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In Vitro Radioisotopic Labeling of Proteins Associated with Purified Polyoma Virions

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An in vitro procedure using [^{14}C]formaldehyde and sodium borohydride was used to label the proteins of purified polyoma virions. This method, which labeled the three capsid proteins and the four internal histones of polyoma virus, did not alter the biological and biophysical characteristics of the virus. In addition to the seven viral proteins, five additional radioactive peaks were resolved on polyacrylamide gels. Four of these peaks could be attributed to growing the virus in the presence of serum. The fifth peak appears to be a nonhistone host-contributed protein made before infection.

Several investigators have reported the use of an in vitro radioiodination method to label the components of mammalian cell membranes (5, 8, 10, 16), as well as the proteins of purified virions (4, 12, 14, 17, 19). In addition, a pyridoxal phosphate-sodium borotritide reduction method has been demonstrated to label selected virion proteins in vitro (11). These techniques have all been shown to be useful for labeling only the exposed surface proteins. In the present communication, an in vitro procedure was found to label all proteins (external and internal) of purified polyoma virions with no loss of viral infectivity and no alteration in biophysical characteristics of the virus.

Virus to be used for the in vitro labeling procedure was grown in mouse embryo cells (6, 13) which were maintained in Eagle minimal essential medium plus 5% of dialyzed fetal calf serum and [^{3}H]thymidine (10 μCi/ml of media; Schwarz/Mann). The in vivo-labeled virus was grown in mouse embryo cultures which were maintained in serum-free Dulbecco's modified Eagle minimal essential medium with a 10% concentration of amino acids and supplemented with 10 μCi of [^{3}H]protein hydrolysate (Schwarz/Mann) per ml of media to label the virion proteins. The virus purification procedure (R. Consigli, J. Zabielski, and R. Weil, submitted for publication) included a CsCl equilibrium centrifugation followed by CsCl velocity sedimentation of the complete virus band.

The virus was labeled in vitro essentially according to a procedure described by Oliphant and Brackett (9) for labeling immunoglobulins. The purified pelleted virus was resuspended in water and diluted in sodium borate (pH 9.0) (0.1 M final concentration), or in Tris buffer (pH 7.4) (0.01 M final concentration) for the untreated control. Protein concentration was determined from a ratio of 260/280-nm readings in a spectrophotometer (2). The virus sample in sodium borate was then subjected to reductive alkylation by the addition of sodium borohydride (160 μg/mg of viral protein) and 250 μCi of [^{14}C]formaldehyde (New England Nuclear Corp.; specific activity, 40 mCi/mmol). The reaction mixture was incubated at 37 C for 2 h with occasional mixing, followed by exhaustive dialysis against 0.01 M Tris buffer, pH 7.4, at 4 C.

TABLE I. *Biological and biophysical activity of in vitro labeled virus*[a]

Composition of reaction mixture	PFU/ml[b]	Buoyant density (g/cm^3)[c]
Complete mix	8.5×10^9	1.335
Minus [^{14}C]formaldehyde	3.8×10^9	1.335
Minus [^{14}C]formaldehyde, Sodium borohydride	14.0×10^9	1.333
Tris buffer	6.5×10^9	1.333

[a] The complete reaction mixture contained virus in 0.1 M sodium borate, pH 9.0, sodium borohydride, and [^{14}C]formaldehyde; Tris buffer indicates virus suspended in 0.01 M Tris, pH 7.4, as the untreated control.

[b] Infectivity of the virus preparations was determined by the plaque assay (PFU; 1).

[c] Buoyant density determinations were made by centrifuging the virus samples in an equilibrium CsCl gradient. Fractions were collected from the bottom of the tube and the radioactivity and refractive index were determined.

The purpose of this work was to determine if the [^{14}C]formaldehyde-sodium borohydride procedure would label all the proteins of polyoma virus while maintaining the biological and biophysical integrity of the virus. The basis for the reaction is the formation of a Schiffs base intermediate between the formaldehyde and the lysine residues in the proteins, followed by reduction yielding alkyl-lysine (9). The data presented in Table 1 demonstrate that there was no loss of viral infectivity after the 2-h in vitro labeling reaction. Further, the hemagglutinating ability of the virus was not reduced (data not shown). The average specific activity from four separate labeling reactions was 5,100 counts/min per μg of protein. In a separate experiment, in which the reaction was allowed to proceed for 24 h, total viral infectivity was retained and there was increased incorporation of the label. Buoyant density determinations made on the virus samples revealed there was no change, suggesting that this in vitro labeling procedure does not weaken the structure of the virion. To determine whether this method had an adverse effect on the virion supercoiled DNA, the DNA was analyzed in alkaline sucrose (Fig. 1). It was found that the ratio of

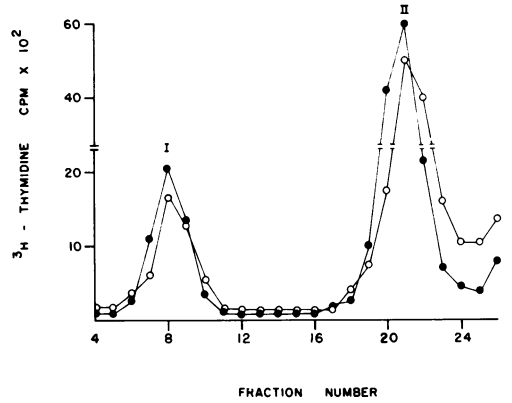

FIG. 1. *Analysis of [^{3}H]DNA extracted from in vitro-labeled virions on alkaline sucrose gradients. In vitro-labeled (●) and untreated (○) virions were disrupted by incubating the samples for 2 h in carbonate-bicarbonate buffer (0.1 M; pH 10.5) containing 5 × 10^{-3} M dithiothreitol. These samples were then layered over a 5 to 20% linear alkaline sucrose gradient (0.25 N NaOH, 1 M NaCl, pH 11.5) and centrifuged at 35,000 rpm for 3 h in an SW 50.1 rotor. Fractions were collected from the bottom of the tube and assayed for radioactivity. The alkaline sucrose profiles from the in vitro-labeled and untreated DNA samples are superimposed in this figure.*

