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Process of Infection with Bacteriophage ϕX174

XXXVI. Measurement of Virus-Specific Proteins During a Normal Cycle of Infection

ROBERT F. MAYOL AND ROBERT L. SINSHEIMER
Division of Biology, California Institute of Technology, Pasadena, California 91109

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Double-labeling techniques in which ^{14}C-labeled, ϕX174-infected cells and ^{3}H-labeled, uninfected cells were used permitted the identification of the virus-specific proteins after separation by sodium dodecyl sulfate-polyacrylamide gel electrophoresis without prior inhibition of host-cell protein synthesis by ultraviolet irradiation. It was also possible to detect previously undescribed components of high molecular weight which may represent induced host proteins. The gel regions specifically corresponding to cistron II protein and the chloramphenicol-resistant VI protein were identified, and a third new, small peak of unknown origin was detected. Studies of the rate of synthesis of virus-specific proteins at various times after infection indicated that the product of cistron I (lysis) is made only late in infection, but the other proteins seemed to be synthesized at the same relative rates throughout infection (although in different amounts). Studies of the proteins obtained from uniformly labeled ϕX virus particles indicated that all of the spikes are identical and allowed a formulation of the structure of the phage capsid.

The deoxyribonucleic acid (DNA) of bacteriophage ϕX174 is a single-stranded ring with a molecular weight of 1.7×10^6. Eight complementation groups have been determined, suggesting that the viral DNA can code for at least eight proteins. Four cistrons (II, III, VII, and probably IV) specify structural components of the phage coat (19), and one (cistron I) is responsible for lysis of the cell (9). The protein of cistron VI is necessary for replicative form (RF) replication, and the products of cistrons V and VIII appear to be necessary for synthesis of single-stranded DNA (14; F. Funk, *personal communication*).

Previous reports (2, 7) have described the electrophoretic patterns in polyacrylamide gels of the proteins made during infection with ϕX174 and with the related phage S13 (17); these results were obtained by use of ultraviolet (UV)-irradiated cells to suppress the incorporation of labeled amino acids into host proteins. In this paper, we describe experiments performed with nonirradiated cells. These experiments allowed shorter pulses, higher specific activities of the proteins, and conditions which can be correlated more closely with the known sequence of events in ϕX replication. Some new components have been observed by use of these methods, and identification of the cistrons corresponding to other gel components has been made.

MATERIALS AND METHODS

Phage and bacteria. All of the *amber* mutants used in these studies were isolated by Clyde Hutchison or Fred Funk. Stocks were grown from single plaques according to the method described by Hutchison and Sinsheimer (9) and were concentrated by precipitation with 5% polyethylene glycol. Labeled phage particles were purified by repeated sedimentation in 5 to 20% sucrose gradients followed by CsCl equilibrium banding (10). Phage spikes were removed by treatment of phage particles with 4 M urea as described by Edgell, Hutchison, and Sinsheimer (6).

Escherichia coli C was used as the nonpermissive host in these experiments.

Media. The medium used was TPG (21) plus adenine (10 μg/ml).

Labeling procedure for detection of phage-specific proteins. Cells were grown with aeration to 3×10^8 to 4×10^8/ml, and 5-ml portions were transferred to small bubbler tubes and infected with ϕX174 *wt* (or *am* mutant) at a multiplicity of 3 to 5. The infected cultures were labeled with ^{14}C-leucine (1 μCi/ml; 312 mCi/mmole, Schwarz BioResearch Inc.), usually for 5 min at 15 to 20 min after infection. Short pulses were terminated by the addition of 10 μmoles of unlabeled leucine and quick chilling in ice. The uptake of ^{14}C-leucine into acid-precipitable components under these conditions is linear for approximately 7 min of pulse. An uninfected control culture was labeled for an equal time interval with ^{3}H-leucine (20 μCi/ml; 2 Ci/mmole, Schwarz BioResearch Inc.). At the end of the labeling period, 50-μliter portions of each culture were

precipitated with 5% trichloroacetic acid onto membrane filters (type HA, Millipore Corp.) and counted.

The cells were centrifuged at low speed, and the pellets were suspended in 0.2 ml of 0.05 M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride, *p*H 8.0; 25 μliters of freshly prepared 1 mg/ml lysozyme (Sigma Chemical Co.) in 0.1 M Na_2-ethylenediaminetetraacetate was added. After incubation for 5 min at 37 C, the cells were disrupted by freezing and thawing; 10 μliters of deoxyribonuclease (0.1 mg/ml in 1 M $MgSO_4$) was added, and the cells were incubated for an additional 1 min at 37 C. The entire extracts were dialyzed against 0.05 M Tris-hydrochloride, *p*H 8.0.

Solubilization of cell extracts and phage particles. Samples of each of the ^{14}C-labeled, ϕX-infected cell extracts were mixed with an equal volume of the ^3H-labeled, uninfected cell extract. This solution was made 0.1% sodium dodecyl sulfate (SDS), 0.5% 2-mercaptoethanol, and 9 M by the addition of solid urea (ultrapure, Mann Fine Chemicals, Inc.). The solution was incubated at 37 C for 3 hr and was heated to 80 C for 15 min just prior to electrophoresis. This treatment was necessary for elimination of protein aggregates which were present and for complete disruption of purified protein-labeled phage particles so that no counts remained at the top of the gel after electrophoresis.

