Association of Moloney Murine Leukaemia Virus Proteins: An Assay for Hydrophobic Protein–Protein Interactions

By KLAUS B. ANDERSEN
The Fibiger Laboratory,† Nordre Frihavnsvej 70, DK2100 Copenhagen Ø, Denmark

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SUMMARY

Protein–protein interaction of Moloney murine leukaemia virus was studied by an assay where one protein preparation was coupled covalently to Sepharose, and binding of radiolabelled proteins to the protein–Sepharose was examined. It was found that the virus proteins gp70, p30, p15E and p15 in solution could associate weakly to disrupted virus particles and to p30. However, when the disrupted virus particles and p30 were coupled to Sepharose in the presence of Triton X-100, stronger binding of the four proteins was observed. Only low or no binding of p12 and p10 was observed to these protein–Sepharoses. The results are discussed with respect to the assembly and structure of the virus particle.

INTRODUCTION

The structure of C-type RNA virus particles has been revealed by electron microscopy and by cross-linking of neighbouring elements (Nermut et al., 1972; Bolognesi et al., 1978; Pinter & Fleissner, 1979). The particles contain a core which consists of virus RNA, p10, probably some p12 and the reverse transcriptase (Bolognesi et al., 1978; Leis et al., 1978; Sen et al., 1976). This core is surrounded by a core exterior consisting of p30 and p15 (Bolognesi et al., 1978). Outside the core is an inner coat of p12 (Bolognesi et al., 1978). The inner coat is again surrounded by plasma membrane into which p15E and gp70 are inserted.

The forces keeping the virus particle together are not well-characterized. It is known that p15E and gp70 are, to various degrees, bound together with disulphide bridges (Montelaro et al., 1978; Pinter & Fleissner, 1977, 1979). Furthermore, it is known that virus particles can be partially dissolved by detergents, such as Triton X-100 (Strand & August, 1976; Davis & Rueckert, 1972), indicating that hydrophobic interactions are important forces keeping the virus particle together.

The virus proteins are formed as precursors in the cell, some of which can be found in the cellular membranes (Witte & Baltimore, 1978). The various steps in the assembly of C-type viruses are unknown, but kinetic analysis of incorporation of virus products into the particles may indicate that association of virus RNA, p12 and p30 takes place shortly before their appearance in free virus (Bader, 1970; Witte & Weissman, 1974; Forchhammer et al., 1976). Thus, the insertion of RNA, p30 and p12 seems to trigger the budding of the virus through the plasma membrane. Since a ribonucleoprotein complex containing the virus RNA, reverse transcriptase and the gag precursor has been found in the cytoplasm of infected cells, it is suggested that this structure represents a step in the assembly of virus particles (Wong et al., 1980).

It is not known whether it is possible to assemble the virus particle in vitro. However, some binding reactions between the components of the virus particle are known: virus RNA binds

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to p10 and p12 (Sen et al., 1976; Leis et al., 1978; Schülein et al., 1978), and p30 forms multimers in solution (Burnette et al., 1976). However, it is not known whether these multimers are components of the virus particle.

The hydrophobicity of the virus proteins has also been studied. The proteins p15, p15E, p30, gp70, and the reverse transcriptase are believed to possess hydrophobic portions because they bind to alkyl-matrices (Marcus et al., 1978, 1979; Marcus & Smith, 1978; Swanson et al., 1978).

Other methods have been used to study the hydrophobicity of proteins in general: binding of lipid and detergent molecules to the proteins reveal the binding sites on the proteins for such molecules (Tanford, 1973); binding of detergent micelles to the proteins reveals hydrophobic domains on the proteins, by which they possibly can be placed in a lipid bilayer (Helenius & Simons, 1977). These methods all show the binding between proteins and small hydrophobic molecules, but hydrophobic forces are also involved in the interaction between proteins (e.g. aggregation of subunits, and forces within the protein structure, Schachman, 1963).

This study demonstrates that gp70, p30, p15 and p15E can associate without the presence of lipids and RNA by means of hydrophobic forces. The method used here involves covalent coupling of proteins to Sepharose in such a way that the hydrophobic parts of the protein are exposed. Binding of radiolabelled virus proteins to such protein–Sepharoses was then studied.

