On the Use of Chloramine-T to Iodinate Specifically the Surface Proteins of Intact Enveloped Viruses

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SUMMARY

Rous-associated virus-61 was used as a model for studying the labelling specificity of the chloramine-T iodination procedure when applied to intact enveloped viruses. The specificity of labelling depended markedly on the concentration of iodide in the reaction mixture. At low concentrations of iodide (below 0.5 μM) only the surface proteins and lipid of intact virions were iodinated; there was no detectable labelling of internal proteins. At 10 μM-iodide, however, both internal and external proteins were iodinated; moreover there was a marked change in the reactivity of the surface proteins. It appears that the lipid envelope provides an effective barrier to the iodinating complex generated at low, but not at high, concentrations of iodide. These and other observations suggest that the chloramine-T procedure has a previously unrecognized potential for specifically labelling the surface proteins of lipid-enveloped structures.

Radioiodination mediated by chloramine-T (Greenwood & Hunter, 1963) has become a widely used procedure for probing the organization of proteins in enveloped and non-enveloped viruses. It has been reported that both the external and internal proteins of intact enveloped viruses are readily labelled by the chloramine-T (Stanley & Haslam, 1971; Poláková & Russ, 1974). Hence the general assumption has been that the iodinating complex must diffuse freely throughout the intact enveloped virions. An exception to this generalization, however, is the recent report of Moore, Kelley & Wagner (1974) that only the surface proteins of another enveloped virus, vesicular stomatitis virus, were labelled by the chloramine-T procedure. The authors made no attempt to explain this surprising result, which they may have attributed to differences in the intactness or structure of the different viruses studied in the various laboratories.

We report here a similar case of specific labelling of surface proteins using yet another enveloped virus, Rous-associated virus-61 (RAV-61), an oncornavirus. We show further that the specificity of labelling obtained with the chloramine-T procedure, either surface or general labelling, depends on the concentration of iodide used in the radioiodination.

RAV-61 was grown in secondary cultures of chicken embryo fibroblasts and purified as described previously (Davis & Rueckert, 1972). The concentration of virus protein was determined by the method of Lowry et al. (1951) using lysozyme as a standard. Iodination catalysed by lactoperoxidase (Phillips & Morrison, 1971) was carried out by adding 30 μg of intact RAV-61 in 0.3 ml of 0.1 M-sodium phosphate buffer, pH 7.2, to a 0.2 ml bed vol. of Sepharose-bound lactoperoxidase (Worthington, 420 units/g dry weight), followed by 0.5 mCi of carrier-free 125I and 5 μl of a 1 M solution of sodium iodide. The iodination was initiated by adding 5 μl of freshly prepared 0.01 M-hydrogen peroxide, followed by a second 5 μl after 15 min. The reaction was carried out at 25 °C with constant agitation of the vial to ensure proper mixing of the reactants. After a total of 30 min, the iodination was terminated by adding 5 μl of 10% 2-mercaptoethanol to reduce residual peroxide. The Sepharose beads
Fig. 1. Electrophoretic profile of radioactively labelled RAV-61 proteins on SDS-containing polyacrylamide gels. (a) Virus purified from infected chick cells after labelling with [3H]-leucine. The protein nomenclature is based on the apparent mol. wt. (in thousands) of the virus proteins as recommended by August et al. (1974). (b) Purified RAV-61 virions iodinated with lactoperoxidase and 125I. Electrophoresis was on 25 cm gels for 16 h at 8 mA/gel. Protein migration was from left to right; bromphenol blue (H) was used as a visual tracking dye during electrophoresis.

were then removed by filtration, and the labelled virus purified from unreacted 125I by centrifuging on sucrose density gradients. Iodination mediated by chloramine-T was performed by adding 0.5 mCi of carrier-free 125I to 30 µg of intact or SDS-disrupted virus in 0.5 ml of the phosphate buffer. When necessary, carrier sodium iodide also was added to a final concentration of 10 µM. The iodination was initiated by adding 5 µl portions of freshly prepared chloramine-T (1 mg/ml) at 10 min intervals. The reaction was allowed to proceed for 30 min at 5 °C, when 5 µl of 10%, 2-mercaptoethanol was added to exhaust any unreacted chloramine-T. Radioactively labelled intact virus was purified by centrifuging on sucrose density gradients: SDS-disrupted virus, by gel filtration on a column of Sephadex G25 (Montelaro & Rueckert, 1975). Samples of radioactive virus were prepared for electrophoresis by adjusting the concentration of SDS and 2-mercaptoethanol to 1% each and heating in a boiling water bath for 2 min. The samples then were electrophoresed on SDS-containing polyacrylamide gels, and the gels were analysed for radioactivity as described previously (Medappa, McLean & Rueckert, 1971; Montelaro & Rueckert, 1975).