Electrophoresis. The 12% gels used were prepared according to the method of Davis (4) from a stock solution "C" containing 60 g of acrylamide (Eastman Chemical Products, Inc.) and 0.5 g of *N,N'*-methylenebisacrylamide (Eastman Chemical Products, Inc.) per 100 ml. The gels contained 0.38 M Tris-hydrochloride, *p*H 8.9, and 0.1% SDS. An additional 5 μliters of TEMED (Eastman Chemical Products, Inc.) was added per 8 ml of gel solution. Polymerization was catalyzed by the addition of 1 volume of 0.14% ammonium persulfate per volume of gel solution. The electrode buffer contained 0.6 g of Tris, 2.88 g of glycine per liter, *p*H 8.3, and 0.1% SDS. Gels were usually 6 cm in length and 0.5 cm in diameter.

The heated protein solutions were cooled to room temperature, a small amount of bromophenol blue tracking dye was added, and they were layered onto the top of the gel. The current was adjusted to 2 ma per tube until the dye had completely entered the gel. Electrophoresis was continued at 4 ma per tube (250 v) until the dye had migrated to the bottom (1.5 hr for a 6-cm gel). The gels were removed from the tubes and frozen on dry ice.

Slicing and counting of gels. The frozen gels were sliced with a device consisting of parallel razor blades mounted on threaded rods and separated by washers. Each slice was placed into a scintillation vial, and 5 ml of a 1:10 mixture of NCS Reagent (Nuclear-Chicago Corp.) and toluene scintillation fluid was added. The vials were capped and allowed to stand overnight at room temperature. During this time, the radioactivity is extracted from the gel slice, which becomes swollen and transparent. This method allows quantitative recovery of protein radioactivity from acrylamide gels (15*a*). The samples were counted in a Beckman liquid scintillation spectrometer. Overlap of ^{14}C into the ^3H channel was 18%; of ^3H into the ^{14}C channel, less than 1%. Efficiency for ^{14}C at these settings was 60%; for ^3H, 25%. R_F values for the peaks were determined from the migration of the bromophenol blue tracking dye. Molecular weights were determined by calibrating the gels with proteins of known molecular weights as described by Shapiro et al. (16).

Calculation of phage-specific ^{14}C counts per minute. The amount of ϕX-specific ^{14}C counts/minute was determined by subtracting the host-cell specific ^{14}C counts/minute (obtained from the amount of ^3H counts/minute in the fraction) from the total ^{14}C counts per minute per fraction, according to the equation:

$$\Delta^{14}C = {}^{14}C - {}^{3}H\ (R_{min})$$

where $\Delta^{14}C$ is the activity in the ϕX-specific component, ^{14}C is the total ^{14}C counts per minute per fraction, ^3H is the counts per minute per fraction corrected for overlap of ^{14}C; and R_{min} is the minimum $^{14}C/^3H$ ratio found among the fractions (regions of the gel which contain no ϕX-specific material).

RESULTS

Intracellular ϕX-specific proteins. Infection with ϕX174, unlike some bacteriophages, does not appreciably affect host-cell protein synthesis, although host DNA synthesis is suppressed at late times after infection (13). Because of the high background of incorporation into host proteins, previously mentioned attempts to study the synthesis of intracellular proteins during infection have made use of UV-irradiated cells, which synthesize very little host protein but are able to support the growth of ϕX to a limited extent. The doses of UV radiation employed have reduced the net phage yield from such cells to 5 to 15% of normal (2, 17). We have observed that it is not necessary to suppress host protein synthesis, since the ϕX-specific proteins can be detected directly, by use of double-isotope techniques with uninfected cells as controls.

Figure 1 shows a typical electrophoretic pattern obtained from cells infected with ϕX174 *wt*. The pattern of radioactivity obtained from the sliced gel (Fig. 1a) is very heterogeneous and, although there are regions of relative increases of ^{14}C counts/minute compared with the ^3H counts/minute, interpretation of these differences is difficult because of the large number of apparent peaks present. If, however, the data are presented as the ratio of ^{14}C counts/minute to ^3H counts/minute in each fraction (Fig. 1b), the pattern is more easily interpreted and resembles that obtained previously in UV-treated cells. The $^{14}C/^3H$ ratios obtained under similar double-labeling conditions when neither culture was infected (or when both were infected) show no peaks and deviate no more than ± 5 to 10% from the mean

