leucine, threonine, and thymidine auxotroph) was grown to 2.3×10^8 cells/ml in minimal medium (Salivar and Sinsheimer, personal communication) with maltose (1 mg/ml) instead of glucose and 20 μg/ml each of leucine, threonine, and thymidine. The cell culture was divided so that one half was treated with 30 μg/ml of chloramphenicol for 15 min and the second half with 150 μg/ml of this drug for the same time period. The cells were then centrifuged out of suspension and each culture was resuspended in 2 ml of T-M medium (Salivar and Sinsheimer, personal communication) supplemented with 30 μg/ml chloramphenicol for those cells pretreated with that concentration of the drug or 150 μg/ml for those cells previously treated with the higher concentration. One half of each of the two 2-ml cell suspensions was then infected with λC26 (m.o.i. 5), and ^{3}H-leucine (25 μc/ml) was added to the infected cultures. ^{14}C-leucine (1 μc/ml) was added to the uninfected. After 10 min at 37°C all four cultures received an additional 9 ml of minimal medium (supplemented with 20 μg/ml of threonine and 20 μg/ml of thymidine). The final concentrations of chloramphenicol, ^{3}H-leucine, and ^{14}C-leucine were adjusted to equal those present in the adsorption step. After 40 min at 37°C the four cell cultures were collected by centrifugation. The infected and uninfected cells that were treated with 30 μg/ml of the drug were mixed, treated with lysozyme-EDTA, and lysed by freezing and thawing as described by Levine and Sinsheimer.[5] An identical procedure was employed for the cells labeled in 150 μg/ml of the drug.

The two resultant lysates were dialyzed against 0.01 *M* sodium phosphate buffer at pH 6.5 and chromatographed on $CaHPO_4$ columns as described elsewhere.[5] The

elution profile ($CaHPO_4$ column) of the chloramphenicol-resistant proteins made in 30 μg/ml of this drug by the λC26-infected cells (^{3}H-leucine) and uninfected cells (^{14}C-leucine) is shown in Figure 1. The radioactivity in a component (or components) slightly retarded by the column was preferentially found in the infected cells (^{3}H-leucine-labeled) as reflected in the increased ratio of ^{3}H to ^{14}C. The ratio of ^{3}H to ^{14}C in the extract before fractionation was 5.5. The variations in this ratio from fractions eluted at higher pH and ionic strengths (not shown in Fig. 1) were between 5.0 and 5.9. In contrast, the chromatographic profile shown in Figure 1 yielded up to a threefold increase in the ^{3}H to ^{14}C ratio (to 17.0). When the labels were reversed (^{14}C for the infected cells and ^{3}H for the uninfected cells), a similar result (reciprocal) was obtained. The increased ^{3}H to ^{14}C ratio was also observed when L-lysine was employed instead of leucine as the radioactive amino acid. The portion of the chromatogram shown in Figure 1 represents approximately 3–5% of the total counts.

The elution profile (second $CaHPO_4$ column) of the chloramphenicol-resistant proteins made in 150 μg/ml of this drug by the λC26-infected and uninfected cells is shown in Figure 2. The ^{3}H to ^{14}C ratio of the extract before fractionation was 4.5. The variation in the ^{3}H to ^{14}C ratio presented in Figure 2 is between 4.0 and 5.1. There is no indication of the chloramphenicol-resistant protein synthesized in 30 μg/ml of this drug and detected by $CaHPO_4$ chromatography (Fig. 1).

Two uninfected cell cultures were treated with 30 μg/ml of chloramphenicol for 15 min at 37°C. The first culture was labeled with ^{3}H-leucine and the other with ^{14}C-leucine as previously described. When these cells were mixed, lysed, and chromatographed on $CaHPO_4$ columns, the drug-resistant protein found in Figure 1 was not observed.

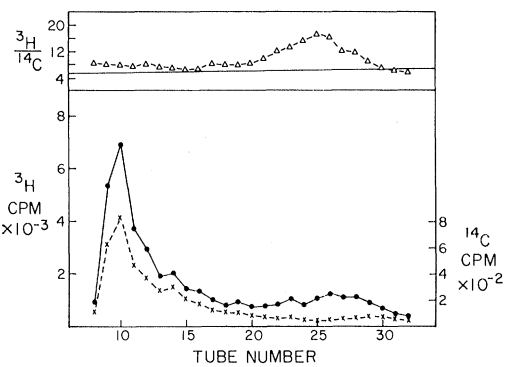

FIG. 1.—Elution profile of CM-resistant proteins (30 μg/ml of CM) from a $CaHPO_4$ column at 0.01 *M* sodium phosphate buffer (pH 6.5). (●—●) ^{3}H-labeled infected culture; (x – – – x) ^{14}C-labeled uninfected culture; (△—△) ^{3}H/^{14}C ratio. The solid line included in the ^{3}H/^{14}C ratio portion of the graph indicates the ratio of the two different radioactive labels found before fractionation by $CaHPO_4$ chromatography.

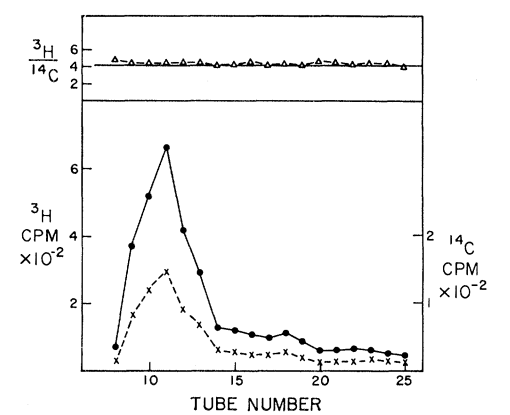

FIG. 2.—Elution profile of CM-resistant proteins (150 μg/ml of CM) from a $CaHPO_4$ column at 0.01 *M* sodium phosphate buffer (pH 6.5). (●—●) ^{3}H-labeled infected culture; (x – – – x) ^{14}C-labeled uninfected culture; (△—△) ^{3}H/^{14}C ratio. The solid line included in the ^{3}H/^{14}C ratio portion of the graph indicates the ratio of the two different radioactive labels found before fractionation by $CaHPO_4$ chromatography.

Discussion.—The results presented here indicate that a λ-specific protein synthesized in 30 μg/ml but not in 150 μg/ml of chloramphenicol can be isolated with the same techniques used to demonstrate an analogous φX-specific protein.[5] The φX-drug-resistant protein (to 30 μg/ml) is required for RF replication.[3, 4] An *E. coli* protein (replicator) required for the initiation of *E. coli* DNA replication has been shown to possess the same differential sensitivity to chloramphenicol levels (DNA synthesis is initiated at 30 μg/ml but not at 200 μg/ml).[6, 7] These facts, taken together with the differential sensitivity of λ DNA replication to the drug concentration, suggest the possibility that the λ protein might be required for λ DNA synthesis. It is possible that the $CaHPO_4$ fractionation techniques employed here could be used to demonstrate additional examples of a class of similar chloramphenicol-resistant proteins.

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