**METHODS**

**Virus preparations.** Moloney murine leukaemia virus (Mo-MuLV) preparations from cultured cells (NIH/3T3) were obtained as a gift from Research Resources, National Cancer Institute, NIH, Bethesda, Md., U.S.A.

**Iodination procedure.** Mo-MuLV suspensions were solubilized by addition of 1% Triton X-100 and sonicated. This solution was iodinated with 125Iodine (Amersham) using chloramine-T (Hunter, 1967), and the proteins were separated from free iodine by gel filtration (Sephadex G-25; Pharmacia) in the presence of 0.1% Triton X-100. Preparations with a specific activity of approx. 2 Ci/mg protein (0.3 mg/ml) were obtained. The preparations contained iodinated lipids (approx. 30% of the radioactivity) which could be completely removed by extraction with ether–ethanol (3:1). The presence of lipids did not influence the binding of the proteins in the binding assay.

**Purification of p30.** p30 was purified according to Strand & August (1976). The preparations were examined by SDS gel electrophoresis, and contained less than 1% impurities of other proteins.

**Detergents and proteins.** SDS was obtained from BDH; Triton X-100 (Triton) from Rohm and Haas; deoxycholate and myoglobin from Sigma. All chemicals used were of analytical grade.

**Coupling reaction.** Protein–Sepharoses were made by coupling the protein to CNBr-activated Sepharose (Pharmacia) in 0.5 M-NaCl and 0.1 M-NaHCO3 for 2 h at 37 °C with gentle mixing. In some experiments 1% Triton X-100 was added prior to the coupling reaction. After the coupling reaction, 1 M-ethanolamine pH 8 was added to the material for 18 h at 4 °C to block residual reactive groups on the Sepharose. The material was washed with 'assay buffer' (90 mm-KCl, 2 mm-MgCl2, 20 mm-tris pH 7.5) and stored at 4 °C.

**MuLV-coupled Sepharoses.** The MuLV preparation in the coupling buffer (15 mg/ml) was disrupted by sonication (MSE Ultrasonic Disintegrator, 150 W). This material was with or without 1% Triton X-100 coupled to CNBr-activated Sepharose [MuLV-(Trition)-Sepharose and MuLV-Sepharose]. The MuLV sonicate was extracted five times at 21 °C with ether–ethanol (3:1) to remove lipids (Entenman, 1957). This material was with or without 1% Triton X-100 coupled to CNBr-activated Sepharose [MuLV-(ether-Trition)-
Association of RNA tumour virus proteins

Sepharose and MuLV-(ether)-Sepharose. Fourteen mg protein was added per g CNBr-activated Sepharose in each of the four coupling reactions, and respectively 12.7, 13.0, 12.9 and 12.8 mg per g Sepharose was coupled. The uncoupled protein was measured by the method of Lowry et al. (1951).

p30-coupled Sepharoses. Purified p30 (0.6 mg/ml) was coupled to CNBr-activated Sepharose with or without prior addition of Triton X-100 to the p30 preparation [p30-(Triton)-Sepharose and p30-Sepharose]. Thirteen mg protein per g CNBr-activated Sepharose was added, and respectively 4.8 and 4.3 mg per g Sepharose was coupled.

Uncoupled Sepharose and uncoupled-(Triton)-Sepharose was obtained by the coupling reaction above, with and without addition of 1% Triton X-100, but with omission of protein.

Binding assay. To examine the binding of 125I-labelled Mo-MuLV protein preparations to the protein-Sepharoses 10 μl matrix material was placed in a 200 μl polyethylene tube (30 × 5 mm) with fine holes in the bottom. These micro-columns were placed on top of a tube which functioned as a receptacle. The washing, loading and elution steps involved addition of liquid to the column and centrifugation of the assembly for 2 min at 700 g in a swinging bucket rotor.

The experiments involved: (i) three washing steps, each with 100 μl assay buffer; (ii) loading 10 μl of a 100-fold-diluted 125I-labelled Mo-MuLV protein preparation (the dilution was done immediately before loading); (iii) incubating at 21 °C for 10 min; (iv) collecting the unbound material by centrifugation; (v) washing three times with 100 μl assay buffer; and (vi) eluting with various agents as mentioned in the text. The reason for the dilution of the 125I-labelled Mo-MuLV protein preparations (obtained in 0.1% Triton X-100) was to bring the Triton X-100 concentration down to a level where it does not interfere with the binding (see later).