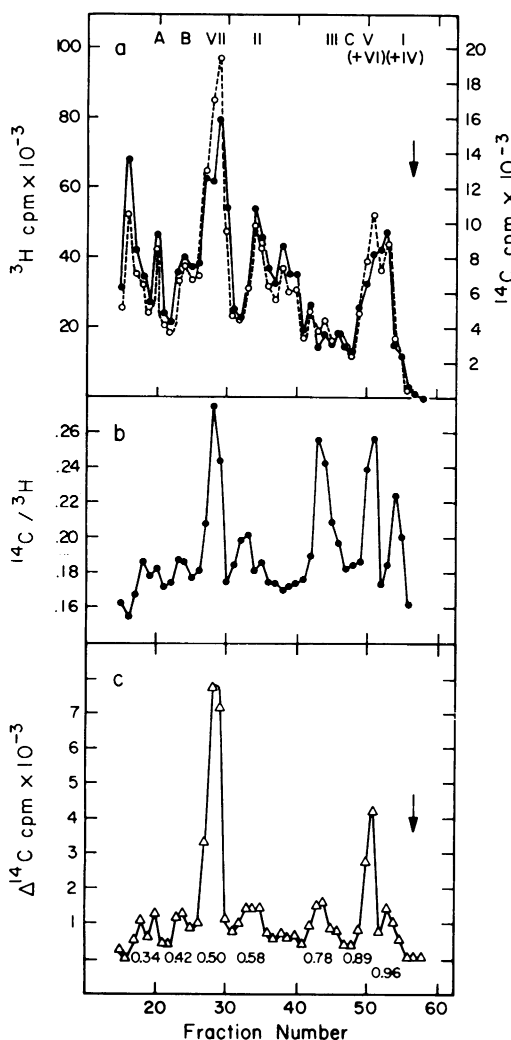

FIG. 1. *Polyacrylamide gel electrophoresis of a mixture of ^{14}C-labeled proteins from φX174 wt-infected cells (multiplicity of infection = 1) and ^{3}H-labeled proteins from uninfected cells. (a) Counts/minute of ^{14}C (○) and ^{3}H (●) per gel slice; (b) ratio of $^{14}C/^{3}H$ per gel slice; (c) phage-specific ^{14}C obtained by subtraction of ^{3}H counts per minute per fraction × $^{14}C/^{3}H$ minimum ratio (fraction 16) from the total ^{14}C counts per minute per fraction (see Materials and Methods). The numbers below each peak indicate the R_F relative to the tracking dye.*

(see also Fig. 6a). If it is assumed that there is no selective termination of host protein synthesis after infection, then the points of lowest $^{14}C/^{3}H$ ratios (fractions 15, 16, and 17) illustrate the true "background" ratio which would have been observed had the cells not been infected. One can then subtract from the total ^{14}C counts/minute in each fraction the amount of ^{14}C which represents host protein incorporation, calculated from the amount of ^{3}H present in the fraction (see Materials and Methods). Performing these calculations on the data in Fig. 1a and 1b allows the phage-specific ^{14}C counts/minute ($\Delta^{14}C$) to be plotted (Fig. 1c).

The size of the $^{14}C/^{3}H$ ratio change was dependent upon the number of ^{3}H counts/minute in the fraction. The difference in the apparent size of the III peak between Fig. 1b and 1c was a consequence of the low ^{3}H counts in these fractions (Fig. 1a), so that the increased ^{14}C counts/minute produced a larger increase in the $^{14}C/^{3}H$ ratio. These peaks changed only slightly in proportion if a mixture of five amino acids was used to label the proteins instead of solely leucine.

Although this gel pattern resembles those reported previously, there are significant differences in minor components which possibly were more easily detected under these conditions because of the greater incorporation of isotope and higher specific activities which were obtained. The minor peaks A and B of high molecular weight have not been reported after infection in UV-irradiated cells; they may be host proteins whose synthesis is induced or stimulated by infection.

The possibility was considered that these peaks could be aggregates of viral coat components. We have observed a large peak migrating in the region of peak A when the double-labeled extract was not subjected to the 9 M urea treatment, but only to SDS, 2-mercaptoethanol, and heat. However, evidence will be presented later that the residual minor components in regions A and B cannot be aggregates.

An additional minor component C was also observed and will be discussed later.

The approximate molecular weights are 48, 40, 23, 18, and 5 × 10^{3} to 15 × 10^{3} for proteins VII, II, III, V, and I, respectively. The gel region containing fractions 1 to 15 has few counts (see Fig. 4) and is usually omitted from the figures.

Determination of cistron assignments for gel peaks. The peaks corresponding to cistrons I, V, and III (Fig. 1c) were previously identified by Burgess and Denhardt (2) on the basis that infection of nonpermissive cells with *am* mutants in these cistrons eliminates these peaks from the gel patterns. We have confirmed these observations using *am*6 (cistron I), *am*10 (cistron V), and *am*9 (cistron III). Most mutants, however, give patterns similar to that seen with *wt* φX.

Burgess and Denhardt also reported that infection with *am*S5-10 (cistron VII) resulted in a peak of capsid protein which was of smaller molecular weight than that seen in *wt*-infected cells; this result suggested that the capsid protein

is coded by cistron VII, and not by cistron IV as previously believed. We have confirmed these observations using mutants *am* S5-4, S5-10, and S5-14 (cistron VII), all of which produce a capsid protein which migrates faster than purified capsid protein obtained from 4 M urea-treated ^{3}H-labeled ϕX particles (6).

We have identified the cistron II peak by observing that it is missing in extracts after infection with *am*S5-6 (cistron II) under nonpermissive conditions. However, the cistron II peak was often difficult to detect under these conditions of double labeling; for unknown reasons, it was more easily detected in extracts from cells infected with *am*3 (cistron I) than from those infected with *wt* ϕX (compare Fig. 1c and 4). In addition, it migrates as a broad peak in extracts from infected cells and may contain more than one component, especially as it migrates as a sharp peak when obtained from disrupted whole phage.

The cistron II product is clearly a component of the phage coat (see Fig. 2b) and is removed by removing the spikes from the capsid. Infection with cistron II mutants under nonpermissive conditions resulted in the production of noninfectious particles which sedimented more slowly than 114*S* ϕX particles (S. G. Krane, Ph.D. Thesis, California Institute of Technology, Pasadena, 1966). The electrophoretic pattern obtained from purified ^{3}H-labeled *am*S5-6 (cistron II) defective particles (Fig. 2a) is missing the same spike protein which was missing in the *am*S5-6 infected extract (*data not shown*). The gel patterns obtained from whole phage and "70*S*" particles (18), which are also noninfectious, are identical. It is interesting that these defective particles contain different relative amounts of VII and III proteins than do whole phage or 70*S* particles. In leucine-labeled whole phage, 70% of the counts/minute were in VII and 19% were in III, whereas in leucine-labeled *am*S5-6 defective particles 62% were in VII and 30% were in III.

