Interaction of poly(A) with different adsorbents for affinity chromatography of nucleic acids

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Chromatography on adsorbents for separation of mRNA containing poly(A) has given interesting results, even if the nature of the occurring interaction was not always well understood. In the present study we report the chromatographic behaviour of poly(A) homopolynucleotides on different substituted matrices, poly(U)-, poly(4)-, phenyl-, octyl- ethanolamine-, acriflavine- and DNA-Sepharose; oligo-dT and MN-cellulose. Using different experimental conditions as ionic strength, salt concentration, pH, temperature, buffer composition it was possible to evaluate the participation of electrostatic, hydrophobic, hydrogen-bonding, and charge-transfer interaction. Furthermore, it is shown that poly(A) interacts non-specifically with matrices like acriflavine or DNA-Sepharose, as well as with oligo-dT cellulose or poly(U)-Sepharose.

Experimental

Matrices and chemicals

3H-poly(A) and 3H-poly(U) (MW 100 000, specific activity 2 × 10^5 c.p.m./mmol of AMP or UMP) were purchased from New England Nuclear Co. (Boston, Mass., USA). Unlabelled poly(A) and poly(U) (MW 100 000) and MN-cellulose were from Sigma (St. Louis, Miss., USA). Phenyl-, octyl- poly(A)-, poly(U)-Sepharose 4B, and Sepharoses 2B and 4B were obtained from Pharmacia Fine Chemicals (Uppsala, Sweden). Oligo-dT-cellulose (about 10 nucleotides chain length) came from Collaborative Res. Inc. (Waltham, Mass., USA). DNA-Sepharose 2B and ethanolamine-Sepharose 2B were prepared as described before. Acriflavin (Fluka AG, Switzerland) was coupled to Sepharose 4B according to the method described in ref 6.

Samples

A stock solution (100 mg/ml) of poly(A) was prepared with 10 mM Tris HCl buffer, pH 7.6, and mixed with 5 μl of 3H-poly(A) (specific activity 20 × 10^5 c.p.m./ml). This mixture (100 μl, about 10 × 10^6 c.p.m.) was applied to each column according to the conditions described in the legends to the respective figures. The radioactivity was measured by liquid scintillation in an ABAC-SL40 Intertechnique scintillation counter using Instagent (Packard Instruments Co., Inc., Downers, Ill., USA). For the study of the pH effect, the stock solution was prepared in the following buffers: 50 mM glycine HCl, pH 3; 50 mM sodium acetate acetic, pH 4; 50 mM Tris HCl, pH 5.6, 7.6, and 8.6. All experiments were run at room temperature unless otherwise stated.

Columns

Pasteur pipettes were packed with 200 μl of gel. A fibreglass cap was attached to the bottom of each column.
The ability of nucleic acids to enhance the solubilization of organic compounds in aqueous systems has been known for several years. Nevertheless, the contribution of DNA to the enhancement of solubility is not well understood. DNA is known to have a strong affinity for nucleic acids, and this affinity is thought to be responsible for the enhancement of solubility. However, the mechanism by which DNA enhances solubility is not well understood.

In this study, we investigated the role of DNA in the enhancement of solubility of organic compounds. We found that DNA enhances the solubility of organic compounds in a concentration-dependent manner. This effect is most pronounced at low ionic strengths, and it is thought to be due to the formation of complexes between DNA and the organic compounds.

The results of this study suggest that DNA can be used as a solvent for organic compounds. This could have important implications for the extraction of organic compounds from aqueous solutions. Further studies are needed to understand the mechanism by which DNA enhances the solubility of organic compounds.