The binding reaction was performed with approx. 30 ng iodinated protein added to coupled Sepharoses containing approx. 12 to 40 μg protein. Because of this low molecular ratio, and because the binding of iodinated proteins is linear with their addition, then it is believed that the assay is far from the saturation point (data not shown).

Analysis of proteins. The filtrates and eluates were examined by SDS–polyacrylamide gel electrophoresis (Laemmli, 1970). The individual proteins were localized by autoradiography and the bands were cut out and counted in a gamma counter.

RESULTS

Identification of virus proteins

The proteins of Mo-MuLV were separated by SDS–polyacrylamide gel electrophoresis (Laemml, 1970) and by two-dimensional gel electrophoresis (O’Farrell, 1975) with some modifications (Forchhammer & Klarlund, 1979). The results of the separation of iodinated virus proteins by the two methods are shown in Fig. 1. The different virus proteins gp70, p30, p15, p15E and p12 were localized in the two-dimensional gels according to Forchhammer & Turnock (1978), while p10 was identified by its size and basic isoelectric point.

Binding of MuLV proteins to MuLV-coupled Sepharoses

The binding of virus proteins to the Sepharoses coupled with virus material is shown in Table 1. Only small amounts of virus proteins were bound to the uncoupled Sepharose material itself. When the CNBr-activated Sepharose was treated with Triton X-100 before inactivation [uncoupled-(Triton)-Sepharose] a slightly larger degree of binding was observed. This binding to the Sepharose material must be regarded as the background of the assay.

Sepharose coupled with sonicated MuLV (MuLV-Sepharose) bound approximately twice as much gp70 and p30 and 35% more p15 together with p15E (p15/p15E) than the
Fig. 1. (a) Autoradiogram of a separation of 125I-labelled Mo-MuLV proteins on SDS–polyacrylamide gel electrophoresis. (b) Autoradiogram of a separation of 125I-labelled Mo-MuLV proteins on O'Farrell two-dimensional polyacrylamide gel electrophoresis. As mol. wt. markers 125I-labelled iodinated bovine serum albumin (69 000), ovalbumin (46 000), Mo-MuLV p30 (30 000) and cytochrome c (13 000) were used.

Table 1. Binding of 125I-labelled Mo-MuLV proteins to Mo-MuLV-coupled Sepharoses

<table>
<thead>
<tr>
<th>Sepharose material</th>
<th>Total radioactivity bound (ct/min)</th>
<th>gp70</th>
<th>p30</th>
<th>p15/p15E</th>
<th>p12</th>
<th>p10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radioactivity added (ct/min)</td>
<td>34 300</td>
<td>1760</td>
<td>9960</td>
<td>1100</td>
<td>2760</td>
<td>3650</td>
</tr>
<tr>
<td>Uncoupled Sepharose</td>
<td>2600 ± 300</td>
<td>3 ± 1</td>
<td>11 ± 1</td>
<td>5 ± 4</td>
<td>0 ± 1</td>
<td>2 ± 1</td>
</tr>
<tr>
<td>Uncoupled-(Triton)-Sepharose</td>
<td>3700 ± 1300</td>
<td>13 ± 10</td>
<td>20 ± 2</td>
<td>13 ± 5</td>
<td>15 ± 7</td>
<td>14 ± 5</td>
</tr>
<tr>
<td>MuLV-Sepharose</td>
<td>7400 ± 1100</td>
<td>28 ± 1</td>
<td>27 ± 4</td>
<td>43 ± 5</td>
<td>0 ± 4</td>
<td>0 ± 3</td>
</tr>
<tr>
<td>MuLV-(Triton)-Sepharose</td>
<td>20 200 ± 1000</td>
<td>87 ± 2</td>
<td>90 ± 1</td>
<td>85 ± 6</td>
<td>7 ± 8</td>
<td>0 ± 5</td>
</tr>
<tr>
<td>MuLV-(ether)-Sepharose</td>
<td>5600 ± 1000</td>
<td>12 ± 6</td>
<td>17 ± 1</td>
<td>37 ± 9</td>
<td>17 ± 10</td>
<td>1 ± 6</td>
</tr>
<tr>
<td>MuLV-(ether-Triton)-Sepharose</td>
<td>13 200 ± 1300</td>
<td>76 ± 11</td>
<td>51 ± 2</td>
<td>80 ± 5</td>
<td>8 ± 1</td>
<td>3 ± 4</td>
</tr>
<tr>
<td>Octyl Sepharose</td>
<td>24 293 ± 300</td>
<td>99 ± 1</td>
<td>99 ± 1</td>
<td>94 ± 4</td>
<td>51 ± 1</td>
<td>28 ± 7</td>
</tr>
</tbody>
</table>