The product of cistron VI is necessary for the synthesis of progeny (but not parental) RF molecules in ϕX-infected cells (11, 13, 21). We have been unable to observe the reduction of any components in our gel patterns after infection with mutants in cistron VI. Gelfand and Hayashi (7) identified a peak corresponding to their cistron C (functionally equivalent to our cistron VI) migrating in the region between peaks II and III (Fig. 1c). We also saw a broad but variable peak in this region of the gel which we suspect to be an aggregate. Burgess and Denhardt (2) did not report any peaks corresponding to cistron VI protein.

The cistron VI product is a chloramphenicol-resistant protein, being made in 30 μg of chloramphenicol per ml, but not in 100 μg/ml. Levine and Sinsheimer (11) utilized this property to detect the cistron VI chloramphenicol-resistant protein in cell extracts, using double-isotope techniques and $CaHPO_4$ chromatography. Concentration and electrophoresis of the chloramphenicol-resistant VI protein obtained from the $CaHPO_4$ chromatography (11) show a single peak migrating in the region of the cistron V peak (Fig. 3).

It is possible that the protein made in chloramphenicol is a smaller (but active) peptide which has an increased mobility in the SDS gels and therefore migrates farther than the protein identified as cistron VI by Gelfand and Hayashi. Alternatively, evidence presented below shows that material taken from the variable region of the gels, comparable to the Gelfand and Hayashi "C" protein, when further disrupted migrates to the region of the chloramphenicol-resistant VI protein (see Fig. 4 and 5), suggesting that this component between the products of cistrons II and III is an aggregate.

We have been unable to detect any significant differences from wild type in the gel patterns produced by mutants in cistrons IV or VIII.

The following experiment was performed to see whether any small protein components (from either virus or host) were being lost during dialysis. Cells were infected with ϕX*wt* and divided in half; one half was labeled with ^{14}C-leucine and the other with ^{3}H-leucine. The cells were lysed, and the ^{14}C-labeled extract was dialyzed. Amounts of 25 μliters each of the dialyzed and undialyzed extracts were combined and subjected to electrophoresis as usual. The gel was stained in 0.25% Coomassie blue in methanol-acetic acid-water (5:1:5) and destained by soaking in 7.5% acetic acid, 5% methanol. The gel was then sliced and counted. If any proteins had been lost during dialysis, they would have been detected by a reduced $^{14}C/^{3}H$ ratio in their respective regions of the gel. No significant changes in the relative amounts of ^{14}C and ^{3}H were observed.

Composition of the phage particle. Phage ϕX *am*3 (lysis defective) was grown for 2 hr in TPG medium containing uniformly labeled ^{14}C-glucose to achieve uniform labeling of the phage particles. The phage were purified by two cycles of sedimentation in sucrose gradients and CsCl equilibrium density gradients. The purified phage were disrupted, and the components were subjected to electrophoresis after each step in the purification (Fig. 2b). The counts/minute in the phage DNA (21% of the total counts/minute) remained at the top of the gel.

The percentages of counts/minute of the phage

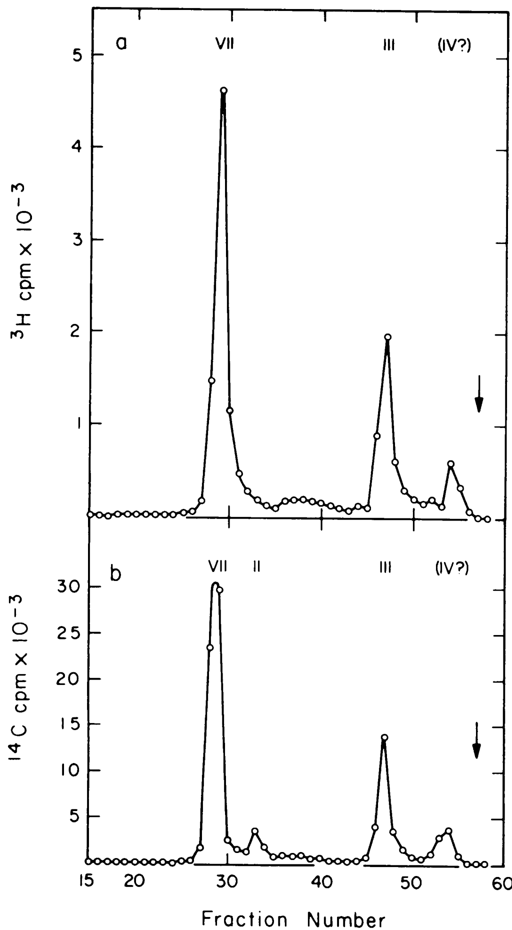

FIG. 2. *(a) Electrophoresis of proteins of amS5-6 (cistron II) defective particles. A 50-ml culture of cells was infected with amS5-6 (cistron II; a late-lysing mutant which produces defective particles), 0.5 mCi of ^{3}H-leucine was added 10 min after infection, and the incubation was continued for an additional 60 min. The cells were harvested and lysed, and the phage was purified by two sedimentations through 5 to 20% sucrose gradients. (b) Electrophoresis of proteins of uniformly labeled φX174 am3 (cistron I). Cells were grown to 3 × 10^8/ml, harvested, and starved for 30 min in 0.5 volume of starvation buffer (5). TPG (2×) medium containing uniformly labeled ^{14}C-glucose (6.6 mCi/mmole; Schwarz BioResearch, Inc.) was added, and the cells were infected with φX am3 (cistron I; lysis defective). The incorporation of radioactivity was followed by precipitation of 50 μliter samples with trichloroacetic acid. At 2 hr after infection, the cells were collected and lysed, and the phage was purified by two cycles of sucrose gradients followed by sedimentation to equilibrium in a CsCl density gradient. Samples were disrupted and the components were subjected to electrophoresis after each purification step. Approximately 25,000 counts/min were found in fractions 1 to 5, which represent 21% of the total counts and are presumably the phage DNA (25% of the phage particle weight).*

