Radioactive Labelling of Viruses: an Iodination Technique Preserving Biological Properties

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Summary

An iodination procedure suitable for the radioactive labelling of viruses to be used in biological experiments is described. It is characterized by the addition of carrier protein to small amounts of virus before iodination with chloramine T, the use of low concentrations of chemicals, and a rapid purification of the labelled virus to minimize radiation inactivation. Using this procedure, polyoma virus was labelled to a specific activity 100 times greater than that which can be obtained with tritiated amino acids, while its sedimentation coefficient, buoyant density, decapsidation and haemagglutinating activity remained unaffected. Reduction in infectivity, possibly due to radiation inactivation, was slight. Similar results were obtained with adenovirus.

Iodination has become a popular method to obtain radioactive polypeptides of high specific activity. Various techniques exist which do not affect protein stability in vivo (McConahey & Dixon, 1966), antigenicity (Thorell & Johansson, 1971) or hormone activity (Stagg et al., 1970; Kurcbart et al., 1972; Lambert, Felix & Jacquemin, 1972). To our knowledge, the effect of iodination on the biological properties of a virus, however, has never been appraised and this is what we have attempted to investigate. Since it had been reported that the lactoperoxidase method labels only external polypeptides (Phillips & Morrison, 1970), whereas large amounts of protein are required by the iodine monochloride (Hunter, 1970) and electrolytic (Rosa et al., 1964) procedures, we initially chose to iodinate virus using chloramine T. The latter technique, which was designed by Hunter & Greenwood (1962) to obtain maximum specific activity, seems to result in some protein denaturation (McConahey & Dixon, 1966; Hunter, 1970). McConahey & Dixon (1966) modified this technique, increasing the protein concentration to reduce the deleterious effects of the chemical reaction. As it is difficult to obtain milligram quantities of any virus, we attempted to minimize protein denaturation by reducing the amounts of some of the reagents used. This approach proved very useful because it permitted labelling, to high specific activities, microgram quantities of virus protein, without detectable damage to the physical or biological properties of the virus itself.

Unlabelled or 32P-labelled polyoma virus was grown and purified as previously described (Frost & Bourgaux, 1975a, b). Its biological properties were assessed by measurements of infectivity and haemagglutinating activity (Roblin, Harle & Dulbecco, 1971). Adenovirus type 5 was grown and titrated as described by Williams (1970).

Standard conditions of iodination. Purified virus (0.1 to 5 μg), suspended in less than 0.1 ml phosphate-buffered saline containing 100 μg bovine serum albumin fraction V (BSA), was added to a mixture containing iodine (200 to 500 μCi carrier-free 125I as NaI or an equal amount of either 131I or non-radioactive NaI in 10 μl 0.1 N-NaOH) and 10 μl portions of 1 M-Na phosphate buffer, pH 6.9, and 0.1 N-HCl. Iodination was initiated by addition of 25 μl of a fresh 0.16 mg/ml solution of chloramine T. After 30 to 45 s at room temperature,
the reaction was stopped with 25 μl potassium metabisulphite at 0.5 mg/ml. Labelled virus was separated from unreacted iodine and iodine-labelled BSA by velocity centrifugation through a 5 to 20% (for polyoma virus) or a 15 to 45% (for adenovirus) sucrose gradient (0.1 M-NaCl, 0.001 M-EDTA, 0.01 M-tris-HCl, pH 7.4) for 42 min at 35000 rev/min in the SB405 rotor of an International ultracentrifuge. Enzymic iodination was performed as described above, except that 5 μl of a 500 μg/ml solution of lactoperoxidase (Calbiochem) was added instead of chloramine T. Iodination was carried out with three additions of 5 μmol H2O2.

In preliminary experiments, when 100 μg chloramine T (Hunter & Greenwood, 1962) were used for iodination, both the sedimentation coefficient and buoyant behaviour of polyoma virus were greatly altered whereas 4 μg produced little or no effect (Frost, 1975). Interestingly, the lower amount of chloramine T also yielded virus of comparable specific activity. Likewise, the percent of iodine bound to BSA was reduced by only 15% when the amount of chloramine T was reduced fivefold (McConahey & Dixon, 1966; Frost, 1975).

To prevent virus adsorption on the walls of containers (Thorne, House & Kisch, 1965) and to minimize the oxidizing effect of chloramine T on virus, 0.1% BSA was added to purified virus stocks. It was found that a maximum of 100 μg BSA could be added to the iodination mixture and still permit satisfactory labelling. In three experiments performed in the absence of BSA, iodination of polyoma virus resulted in 2-, 30- and 150-fold reductions in infectivity (such a wide range of reductions probably results from using different amounts of virus (see Table I). Large reductions, such as the latter two, are unacceptable for biological studies. On the other hand, when iodination was performed in the presence of BSA, infectivity was reduced by about 30% (average of four experiments). This protection was more fully assessed using adenovirus type 5 since large quantities of this virus can be readily prepared and assayed. The addition of 100 μg BSA to the reaction mixture prevented 2- to 20-fold reductions in infectivity that were observed when small amounts of adenovirus were iodinated (see Table I). When iodinating more than 100 μg of virus no inactivation was noted, even without BSA (Table I).