**Results and discussion**

**Salt effect**

The chemical nature of poly(A) suggests that the ionic strength of the medium may be important for the capacity of the molecule to interact with other compounds. At neutral pH and low ionic strength, the poly(A) molecule carries a considerable negative charge. Therefore, and since charges are efficiently neutralized by countercations, it is not surprising that the electrostatic attraction causing adsorption of poly(A) to the positively charged ethanolamine-Sepharose decreases with salt concentration (Figure 1). As has been shown, the activation of polysaccharide matrices by BrCN for the coupling of primary amines is considered to occur via formation of isourea linkages which are positively charged. Such positively charged groups should also be present in poly(U)- and poly(A)-Sepharose, commercially prepared by irradiation, lacks affinity for poly(A) at low ionic strength (Figure 1). Very recently, matrix-bound dyes have been employed for affinity chromatography of nucleic acids taking advantage of their base specificity. Therefore, it was interesting to look at the chromatographic behaviour of poly(A) on this type of adsorbent. For this purpose, acriflavine-Sepharose was prepared by using a coupling procedure that avoids the introduction of charges on the matrix. The data plotted in Figure 1 suggest that the adsorption of poly(A) to acriflavine-Sepharose may be practically salt-independent as a result of mixed effects of electrostatic, hydrophobic, and charge-transfer interactions. Evidence in favor of a charge-transfer association between poly(A) and acriflavine-Sepharose, at moderate salt concentration, has been collected. For instance, free poly(U) is able to displace poly(A) from acriflavine-Sepharose columns provided that conditions for poly(A) poly(U) complex formation are adequate (in our case, 10 mM Tris HCl, pH 7.6, with 0.4 M NaCl are optimal for elution). Competition of poly(U) with poly(A) for adsorption on acriflavine-Sepharose is excluded because poly(U) has no affinity for acriflavine-Sepharose under these conditions (results not shown). Interestingly, similar effects have been observed when cellulose filters have been used in the purification of poly(A)-containing nucleic acids. Although no explanation has been put forward, due to lack of information about the structure of cellulose, some authors have proposed the participation of aromatic interactions between the lignin aromatic groups of cellulose and the bases of polynucleotides. The fact that only polynucleotides possess a significant affinity for these adsorbents supports the idea that aromatic interactions of the type of charge-transfer are not unusual with poly(A).

For the binding of poly(A) to acriflavine-Sepharose, the model for the interaction between this kind of dye and nucleic acids, as proposed by Lerman, seems very similar: that is, both external (binding of the dye directed to the phosphate groups) and intercalated (binding of the dye between the bases) associations between poly(A) and acriflavine-Sepharose are feasible, with accessibility under a given set of conditions being the sole restriction.

**DNA-Sepharose on the other hand, does not possess important adsorption capacity towards poly(A) at low salt concentration, resembling neutral oligo-dT-cellulose in this respect (Figure 1). The low pH employed for the coupling of DNA to BrCN-activated Sepharose is probably the reason why the number of positive charges introduced on the matrix is lower.**

As could be expected, oligo-dT-cellulose commercialized by irradiation, lacks affinity for poly(A) at low ionic strength (Figure 1). This is simply due to repulsion between the negatively charged poly(A) in similar conditions. The same effect would occur between poly(A) and poly(U) complex formation are adequate (in our case, 10 mM Tris HCl, pH 7.6, with 0.4 M NaCl are optimal for elution). Competition of poly(U) with poly(A) for adsorption on acriflavine-Sepharose is excluded because poly(U) has no affinity for acriflavine-Sepharose under these conditions (results not shown). Interestingly, similar effects have been observed when cellulose filters have been used in the purification of poly(A)-containing nucleic acids. Although no explanation has been put forward, due to lack of information about the structure of cellulose, some authors have proposed the participation of aromatic interactions between the lignin aromatic groups of cellulose and the bases of polynucleotides. The fact that only polynucleotides possess a significant affinity for these adsorbents supports the idea that aromatic interactions of the type of charge-transfer are not unusual with poly(A).

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described below. Aromatic interactions of the type of charge-transfcr.

When pH is lowered, the repulsion between negatively
charged polynucleotides is decreased. Yet no tendency of polya forming hydrogen bonds with its complementary
immobilized polynucleotides (oligo-dT-cellulose, poly(U) and DNA-Sepharose) is observed (Figure 2c). This
might be due to protonation of the adenine amino
groups. Only if the pH is above the pK of the basic amino-
group (pK = 3.45), and a certain amount of salt is present,
is the hydrogen-bonding effect observed (Figure 2a). As
pH, and consequently the overall negative charge of the
cells, increases the repulsion between the similarly
charged polymers may account for the decreased capacity
of adsorption at higher pH.

Unfortunately, the chromatographic behaviour of polya on the adsorbents under study at pH lower than
5.6 is dubious. From a control experiment it has been
found that glass wool possesses a strong adsorption
capacity at acidic pH. This effect is particularly
pronounced when salt is present (Figure 2a). The nature of the
adsorption of polya to glass wool under such conditions
remains unclear.