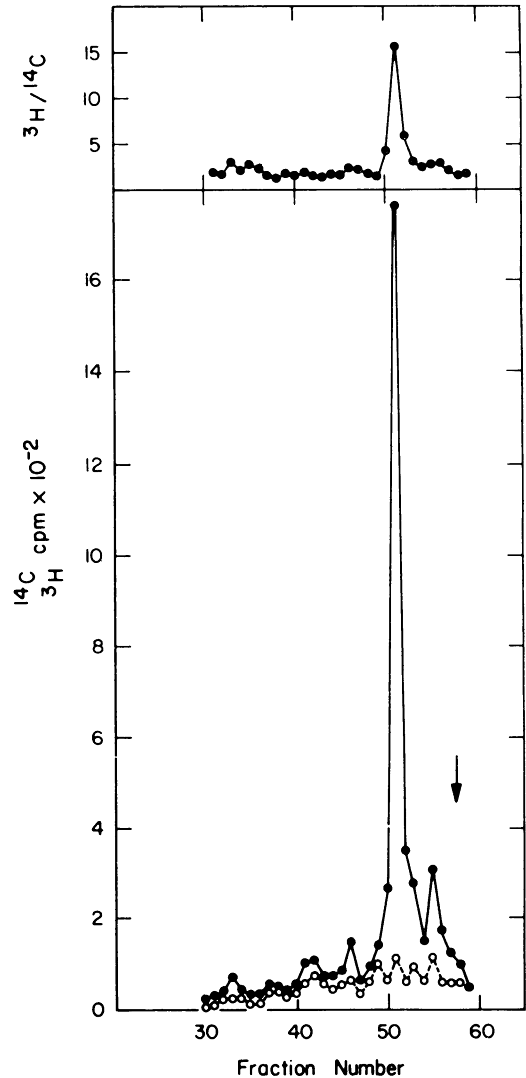

FIG. 3. *Electrophoresis of CM-resistant cistron VI protein. The CM-resistant cistron VI protein was prepared according to the Brij-lysis method described by Levine and Sinsheimer (11) and fractionation on $CaHPO_4$. A single fraction of high ^{3}H/^{14}C ratio was obtained from the column. This fraction was concentrated and subjected to electrophoresis after disruption in urea, SDS, and 2-mercaptoethanol. In this experiment, the infected, CM-treated cells were labeled with ^{3}H-leucine, and the infected cells were labeled with ^{14}C-leucine (11). Symbols; ●, ^{3}H counts/minute; ○, ^{14}C counts/minute.*

proteins were 60:8:23:9 for proteins of cistrons VII:II:III:(IV?). The percentage of counts in each peak was different if histidine or leucine was used to label the phage; the VII peak then contained 77 and 70%, respectively, and the other peaks decreased slightly. If purified 70*S* particles were disrupted and the components subjected to electrophoresis, the pattern obtained was the same as for whole phage, with few counts remaining at the top of the gel, however, which indicates that the 70*S* particles are in large part free from DNA.

During sedimentation of the phage particles to equilibrium in CsCl density gradients, a small number of counts were found at the top of the centrifuge tube. Examination of this material yielded the same composition as the phage particle and excluded the possibility that a minor spike component was removed from the virus particle during the purification. The percentage of counts/minute in each peak under these labeling conditions can be interpreted as the percentage by weight of the proteins in the ϕX particle.

Are some peaks aggregates? The peaks A and B appearing in Fig. 1c are of high molecular weight (>50,000) and could conceivably represent aggregates, as mentioned previously. To examine this possibility, a 25-ml culture was infected with ϕX*am*3 and ^{14}C-labeled for 10 min at 15 to 25 min after infection. The cells were harvested and mixed with 25 ml of cells from a ^{3}H-labeled, uninfected culture in 0.2 ml of Tris buffer; the cells were lysed and dialyzed, and 50 μliters was analyzed by electrophoresis (see Materials and Methods). The gel slices were eluted in 0.2 ml of 0.05 M Tris-0.1% SDS overnight, and 25-μliter samples of each fraction were counted (Fig. 4). (Infection with *am*3 does not result in a diminished peak for the cistron I protein.) Treatment of portions of various fractions with 9 M urea, 0.2% SDS, and 0.5% 2-mercaptoethanol by incubation at 37 C for 3 hr, followed by 15 min at 100 C (in a boiling-water bath) and electrophoresis gave the results shown in Fig. 5. It can be seen that the mobilities of components A and B (fractions 21 and 25) were not changed significantly by the additional treatment.