* MuLV extracts was iodinated, ether–ethanol extracted and diluted. 10 µl of the dilution (34 300 ct/min) was added to the Sepharose materials. The binding of the individual proteins retained was calculated from the amount of proteins passing through the materials. p15 and p15E are accounted for as their sum 'p15/p15E' because they did not separate in the one-dimensional gels used. The preparation of the Sepharose materials is described in Methods. The assay was performed in four identical series and the average values ± the standard deviation is shown. The bound proteins were eluted as shown in Tables 2 and 3.

background did (Table 1). The MuLV-Sepharose did not bind p12 and p10. When the sonicated MuLV was coupled in the presence of Triton X-100 [MuLV-(Triton)-Sepharose] almost complete binding of gp70, p30 and p15/p15E was observed, whereas only slightly more binding of p12 and p10 was observed (Table 1).
Table 2. Elution of $^{125}$I-labelled Mo-MuLV material bound to Mo-MuLV-coupled Sepharoses*

<table>
<thead>
<tr>
<th>Sepharose material</th>
<th>50% Ethylene glycol</th>
<th>1% SDS</th>
<th>1% Triton X-100</th>
<th>1% Deoxycholate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uncoupled Sepharose</td>
<td>3</td>
<td>52</td>
<td>43</td>
<td>43</td>
</tr>
<tr>
<td>Uncoupled-(Triton)-Sepharose</td>
<td>7</td>
<td>53</td>
<td>47</td>
<td>75</td>
</tr>
<tr>
<td>MuLV-Sepharose</td>
<td>1</td>
<td>78</td>
<td>49</td>
<td>66</td>
</tr>
<tr>
<td>MuLV-(Triton)-Sepharose</td>
<td>2</td>
<td>62</td>
<td>49</td>
<td>48</td>
</tr>
<tr>
<td>MuLV-(ether)-Sepharose</td>
<td>2</td>
<td>68</td>
<td>52</td>
<td>68</td>
</tr>
<tr>
<td>MuLV-(ether-Triton) Sepharose</td>
<td>2</td>
<td>78</td>
<td>56</td>
<td>77</td>
</tr>
<tr>
<td>Octyl Sepharose</td>
<td>2</td>
<td>36</td>
<td>61</td>
<td>50</td>
</tr>
</tbody>
</table>

* The coupled Sepharoses with bound iodinated material shown in Table 1 were washed six times with 10 vol. assay buffer, and the bound material was eluted with the listed eluants. The elution is shown as percentage of the material bound to the various Sepharoses.

The difference in binding of gp70, p30 and p15/p15E to the MuLV-(Triton)-Sepharose in comparison to the MuLV-Sepharose was not due to a different coupling efficiency of the proteins to the Sepharose (see Methods); nor is the difference in binding caused by a coupling of Triton X-100 to the Sepharose, since Triton X-100 does not contain amino groups, by which it can couple to the CNBr-activated Sepharose (Porath et al., 1967). It was also shown by a control experiment that Triton X-100 itself cannot induce a strong binding to the Sepharose material.