Temperature effect

The molecular structure of polya is also affected by
variations in temperature. For example, at neutral
pH and moderate salt concentration the polya molecule
possesses a single-stranded helical configuration at
0°C.2°. This helical structure disappears in a non-
cooperative way as temperature increases. For this
reason, one may expect that the interacting abilities of free
poly(A) with other compounds are temperature
dependent.

A typical temperature effect plot for the association of
complementary polynucleotides is shown in Figure 3. The
observed Tm values under these conditions are in good
agreement with those previously reported for the assoca-
tion of this type of polynucleotides. The exceptional
stability of the polya poly(U)-Sepharose com-

poly(A) undergoes conformational changes as a result of
variations in the pH of the medium. Such structural
modifications affect its ability to interact with other
compounds. As shown in Figure 2 the pH effect is
also influenced by the presence or absence of salt (Figures
2a, 2b, 2c and 2d, respectively). In general, it is considered
that these parameters affect directly the overall charge of the
molecule in question. For example, at low pH the
poly(A) molecule is less charged and its tendency to
adsorb hydrophobically to matrices like octyl-Sepharose,
and probably phenyl-Sepharose and MN-cellulose, might
be expected to increase (Figures 2b and 2d).

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Figure 2. pH effect. Adsorption of 3H-poly(A) to different
matrices as a function of the pH. The percent radioactivity
adsorbed with respect to the total amount of material applied is
plotted: (a) and (b) give results of experiments performed with
buffer containing 0.4 M NaCl; (c) and (d) results with the same
buffers but in the absence of salt. All the experiments were run at
20°C. Glass-wool; other symbols as in Figure 1.

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Figure 3. Temperature effect. Adsorption of 3H-poly(A) to
different matrices as a function of temperature. The columns
were calibralcd with 0.4 M NaCI in 10 mM Tris HCI buffer, pH
7.6. in a temperalurc chamber. The percent radioactivity
adsorbed with respect to the total amount of material is plotted.
Symbols as in Figure 1.
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Table 1. Effect of buffer composition*.

<table>
<thead>
<tr>
<th>Gel</th>
<th>0.4 M KCl</th>
<th>0.4 M NaCl</th>
<th>0.4 M CsCl</th>
<th>0.4 M LiCl</th>
<th>4 mM MgCl₂</th>
<th>4 mM CaCl₂</th>
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<tr>
<td>Poly(U)-Sepharose 4B</td>
<td>93</td>
<td>98</td>
<td>94</td>
<td>94</td>
<td>88</td>
<td>92</td>
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<tr>
<td>Acriflavin-Sepharose 4B</td>
<td>95</td>
<td>98</td>
<td>95</td>
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<td>92</td>
<td>96</td>
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<tr>
<td>Oligo-dT-cellulose</td>
<td>90</td>
<td>90</td>
<td>90</td>
<td>86</td>
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<td>87</td>
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<tr>
<td>Mn-cellulose</td>
<td>50</td>
<td>27</td>
<td>73</td>
<td>52</td>
<td>85</td>
<td>35</td>
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<tr>
<td>DNA-Sepharose 2B</td>
<td>20</td>
<td>77</td>
<td>14</td>
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<td>Octyl-Sepharose 4B</td>
<td>39</td>
<td>5</td>
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<td>54</td>
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<td>55</td>
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<tr>
<td>Poly(A)-Sepharose 4B</td>
<td>5</td>
<td>25</td>
<td>21</td>
<td>36</td>
<td>67</td>
<td>34</td>
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<tr>
<td>Phenyl-Sepharose 4B</td>
<td>10</td>
<td>10</td>
<td>21</td>
<td>35</td>
<td>30</td>
<td>36</td>
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<tr>
<td>Ethanolamine-Sepharose 2B</td>
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<tr>
<td>Sepharose 4B</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>12</td>
<td>10</td>
<td>32</td>
</tr>
</tbody>
</table>

* The columns were equilibrated at 20°C with the corresponding saline in 10 mM Tris-HCl buffer, pH 7.6. Figures show the percent radioactivity adsorbed with respect to the total amount of 3H-poly(A) applied in each case. Further details in Experimental section. The salts are arranged according to their increasing chaotropicity: K > Na > Cs > Li > Mg > Ca (refs 30 and 35).

plex is again evidenced by an almost complete absence of a temperature effect in the range 4-60°C, though it decreases at higher temperatures. In other experiments, however, it has been noticed that free poly(U) when it complexes with poly(A)-Sepharose does show a melting point within this range (results not shown here). This fact suggests that the temperature effect for polynucleotide association is related to the nature of the immobilized ligand. Therefore it is conceivable that some restrictions on the mobility of the ligand may occur when it is coupled to a matrix, thus limiting the conformational changes that would be observed with the ligand in solution.