Finally, we verified that BSA could be effectively separated from polyoma virus by sedimentation in a sucrose gradient; we failed to detect 125I-labelled material with the electrophoretic mobility of BSA in 17 different virus preparations purified by velocity centrifugation after iodination (Frost, 1975; Frost & Bourgaux, 1975a,b). Furthermore, when 106 ct/min of 125I-labelled BSA, cold iodine and polyoma virus were mixed before iodination, less than 0.1% of the label was found to sediment as virus (Frost, 1975). Using this assay procedure cytochrome c, lysozyme, myoglobin and ovalbumin were found not to bind to polyoma virus whereas histone (type IIA; Sigma) did (Frost & Bourgaux, unpublished observations).

In two experiments, iodination of polyoma virus was allowed to proceed for 10 min rather than 30 to 45 s. Infectivity was reduced 20 times in one experiment, and was completely abolished in the other.

When compared with the standard technique, neither enzymic iodination nor chemical iodination in the presence of dimethyl sulphoxide (Stagg et al. 1970) were found to be more gentle procedures although both yielded virus with a specific activity lower by 40%.

Characteristics of polyoma virus and adenovirus iodinated under standard conditions. Polyoma virus preparations, iodinated with either 125I or 131I under standard conditions, displayed specific activities ranging from 1 to 5 × 106 ct/min/μg of protein. This can be compared with specific activities not exceeding 104 ct/min/μg of protein which are obtained
Table 1. Effect of iodination on virus infectivity*

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<thead>
<tr>
<th>Expt.</th>
<th>Virus (μg)</th>
<th>Infectivity after iodination</th>
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<tr>
<td>1</td>
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<td>13</td>
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* Varying amounts of adenovirus type 5 were iodinated under standard conditions with or without 100 μg BSA. Infectivity titres were determined for each sample (average counts from four or five Petri dishes containing 25 or more plaques). The ratio of the infectivity of the iodinated sample versus a non-iodinated control is shown.

after labelling polyoma virus in vivo with tritiated amino acids (Roblin et al. 1971; Frost, 1975) or in vitro with 14C-formaldehyde (McMillen & Consigli, 1974). The specific activity of iodinated virus was thus 100 times higher than that of virus labelled otherwise.

As already mentioned, the sedimentation pattern and buoyant density of polyoma virus were unaffected by iodination with 4 μg chloramine T, even when carrier BSA was omitted. In those experiments, however, the marker, but not the iodinated virus, was labelled with 32P. In order to assess precisely the effects of iodination, 32P-labelled polyoma virus and 3H-labelled adenovirus were iodinated. The sedimentation pattern and buoyant density of the iodinated virus were identical to those of the corresponding non-iodinated virus (Fig. 1). We can thus conclude that, in this respect at least, iodination affects neither polyoma virus nor adenovirus.

In five experiments we observed that standard conditions of iodination did not affect the haemagglutinating activity of polyoma virus.

As expected, infectivity was the virus property which appeared to be most sensitive to the effects of iodination. When polyoma or adenovirus were labelled under standard conditions reductions in titre, if any, were slight. Slight effects could be due to radiation inactivation, a phenomenon which has been well documented for 3H- and 32P-labelled virus (Henry, 1967), rather than to chemical inactivation. After storage for 10 weeks, a 10-fold reduction in the infectious titre of 25I-labelled preparations was observed, while the titre of the unlabelled control was unchanged.

Finally, we observed that the intracellular fate of 32P-labelled polyoma virus was not affected by the iodination of the virus (Frost, 1975).

In this report, we have described a simple iodination technique which can be used to effectively label small amounts of virus, and requires neither enzymes nor large amounts of protein. Using carrier protein, as little as 0.01 μg virus protein can be labelled to specific activities greater than 106 c/min/μg. Using the same conditions larger amounts of virus can be iodinated safely without carrier protein. The usefulness of this technique for studies of
Fig. 1. Effect of iodination on the physical properties of polyoma virus and adenovirus. Preparations of $^{32}$P-labelled polyoma virus and adenovirus labelled with $^3$H-thymidine, were iodinated under standard conditions. As a marker, an excess of $^{32}$P-labelled polyoma virus (top panels) or $^3$H-labelled adenovirus (bottom panels), unreacted with iodine, was added to the $^{125}$I-labelled virus before determination of its buoyant density in CsCl solution (left panels) or sedimentation coefficient in sucrose solution (right panels). Centrifugation in the CsCl solution (0.03 % BSA, 0.02 M-EDTA, 0.02 M-tris-HCl, pH 7.4) of density 1.35 g/ml, was for 16 h at 30000 rev/min in the SB 405 rotor of an International B60 ultracentrifuge. Sedimentation in the 5 to 20% (top right panel) or a 15 to 45% (bottom right panel) sucrose solution (0.1 M-NaCl, 0.001 M-EDTA, 0.01 M-tris-HCl, pH 7.4) was for 42 min at 35000 rev/min in the SB 405 rotor. The radioactivity found outside the virus band (top panel) is due to the fact that after CsCl banding, the $^{32}$P-labelled polyoma virus had not been purified by velocity centrifugation, as routinely performed for iodinated virus. (-----), $^3$H or $^{32}$P radioactivity; (---), $^{125}$I radioactivity; (■■■■■), density.

virus decapsidation has already been demonstrated (Frost & Bourgaux, 1975b). Its application to the study of viruses that grow poorly or not at all in tissue culture will no doubt prove very interesting.

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REFERENCES


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