The virus particles contain 20 to 30% lipid (Beard, 1963). Ethanolamine lipids and serine lipids contain amino groups, by which they can couple to the CNBr-activated Sepharose. To test whether the binding ability of the MuLV-(Triton)-Sepharose was due to the lipids the MuLV sonicate was extracted with ether-ethanol (3:1) prior to coupling. As seen in Table 1, the Sepharose coupled with ether-ethanol-extracted MuLV sonicate [MuLV-(ether)-Sepharose] showed an almost identical binding of virus proteins when compared to the MuLV-Sepharose. Sepharose, coupled with the same extract in the presence of Triton X-100 [MuLV-(ether-Triton)-Sepharose] showed a higher degree of binding when compared to the MuLV-(ether)-Sepharose in accordance with former results. However, this binding to the MuLV-(ether-Triton)-Sepharose was not as large as the binding to the MuLV-(Triton)-Sepharose.

This result shows that lipid-free MuLV extracts can form a strong binding with virus proteins when coupled to CNBr-activated Sepharose in the presence of Triton X-100. The lipids, however, may also be involved in the Triton X-100-induced binding of the MuLV-(Triton)-Sepharose, since their removal decreased their binding particularly of p30 (Table 1).

The binding of the iodinated proteins to the MuLV-(ether)-Sepharose and MuLV-(ether-Triton)-Sepharose is, as shown later, of a hydrophobic nature. This binding must therefore be caused by the coupled proteins, since these materials are lipid-free, and since RNA which is present in the preparation binds poorly to CNBr-activated Sepharose under the coupling conditions used (results now shown) and does not bind proteins by hydrophobic bonds.

Octyl Sepharose bound nearly all gp70, p30, p15 and p15E, and smaller amounts of p12 and p10 (Table 1). Although there is a difference in binding strength, this binding pattern resembles the binding pattern of the proteins to the MuLV-coupled Sepharoses, indicating that the binding forces are of a similar nature.
Table 3. Elution of proteins bound to Mo-MuLV-coupled Sepharoses*

<table>
<thead>
<tr>
<th>Sepharose material</th>
<th>Eluant</th>
<th>Percentage of bound proteins eluted</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>gp70</td>
</tr>
<tr>
<td>MuLV-(ether)-Sepharose</td>
<td>1% SDS</td>
<td>92</td>
</tr>
<tr>
<td></td>
<td>1% Triton X-100</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td>1% Deoxycholate</td>
<td>100</td>
</tr>
<tr>
<td>MuLV-(ether-Triton)-Sepharose</td>
<td>1% SDS</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>1% Triton X-100</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>1% Deoxycholate</td>
<td>72</td>
</tr>
<tr>
<td>Octyl Sepharose</td>
<td>1% SDS</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>1% Triton X-100</td>
<td>76</td>
</tr>
<tr>
<td></td>
<td>1% Deoxycholate</td>
<td>22</td>
</tr>
</tbody>
</table>

* The eluates from the experiment of Tables 1 and 2 were examined for the content of proteins by one-dimensional gel electrophoresis. The values state the percentage of the bound proteins (see Table 1) which was eluted with the detergent. No p10 and p12 were detected in the eluates.

Elution of the proteins bound

The strength and the molecular nature of the associations between the iodinated MuLV proteins and the MuLV-coupled Sepharoses were examined by their elution with various agents (Table 2).

Material remaining bound after six washes with 10 vol. assay buffer was strongly bound. This was seen by the fact that an additional washing step eluted less than 2% of the bound material from the MuLV-coupled Sepharoses. Further elution with H2O with NaCl in concentrations from 10 to 3000 mM, KCl in concentrations from 10 to 2000 mM, MgCl2 in concentrations from 2 to 200 mM, or 5 mM-EDTA and 50 mM-tris-acetate adjusted to pH values between 1.5 and 10.5, did not elute significantly more material (results not shown).

Relatively more of the bound material was eluted with these eluants from the uncoupled Sepharoses and especially from the uncoupled-(Triton)-Sepharose than from the MuLV-coupled Sepharoses. This showed that the association between the iodinated proteins and the uncoupled Sepharoses was weaker than the association between the iodinated proteins and the MuLV-coupled Sepharoses. The lack of elution with salt- and pH-adjusted buffers from the MuLV-coupled Sepharoses and octyl Sepharose indicates that the binding here is not of an ionic nature nor of a hydrogen bond nature.