Fraction 40 appears to be the only component tested which can be further disrupted into smaller elements. After the above treatment, 60% of the counts in fraction 40 migrated near the cistron V region of the gel, where the chloramphenicol-resistant VI protein also migrates. After similar treatment, fraction 47 (peak C) migrated as a smaller but distinct peak between two components whose R_F values compare with

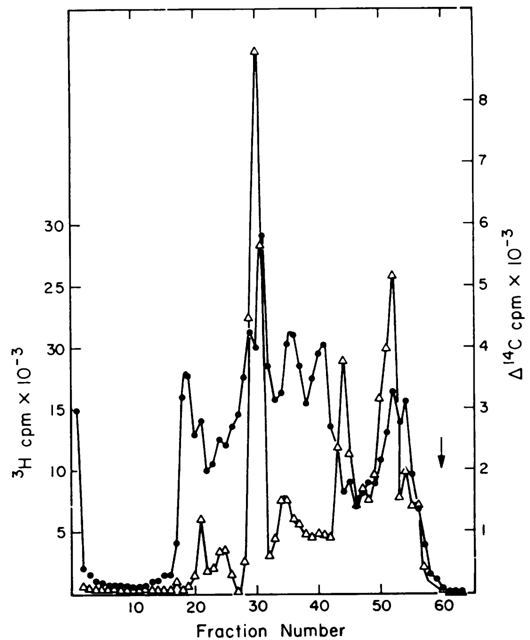

FIG. 4. *Electrophoresis of proteins of concentrated extracts of ϕX am3-infected cells (^{14}C) and uninfected (^{3}H) cells. The gel slices were eluted in 0.2 ml of 0.05 M Tris-hydrochloride, pH 8.0, 0.1% SDS; 25 μliter samples of each fraction were counted in NCS counting solution. Symbols: ^{3}H counts/minute; Δ, Δ^{14}C counts/minute. The ^{14}C/^{3}H ratios of fractions 18 and 27 were used as R_{min}.*

III and V proteins, which apparently overlap into fraction 47. The cistron assignment of this peak has not been made.

The mobilities of the components in fractions 30, 35, 44, 50, and 52 (Fig. 4) were not changed by further treatment and electrophoresis.

The V peak frequently displayed a shoulder of slower mobility which could not be resolved on longer gels. Such asymmetry might be an artifact of the method of slicing the gels, however.

Temporal course of ϕX-specific protein synthesis. Pulse-labeling of *wt*-infected cultures at various times after infection and determination of the phage-specific proteins (Fig. 6) showed that very little phage protein synthesis is detectable before 10 min after infection, and that all of the major proteins increase at approximately the same rate at later times. In Fig. 6b, some capsid protein can be seen at 5 to 10 min after infection, as well as a component which has a slightly greater mobility than is usually observed for the V protein. It is interesting that the chloramphenicol-resistant VI protein, which is required for production of progeny RF molecules

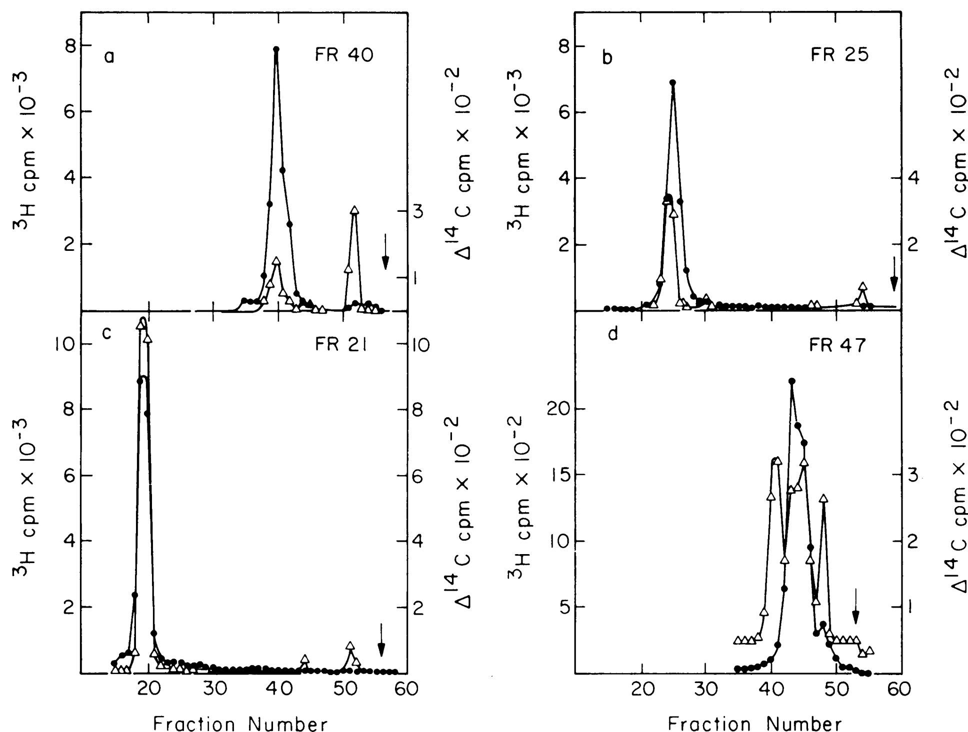

FIG. 5. *Electrophoresis of fractions from Fig. 4. Samples (50 μliters) of fractions 21, 25, 40, and 47 were disrupted further by heating to 100 C in urea, SDS, 2-mercaptoethanol, and were subjected to electrophoresis. Symbols:* ●, *^{3}H counts/minute;* △, *$\Delta^{14}C$ counts/minute. The value for R_{min} was the same as for Fig. 4.*

and must be synthesized early in infection, migrates in this region of the gel. Also of interest in Fig. 6c is the failure to observe the cistron I protein (lysis) until the 15- to 20-min interval. When shorter (2 min) pulses were used (Fig. 7), the cistron I peak was not detected until the 19- to 21-min interval.