Hydrophobic interactions are favoured by increasing temperature. Therefore, the temperature effect is also good evidence against hydrophobic bonding as the main reason for adsorption of free poly(A) to MN-cellulose and poly(A)-Sepharose (Figure 3). In such cases, there is a tendency for adsorption to decrease as a function of temperature (Figure 3). In contrast, hydrophobic adsorbents such as octyl-Sepharose become more efficient with increasing temperature (Figure 3).

The inset of Figure 3 shows the effect of temperature on the adsorption of free poly(A) to ethanolamine-Sepharose in the absence of salt, supporting the previously proposed electrostatic mechanism of adsorption of free poly(A) to this gel.

Although charge-transfer and electrostatic interactions should be reduced by increasing temperature, acriflavin-Sepharose is still a strong adsorbent for free poly(A) over the full range of 4-60°C (Figure 3). This observation suggests that the increasingly favoured hydrophobic interaction between free poly(A) and acriflavin-Sepharose following a rise in temperature dominates.

Buffer composition effect

A large number of reports have put in evidence the effect of the buffer composition on molecular interactions of nucleic acids. The role of certain ions regarded as chaotropes, for example, has been related to denaturation, dissociation and extension of these polymers. From Table 1 it appears that a generalization about a cationic effect on the chromatographic behaviour of free poly(A) is not possible. While with some adsorbent binding is favoured as chaotropicity increases, with others the result is the opposite. This might be due to several factors acting on the molecules in question: changes in conformation, the water structure, neutralization of charges, chelate binding and possibly 'sandwich' complexes between aromatic structures and metals (Figure 4).

An interesting observation that arises from Table 1 concerns the effect of Ca²⁺ and Mg²⁺ on the tendency of poly(A) to bind to those adsorbents. For instance, a hydrogen bond mechanism for the adsorption of poly(A) to oligo-dT-cellulose, and DNA-Sepharose, appears very unlikely in view of the low ionic strength of the buffer. Neither can the large adsorption capacity of MN-cellulose towards poly(A) in this case be ascribed to

potentially important in biomolecular interactions, e.g. protein synthesis, suggests that the thymidine-rich 5' sequences on DNA cannot always be accessible for complexing through hydrogen bonding with poly(A), and that the binding of poly(A) to DNA-Sepharose is to a large extent non-specific.

There are many examples of metal-π-complexes in biological systems and their participation in maintaining biological structures. Moreover, the fact that Mg²⁺ and Cu²⁺ are essential for the complex reactions involving polynucleotides, e.g. protein synthesis, suggests that they are especially important in biomolecular interactions between aromatic structures.

Both acceptor capacity and chaotropicity of cations certainly contribute to the effect expected from the ionic strength of their solutions alone. Hence, it is very difficult to draw any conclusion about their action, particularly with molecules (proteins and polynucleotides) whose structural conformations depend on these parameters.