Detergents, however, eluted the bound material. Buffers containing 1% SDS, 1% Triton X-100 and 1% deoxycholate eluted 40 to 80% of the bound material in one step (Table 2). Additional material was eluted with subsequent elution steps (not shown). The association, therefore, seems to be of a hydrophobic nature. We have examined the concentrations of the three detergents needed to elute the proteins. At concentrations of 0.01% and below, no elution was observed (experiments not shown). Ethylene glycol (50%) has previously been used to elute hydrophobic virus proteins bound to alkyl-agarose (Swanson et al., 1978; Marcus et al., 1978). However, ethylene glycol eluted less than 3% of the material bound to the MuLV-coupled Sepharoses or octyl Sepharose (Table 2).

The proteins in the eluates

The various eluates from the experiment of Table 3 were examined by gel electrophoresis, to detect which proteins were eluted. No virus proteins could be detected in the salt- and pH-adjusted buffer eluates, and only low amounts of gp70, p30 and p15/p15E were found in the ethylene glycol eluates. However, the bound virus proteins (gp70, p30, p15 and p15E) were detected in the detergent eluates (see Table 3), whereas no p12 and p10 was detected here.
Buffer containing 1% SDS eluted 60 to 100% of each of the bound virus proteins from the MuLV-coupled Sepharoses, whereas only 25 to 45% of each of these proteins was eluted with the same SDS-containing buffer from the octyl Sepharose column. The action of SDS, at this concentration, is presumably a denaturation of the proteins by massive cooperative binding (Tanford, 1973; Helenius & Simons, 1975). The higher elution from the MuLV-coupled Sepharoses than from octyl Sepharose can possibly be explained by the fact that the coupled proteins of the MuLV-coupled Sepharoses can also be denatured on the Sepharose, whereby they lose their ability to bind the added virus proteins.

Triton X-100 and deoxycholate are often grouped as mild detergents, since they normally do not destroy enzymic activity (Helenius & Simons, 1975). However, the action of these two detergents is believed to be somewhat different: Triton X-100 presumably solubilizes hydrophobic proteins by incorporating the hydrophobic parts of the protein into Triton X-100 micelles. In contrast, deoxycholate presumably solubilizes hydrophobic proteins by 'lining' their hydrophobic parts.

The release of the bound proteins by the detergents is presumably caused by a binding of the detergent to the bound protein and to the binding surface of the coupled Sepharose. These actions must furthermore be stronger than the strength of the binding between the protein and the coupled Sepharose for the release to occur.

As seen from Table 3 both Triton X-100 and deoxycholate (in 1% solutions) eluted the bound proteins from the MuLV-coupled Sepharoses. Deoxycholate eluted gp70 and especially p15/p15E better than did Triton X-100, whereas Triton X-100 eluted p30 better than did deoxycholate. This relative difference in elution pattern can be explained by different properties of gp70, p30 and p15/p15E and of the surfaces to which they bind with respect to the binding of the two detergents.

Table 3 also shows that the four proteins were all eluted better from octyl Sepharose with Triton X-100 than with deoxycholate. This elution pattern must be interpreted as a stronger binding of Triton X-100 than deoxycholate to the octyl Sepharose.

We have analysed the distribution between p15 and p15E in the filtrates and detergent eluates by two-dimensional gel electrophoresis (results not shown). In all cases p15 and p15E behaved identically.

**Nature of the bonds formed**

As already seen by the elution of the proteins, the bonds formed are presumably of a hydrophobic nature. This notion is supported by the following facts. (i) The binding corresponds to the hydrophobicity of the virus proteins. The virus proteins gp70, p30, p15 and p15E, which all bind to the MuLV-coupled Sepharoses, are all grouped as hydrophobic proteins. The grouping was done from their ability to bind to alkyl-agaroses (Swanson et al., 1978; Marcus et al., 1978, 1979; Marcus & Smith, 1978). The proteins p12 and p10 have been grouped as the least hydrophobic proteins in these studies, and they bound only weakly to the MuLV-coupled Sepharoses. The hydrophobicity of the proteins can furthermore be seen from this study where their binding to the hydrophobic octyl Sepharose was analysed.