Fig. 1(a) shows the electrophoretic profile of purified [3H]-leucine-labelled RAV-61 virus. The virion contains two glycoproteins, gp73 and gp32, and at least seven non-glycosylated proteins. As shown in Fig. 1(b), only the viral glycoproteins and lipid were susceptible to iodination when intact virions were treated with the immobilized lactoperoxidase. These results clearly demonstrate the location of the two glycoproteins on the outer surface of the virion and the location of the remaining proteins in the virion core enclosed by the lipid envelope. In addition, the lack of detectable label in any of the internal viral proteins is strong evidence for the intactness of the virus used in these iodination studies.

Evidence that the chloramine-T-mediated iodination is specific for the surface proteins of
intact RAV-61 virions at ‘low’, but not at ‘high’, iodide concentrations is summarized in Fig. 2. When intact virions were labelled with carrier-free $^{125}$I ($1\times10^{-2}$ mCi/ml; $0.5\mu$M), only the viral lipid and the two surface glycoproteins were labelled (Fig. 2a). That this labelling pattern is not simply due to a lack of reactive residues in the internal viral proteins is shown by the profile of virions iodinated after disruption with SDS (Fig. 2c). Thus the lack of internal protein labelling in the intact virions was apparently due to the inability of the iodinating intermediate to penetrate the virus lipid envelope.

Addition to carrier iodide (to $10\mu$M) changed the radioactivity profile of the proteins in intact virus in two important ways (Fig. 2b): (a) most, if not all, of the internal proteins of intact virions were labelled, implying that at this concentration of iodide, the lipid envelope was no longer a completely effective barrier to iodination, and (b) there was a marked enhancement in the amount of label in protein gp73 relative to that in gp32 and lipid. For example, substitution with iodine atoms was enhanced 20- to 30-fold in gp32 and lipid, but over 600-fold in gp73. A similar effect of iodide concentration on relative reactivities of proteins was also observed with the disrupted virions (Fig. 2d). In this case, however, substitution of lipid was enhanced only 11-fold, while that of gp32 was enhanced 130-fold; that of gp73, 250-fold. Therefore iodide concentration affected not only the apparent permeability of the membrane barrier to iodination, but also the relative reactivity of residues within the individual protein molecules. Intact virions iodinated at low and high iodide concentrations banded quantitatively at the expected density of 1.16 g/ml in sucrose density gradients. Thus there was no evidence of alterations in the virion integrity at the degree of iodination obtained here. The same results were observed with other preparations of RAV-61 and when $^{131}$I was substituted for $^{125}$I.

This finding that iodination mediated by chloramine-T is specific for the external proteins of RAV-61 at low, but not at high, iodide concentrations is probably not a peculiarity of this particular virus or of oncornaviruses in general. As noted earlier, a similar selective labelling of surface proteins was reported for vesicular stomatitis virus, a rhabdovirus (Moore et al. 1974). This result suggested that these investigators may have carried out the
iodination at a low concentration of iodide. Indeed re-examination of their reaction conditions and a simple calculation reveal that carrier-free $^{125}$I was used at a concentration of 0.5 μM, the same as that in which the exterior proteins of RAV-6I were selectively labelled. On the other hand, the labelling of both the internal and external proteins of intact influenza virions (Stanley & Haslam, 1971) can be rationalized in terms of the high concentrations of carrier iodide (greater than 50 μM) used by these workers. This effect of iodide concentration on the labelling specificity of iodination by chloramine-T does not appear to have been recognized previously, although it is of obvious importance in interpreting labelling profiles obtained with this method.

The mechanism for this effect is unclear. It seems unlikely that an iodide concentration of only 10 μM would disrupt the integrity of the membrane and thus alter its permeability. Similarly, simple kinetic explanations cannot account for the changes in the relative reactivity of the viral proteins. In seeking an alternative explanation we were unable to find much specific information on the nature of the active iodination species. Hunter (1966) has suggested that iodination proceeds via a complex of the iodine with the sulphamid; others (Hallaba & Drouet, 1971) have proposed free iodine as the reactive intermediate. Actually, our results are best explained by postulating the existence of two different iodinating complexes, the relative amounts of which depend on iodide concentration. Following this idea, the proposed iodinating species (I) predominating at low iodide concentrations would be membrane impermeable, perhaps reflecting an ionic and hydrophilic nature, while the second iodinating species (II) would be membrane permeable, implying a more hydrophobic character than species I. Such a dual labelling pathway accounts for the labelling of internal viral proteins as the iodide concentration is increased. The changes in the relative reactivity of the RAV-6I glycoproteins may also be explained in terms of the differences in target specificity of the two iodinating intermediates.

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