Table 2 shows the action of detergents, chaotropic anions, polyanion bridging agents and hydrogen bond-breaking agents on the adsorption of poly(A) to the matrices under study. In the cases where hydrogen bond formation is the main reason for binding, urea and formamide are very efficient in preventing adsorption of poly(A) (oligo-dT-cellulose and DNA-Sepharose) (Table 2). In contrast, the failure of urea to suppress the adsorption of poly(A) to poly(U)-Sepharose emphasizes again the participation of types of interactions other than hydrogen bonding. Since SDS is an excellent eluant for polynucleotide matrices under study, it is thought to occur with a significant contribution from hydrophobic bonding. This explains the larger efficiency of formamide compared to urea in preventing the formation of a poly(A)-poly(U) complex (Table 2). In other words, formamide owes its effect both to disruption of hydrogen bonds and to polyanion-reducing properties that weaken hydrophobic interaction. As shown in the case of octyl-Sepharose, urea only partially decreases the tendency of poly(A) to adsorb to this matrix hydrophobically (Table 2), while formamide (90°, v/v) is again more efficient than urea as a hydrophobic-suppressing agent in this example. The chaotropic anion SCN⁻ affects mainly the adsorption of poly(A) to hydrophobic matrices (octyl-Sepharose, phenyl-Sepharose), charge-transfer adsorbents (acriflavine-Sepharose, MN-cellulose) and the complementary DNA-Sepharose, but shows no effect in the cases of poly(U)-Sepharose and oligo-dT-cellulose. This seems to indicate that the hydrogen bonding between these two matrices and poly(A) is strong enough to resist the action of SCN⁻. In the case of DNA-Sepharose, it is probable that the thymidine-rich 5' sequences on DNA cannot always be accessible for complexing through hydrogen bonding with poly(A), and that the binding of poly(A) to DNA-Sepharose is to a large extent non-specific.

The remarkable ability of urea to prevent the adsorption of poly(A) on acriflavine-Sepharose, indicated the electron donor nature of urea. The action of urea consists of providing the necessary electronic cloud for the electron-deficient acrilavin ring system, thus preventing the adsorption of poly(A) by this mechanism. The presence of salt is only required to eliminate electrostatic effects between poly(A) and acriflavine-Sepharose. On the other hand, the efficiency of SDS in disrupting the acrilavin poly(A) complex could be interpreted as evidence for participation of a hydrophobic bonding mechanism in the adsorption of poly(A) to acriflavine-Sepharose. Even if hydrophobic contributions are not fully eliminated, it appears difficult to ascribe the ability of free poly(U) to displace poly(A) from acrilavin-Sepharose columns to a detergent-like action. The SDS effect might be explained simply in terms of the formation of a micelle structure around the hydrophobic acrilavin molecule causing a strong repulsion towards poly(A).

Conclusions

The chemical structure of poly(A) enables the molecule to interact with different compounds electrostatically, hy-

Table 2. Effect of buffer composition on the adsorption of poly(A) to various affinity adsorbents

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</tr>
</thead>
<tbody>
<tr>
<td>NaCl concentration (M)</td>
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<td>1.0</td>
<td>0</td>
<td>0.4</td>
<td>1.0</td>
<td>0</td>
<td>0.4</td>
<td>1.0</td>
<td>0</td>
</tr>
<tr>
<td>Radioactivity adsorbed</td>
<td>95</td>
<td>96</td>
<td>98</td>
<td>96</td>
<td>96</td>
<td>98</td>
<td>96</td>
<td>90</td>
<td>80</td>
<td>90</td>
</tr>
<tr>
<td>6.0 M Urea</td>
<td>90</td>
<td>80</td>
<td>80</td>
<td>80</td>
<td>80</td>
<td>80</td>
<td>80</td>
<td>80</td>
<td>80</td>
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<tr>
<td>90°, Formamide</td>
<td>90</td>
<td>13</td>
<td>18</td>
<td>90</td>
<td>13</td>
<td>18</td>
<td>90</td>
<td>13</td>
<td>18</td>
<td>90</td>
</tr>
</tbody>
</table>

*The columns were equilibrated at 20°C with 10 mM Tris·HCL buffer, pH 7.6 containing the compound or salt as indicated. The percentages of radioactivity adsorbed with respect to the total amount of 3H-poly(A) applied are shown.
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drophiobically, by hydrogen bonding, and or charge-transfer mechanisms. Of interest for polynucleotide associations, these possibilities have been estimated with synthetic poly(A) and different adsorbents under various conditions of pH, temperature, salt concentration and buffer composition. For practical purposes, several recommendations are suggested for chromatography of poly(A)-containing nucleic acids on affinity adsorbents containing polynucleotide- or dye-bound matrices: apart from reduction of salt concentration, poly(A)-nucleic acids can be eluted from oligo-dT-cellulose by increasing the temperature. Urea, formamide and detergents can be useful when strong binding is present as a result of the participation of other less specific interactions than hydrogen bonding (in cases like polylU-Sepharose, DNA-Sepharose, etc.). Chaotropic acids can be eluted from poly(A)-containing nucleic acids on affinity adsorbents under various buffer compositions.

Acknowledgements

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