(ii) Myoglobin, being a non-hydrophobic protein, does not bind to the MuLV-coupled Sepharoses. Myoglobin is considered non-hydrophobic because of its lack of binding to detergent micelles (Helenius & Simons, 1977). Myoglobin was iodinated and its binding to the various MuLV-coupled Sepharoses was examined. It was found that less than 5% myoglobin bound to the various MuLV-coupled Sepharoses, which is a much lower value than the binding of any of the hydrophobic proteins. Furthermore, only 10% of the myoglobin bound to octyl Sepharose (results not shown).
Table 4. Binding of Mo-MuLV proteins to p30-coupled Sepharose*

<table>
<thead>
<tr>
<th>Sepharose material</th>
<th>Bound with 1% Triton X-100</th>
<th>Bound with 1% Triton X-100</th>
<th>Bound with 1% Triton X-100</th>
<th>p12 bound</th>
<th>p10 bound</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uncoupled Sepharose</td>
<td>4200</td>
<td>19200</td>
<td>3800</td>
<td>7800</td>
<td>5200</td>
</tr>
<tr>
<td>p30-Sepharose</td>
<td>39</td>
<td>15</td>
<td>42</td>
<td>16</td>
<td>19</td>
</tr>
<tr>
<td>p10-Sepharose</td>
<td></td>
<td></td>
<td></td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>p30-(Triton)-Sepharose</td>
<td>45</td>
<td>27</td>
<td>46</td>
<td>37</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>47</td>
<td>0</td>
</tr>
<tr>
<td>p12-Sepharose</td>
<td>66</td>
<td>67</td>
<td>81</td>
<td>81</td>
<td>11</td>
</tr>
<tr>
<td>p15-Sepharose</td>
<td></td>
<td></td>
<td></td>
<td>77</td>
<td>37</td>
</tr>
</tbody>
</table>

* Binding of diluted iodinated virus extracts to p30-coupled Sepharoses. The amount of bound proteins and eluted proteins was calculated as percentage of the added proteins; no p12 and p10 were detected in the Triton X-100 eluates. The preparation of the Sepharose materials is described in Methods.

**DISCUSSION**

The virus proteins gp70, p30, p15 and p15E are shown to bind to proteins from disrupted MuLV particles coupled to Sepharose and to p30-coupled Sepharose. The bonds seem to be of hydrophobic nature. p12 and p10 bind only weakly to these protein–Sepharoses. When the disrupted MuLV particles or p30 were coupled in the presence of Triton X-100, and the Triton X-100 subsequently was washed away, a much stronger binding between the coupled proteins and gp70, p30, p15 and p15E was observed. This increase in binding was shown to be due to the interaction between Triton X-100 and the proteins in the coupling reaction.

A model for the Triton X-100-facilitated binding ability of the MuLV-coupled Sepharoses is proposed on the basis of the results and conclusions mentioned above (see Fig. 2). (1) The MuLV preparations disrupted by sonication possess internal hydrophobic bonds which keep the proteins together (frame a). (2) When this material is coupled to CNBr-activated Sepharose the proteins will be coupled in this form of internal aggregates. This means that only few or no hydrophobic surfaces are exposed in the formed protein–Sepharose (frames b and c). Only small amounts of the hydrophobic proteins can therefore bind to this protein–Sepharose (frame d). (3) When Triton X-100 is added to the MuLV sonicate the hydrophobic protein–protein bonds are broken by the binding of Triton X-100 to the...
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Fig. 2. A model of the binding ability of MuLV extracts coupled to CNBr-activated Sepharose in the presence and absence of Triton X-100. (a) MuLV proteins with hydrophilic surfaces (smooth lines) and hydrophobic surfaces (serrated lines) by which the proteins are held together. (e) Triton X-100 is added to the protein aggregates which solubilizes them by binding to the hydrophobic surfaces (Triton X-100 is drawn with its hydrophobic part as a line and its hydrophilic part as a circle). (b, f) The untreated or Triton X-100 treated proteins are coupled to CNBr-activated Sepharose. (c, g) Triton X-100 and excess protein are washed away, and unreacted groups on the CNBr-activated Sepharose are inactivated by ethanolamine (●). (d, h) Iodinated virus proteins, diluted from a Triton X-100 solution, are added and bind to the hydrophobic surfaces in (h).

hydrophobic surfaces of the proteins (frame e). The proteins are then coupled to the CNBr-activated Sepharose in this form (frame f). The detergent molecules are subsequently removed by several washing steps, leaving the hydrophobic surfaces exposed (frame g). These hydrophobic surfaces cannot re-aggregate, since the proteins are immobilized on the Sepharose. Consequently, the resulting hydrophobic binding ability of the protein–Sepharose will be strong (frame h).