DISCUSSION

Identification of peaks. Using the double-labeling procedures described in this paper, we were able to detect all of the peaks reported to be seen after infection of UV-irradiated cells, which allow labeling of only the ϕX proteins specifically. We have seen other peaks of higher molecular weight (peaks A and B), and, since the sum of the molecular weights of the other components comes close to the coding limit imposed by the small size of the viral DNA, these might be host proteins which are induced by viral infection, and might not have been detectable in UV-treated cells. An additional component C has also been observed, but its corresponding cistron has not been identified.

Heat-inactivation data indicate that cistron IV codes for a protein of the phage particle (18, 19). We believe that the cistron IV product must, by elimination, be the minor fast-moving spike component (see Fig. 2). However, the genetic map of cistron IV (R. Benbow, *personal communication*) suggests that the cistron IV product is large and the protein should be in the high molecular weight region of the gel. This would imply that either the cistron IV protein is not a coat protein component, since the cistrons of the higher molecular weight components of the particle have been identified as VII and II, or that the cistron IV peak is undergoing co-electrophoresis with one of the other peaks from the disrupted particle. Cistron VIII mutants show no evidence of being coat protein components (F. Funk, *personal communication*).

Several components are found in or near the region of the cistron V protein: the chloramphenicol-resistant cistron VI protein, the cistron V protein, and the minor spike component (IV?). Attempts to elute these fractions, remove the SDS, and rerun the proteins in a non-SDS gel system have been unsuccessful at resolving additional components other than the V protein itself. Unfortunately, the other proteins are present in very small amounts. Calculation of

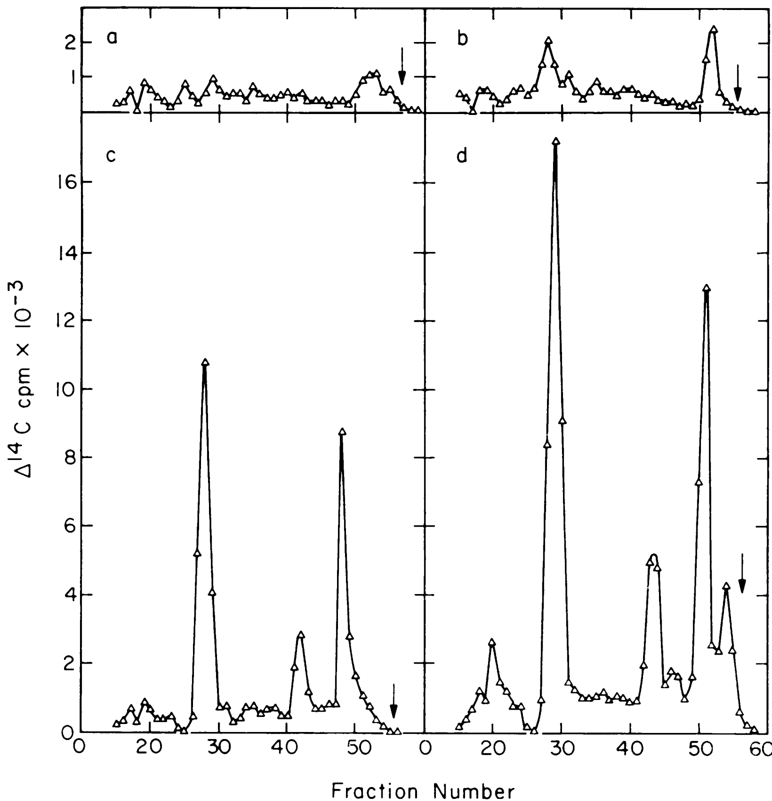

FIG. 6. *Rate of synthesis of ϕX-specific proteins at times after infection. A 30-ml culture of E. coli C was infected with ϕX wt (multiplicity of infection = 3), and 5-ml samples were removed and pulse-labeled for 5 min with ^{14}C-leucine at various times. A single 5-ml sample was removed prior to infection and pulsed for 5 min with ^{3}H-leucine. The pulses were stopped by addition of cold leucine; the cultures were chilled rapidly and centrifuged. Each was lysed, deoxyribonuclease-treated, and dialyzed separately. Equal (25 µliter) samples of each of the ^{14}C-labeled solutions were mixed with 25 µliters of the ^{3}H-labeled control solution prior to electrophoresis. Data are presented as Δ^{14}C: (a) 0 to 5 min after infection; (b) 5 to 10 min; (c) 10 to 15 min; (d) 15 to 20 min.*

the amounts of cistron VI protein made in the presence of chloramphenicol indicate that, if the rate of synthesis of cistron VI protein were unaffected by chloramphenicol, it would comprise less than 5% of the counts in the cistron V region of the gel.

Structure of the phage particle. Electrophoresis of ϕX174 labeled with ^{14}C-glucose has provided an estimate of the weight-percentage of each protein in the phage particle (see Fig. 2b). The average mole-ratio of proteins in the phage particle, based upon the molecular weights obtained by SDS-acrylamide gel electrophoresis, is approximately 62:10:50:(30) for proteins of cistrons VII, II, III, and IV(?) respectively. The molecular weight of the IV(?) protein is difficult to estimate because of its high mobility, and has been taken to be 15,000 for these calculations. These mole-ratios agree with those reported by Burgess (1), which were obtained from phage labeled with an amino acid mixture, and further suggest that all of the spikes contain one molecule of II protein and are probably identical. However, to account for the ratios, the assumption must be made that some of the spikes have been lost during the purification procedure (1). We have not rigorously excluded the possibility that a minor spike component (especially if it were restricted to a unique spike) may have remained undetected.