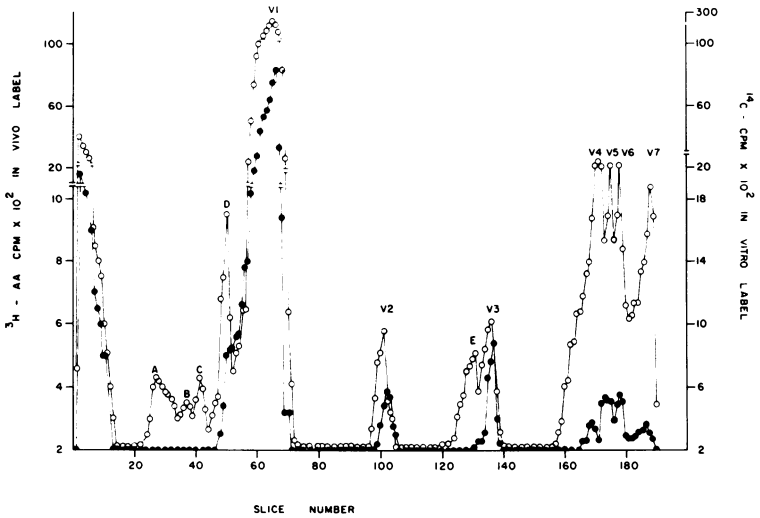

FIG. 2. *Co-electrophoresis of in vivo-labeled (●) and in vitro-labeled (○) polyoma proteins on sodium dodecyl sulfate-15% polyacrylamide gels. The in vitro ^{14}C-labeled virions were mixed with in vivo [^{3}H]amino acid-labeled virions (approximately 100 μg of viral protein for each sample), precipitated with 10% trichloroacetic acid, washed in ethanol-ether (1:1), ethanol and air-dried. The preparation was resuspended in 10^{-3} M phosphate buffer, pH 7.2, and solubilized for electrophoresis by boiling for 5 min in 2% sodium dodecyl sulfate, 5% 2-mercaptoethanol and 10% glycerol. Electrophoresis was carried out at 5 mA/gel for 17 h on 15-cm columns of 15% polyacrylamide gels with 0.1 M phosphate buffer, pH 7.2, containing 0.1% sodium dodecyl sulfate as the running buffer (7). Following fixation-staining and destaining, the gels were frozen and fractionated into 1-mm slices, solubilized in NCS (Amersham/Searle) and the radioactivity was determined for each gel slice. All results were corrected for the overlap of ^{14}C into the [^{3}H] channel. Molecular weights were determined by the method of Weber and Osborn (18).*

supercoiled component I (53S) DNA to component II (17S) DNA was the same in the complete reaction mix as in the untreated viral preparation. Further, it was noted that there were no ^{14}C counts associated with the viral DNA, indicating that under these conditions, the nucleic acid is not labeled.

A comparison of the electrophoretic profile of the in vitro-labeled and in vivo-labeled polyoma proteins is shown in Fig. 2. The in vivo-labeled virus preparation demonstrates the three viral capsid proteins, V1, V2, and V3, with molecular weights of 43,000, 34,000, and 22,800, respectively. In addition there are four host-contributed histones, V4-V7, with molecular weights of 17,200, 15,800, 14,600, and 13,200. The profile of the in vitro-labeled virus demonstrates that all seven of the virion proteins were labeled by this method. The extent of labeling of the histones is much greater than is found for the three viral capsid proteins. This is to be expected, since histones are rich in lysine. In addition to the seven virion proteins, five additional protein peaks were resolved on the gel, labeled A to E. Peaks A to D, having molecular weights greater than 43,000, apparently result from growing the virus in media containing serum. This conclusion is based on the fact that in separate experiments in which the infected cells were maintained in serum-free media, these peaks were absent from the gel. The protein peak E, which has a molecular weight of 24,000, is seen in in vivo-labeled virus preparations only when the radioactive label is present both pre- and post-infection (unpublished data). It appears that peak E represents a virion-associated protein which is made before infection, consistent with a previously reported observation (3).

There are several advantages in using this in vitro-labeling technique. The procedure allows the labeling of all virion proteins. Further, it serves to identify those virion-associated proteins made before infection and thus not ordinarily labeled in conventional in vivo postlabeling procedures. The method is readily carried out and the product is stable for long periods of time. This is in contrast to a previously reported procedure for the in vitro labeling of another of the papova viruses (15). In addition, the conditions used are sufficiently mild so that viral infectivity is maintained and electrophoretic mobility of the proteins is not altered. The use of this method should be particularly useful in labeling lysine-rich proteins, such as histones.

ADDENDUM

Since this paper was submitted, a pre-print of a paper to appear in this journal was received from L. F. Velicer and D. G. Graves describing a similar reductive alkylation technique used to label the polypeptides of disrupted feline leukemia virus.

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