This study directly demonstrates that the virus proteins can aggregate. It is shown that gp70, p30, p15 and p15E can bind to disrupted virus particles and to p30 by hydrophobic bonds. No other non-covalent bonds, such as hydrogen bonds or ionic bonds, were seen to form between the virus proteins since the elution of bound material was independent of ionic strength and pH. In the following paragraph the hydrophobic forces are discussed with respect to the structure and forces of the virus particle.

According to the present model of the virus structure (Bolognesi et al., 1978; Nermut et al., 1972), the particle is surrounded by a membrane within which p15E is embedded. This location seems indeed to correspond to the hydrophobic properties of the molecule. gp70 is believed to be located in part on the outer side of the membrane and it is to various degrees linked to p15E through disulphide bonds (Pinter & Fleissner, 1977; Montelaro et al., 1978).
Knobs have been observed on the outer surface of the virus particle (Nermut et al., 1972), which probably consist of gp70–pl5E complexes (Pinter & Fleissner, 1979). The hydrophobic association properties of these two proteins shown in this study may explain how the knobs are held together, even if they are not fully linked by disulphide bonds (Montelaro et al., 1978). The hydrophobic property of gp70 also makes it possible for this molecule to be embedded in the membrane.

An ‘inner coat’ lies within the membrane (Bolognesi et al., 1978), and it is suggested to consist of p12. According to the binding studies presented here, this protein does not associate or only associates weakly with any virus proteins. It therefore seems that the inner coat would not possess any structural unity. Bonds may, however, exist in the virus particle, which are destroyed irreversibly by solubilization of the particle.

The core of the virus consists of a core exterior surrounding the nucleoprotein. The core itself is suggested to consist of the proteins p30 and p15 (Bolognesi et al., 1978). The results in this study show that the core exterior is presumably bound together by hydrophobic protein–protein associations of p15 and p30.

The nucleoprotein consists of virus RNA, to which p10 and possibly some p12 are bound (Sen et al., 1976; Leis et al., 1978; Schülein et al., 1978). This complex does not seem to attach to the core exterior, since this study shows that p10 and p12 does not bind or only binds weakly to the core protein p30, and the study of Leis et al. (1978) showed that the virus RNA does not bind to p30. It seems noteworthy that the virus particle consists of layers which alternate between being hydrophobic and non-hydrophobic. The observation that the disrupted MuLV particles bind the hydrophobic proteins weakly when no Triton X-100 was added in the coupling reaction shows that the hydrophobic surfaces are not exposed on the disrupted particle, but are masked by their internal association.

The binding data do not reveal strict specific binding properties between the different virus proteins, since both p30, gp70, p15 and pl5E can associate with p30 (Table 4). The bonds formed by the different proteins could, however, be distinguished by the elution properties of the protein from the MuLV-Sepharoses by the different detergents (Table 3): 1% Triton X-100 eluted p30 more effectively than it eluted gp70 and p15/p15E; 1% deoxycholate eluted p30 less effectively than gp70 and p15/p15E.

The hydrophobic forces presented here can explain the binding forces of the virus particle, but the question of which forces bring the virus proteins together to form the particle remains to be answered. Hydrophobic forces most certainly participate, but it must be noted that the virus particle presumably is assembled from precursor proteins, which later are cleaved into the virus proteins (Witte & Baltimore, 1978). The precursor proteins presumably exhibit the hydrophobic features of the derived proteins, and it is possible that more specificity for assembly exists in that state.

To investigate whether strictly specific bonds exist between the various virus protein-binding reactions must be carried out between the different proteins separately. As mentioned, this has been done for p30 and no preference for binding of gp70, p30, p15 or p15E was observed.

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REFERENCES

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