The molecular weight of the capsid protein was reported by Carusi and Sinsheimer (3) to be 25,000, and this led to a proposal by Edgell, Hutchison, and Sinsheimer (6) of a capsid structure composed of 20 hexamers of capsid protein. However, the molecular weight of the capsid protein, as measured on SDS-acrylamide gels, is 48,000. This capsid material cannot be further disrupted by vigorous heating in the presence of dithiothreitol and electrophoresis in

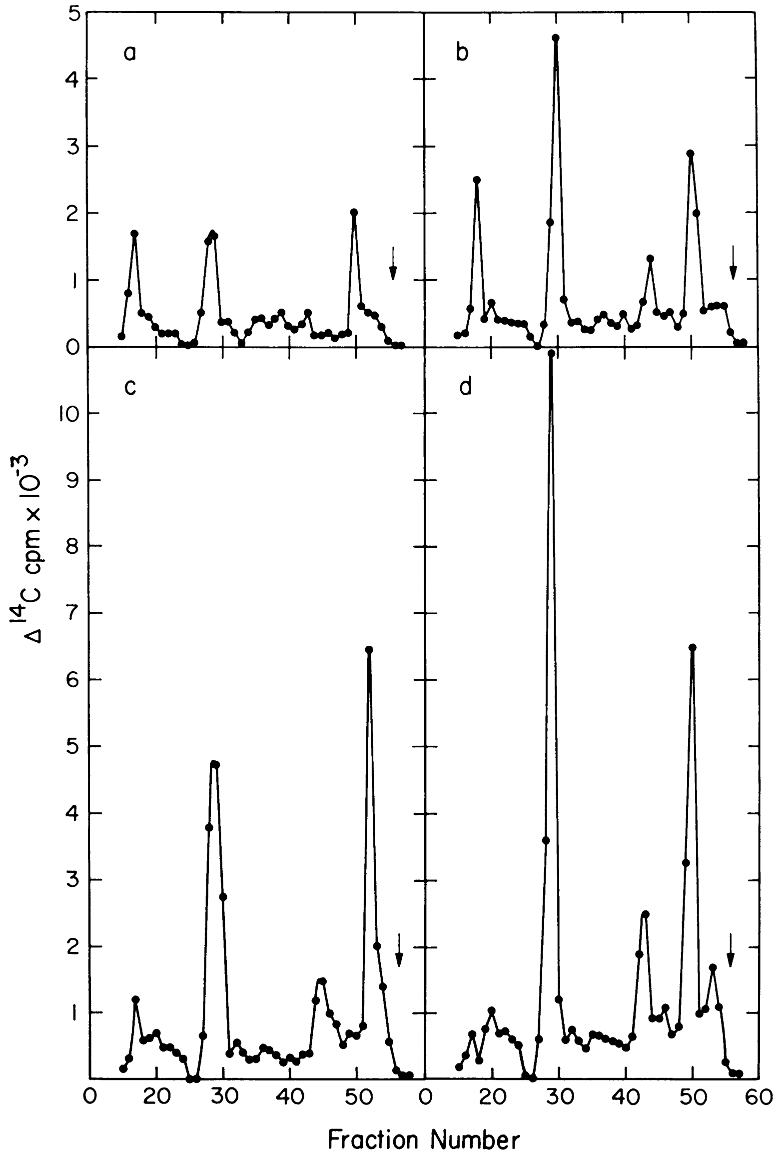

FIG. 7. *Rate of synthesis of φX-specific proteins at times after infection. Same as Fig. 6 except that 2-min pulses were used: (a) 10 to 12 min after infection; (b) 13 to 15 min; (c) 16 to 18 min; (d) 19 to 21 min.*

systems containing dithiothreitol in both buffers and gel.

It is possible to construct a capsid consisting of 20 trimers of coat protein (which would require 60 molecules of VII protein per capsid) with the spikes located on fivefold symmetry axes (1, 6). A trimer of coat protein would have a molecular weight of 144,000. We have observed particles in infected cell lysates which sediment at about 9*S* and contain only VII capsid protein as detectable phage components when subjected to SDS-acrylamide gel electrophoresis. Similar particles have recently been reported in UV-irradiated cells by Tonegawa and Hayashi (22). The composition of these particles, as well as the "6*S*" particles (8), will be described in a subsequent publication.

Temporal studies. Temporal studies indicate that little phage-specific protein synthesis can be detected by these methods prior to 10 min after infection. A small peak was observed between 5 and 10 min which seemed to have a greater

mobility than that usually observed for the cistron V protein occupying this region of the gel, as do both a spike component and the chloramphenicol resistant VI protein. Although Levine and Sinsheimer (12) have demonstrated that the chloramphenicol-resistant VI protein can be detected throughout infection, it is especially required in the early stages for production of progeny RF molecules and must be coded by the parental RF molecule. The identification of this early peak will require more sensitive methods of detection and identification.

At later times in infection, the peak associated with the cistron I product (lysis) does not appear as soon after infection as the other peaks; this observation reinforces the possibility that this might be a "late" protein (15). The rate of synthesis of all of the other proteins seems to increase proportionately throughout infection, although they are being made in different molar amounts (V > VII > III > II). It is interesting that these cistrons are contiguous in this order on the ϕX genetic map (R. Benbow, *personal communication*).

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