m-Aminophenylboronate agarose specifically binds capped snRNA and mRNA

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ABSTRACT
m-Aminophenylboronate-substituted agarose binds specifically RNA chains carrying a mature 5' cap. The binding occurs most effectively at pH > 8 and involves diester formation between the negatively charged tetrahedric boronate group and the cis-diol of the ribose of the cap. The positive charge introduced by the m^G methylation is necessary for efficient binding although two closely spaced cis-diol groups alone (as in the cap analog NADH) are sufficient for binding. Non-capped RNA (like poly(U) and rRNA) or decapped RNA are not bound. It is shown that the matrix can be used for the isolation of capped small nuclear RNA and mRNA.

INTRODUCTION
The family of RNAs wearing a 5' cap (m^G(5')ppp(5')Nmp....) comprises the cytoplasmic mRNAs and their nuclear hnRNA precursors as well as the small nuclear RNAs (snRNAs) (1, 2). Formation of the cap introduces a positive charge at the m^G and creates a second 3' end with an open 2', 3' cis-diol group. The presence of cis-diol groups at the 3' end has been used for the isolation of RNA, and oligo- or mononucleotides by affinity chromatography via matrix-coupled boronate groups (3 - 7). The binding involves the formation of a cyclic diester between the two hydroxyl groups and the boric acid (8). We reasoned, that mRNAs and snRNAs, having two 3' ends per RNA chain and hence two cis-diol groups per molecule, should have a higher chance of being bound to a boronate matrix than RNAs without a cap, i.e. with only one 3' end. This kind of discrimination, however, may not be sufficient to allow a satisfactory separation of capped and non-capped RNAs. On the other hand, if conditions could be found under which binding becomes dependent on the presence of
a positive charge close to the cis-diol group, only capped RNA species would be retained. In this communication we show that such specific binding conditions can in fact be found and that boronate substituted agarose can be used for the rapid and efficient purification of capped snRNAs and mRNAs out of complex mixtures. A number of applications for this technique is immediately obvious and some of them will be discussed.

MATERIALS AND METHODS

Preparation of the boronate matrix. CM sepharose 6B-CL (Pharmacia) was coupled to m-aminophenylboronic acid (Sigma) using the carbodiimide coupling procedure as described by Pace and Pace (7). After extensive washing with water the hydroxyboryl Sepharose was washed with 10 vol. of 0.1 M Na-acetate and equilibrated with binding buffer (BB : 50 mM HEPES, pH 8.5; 1 M NaCl; 100 mM MgCl₂). The gel was stored between runs in elution buffer (see below).

Operation of the columns (Standard conditions). The boronate column was used at room temperature to achieve a high specificity of binding (4). 2 to 4 ml of bed volume were routinely used. 1 ml of the gel binds more than 5 A₂₆₀ units of NAD⁺. Samples of 2 to 10 A₂₆₀ units (or 10⁶ to 5 x 10⁶ cpm) of RNA in 50 to 100 μl water were mixed with 9 vol. of BB and applied to the column at a flow rate of 10 ml/h. Washing was done with BB until no A₂₆₀ material or radioactivity could any longer be detected. The bound RNA was eluted with elution buffer (EB : 50 mM Na-acetate, pH 5; 200 mM NaCl). Fractions of 3 ml were collected through all steps. Experiments with cap analogs were done under identical conditions using 1 - 2.5 A₂₆₀ units per run. Before the first use a mock run with crude tRNA was routinely performed since it improved specificity of binding in the subsequent runs. Probably due to the high concentration of Mg²⁺ in the binding buffer, the columns show some bleeding of the boronate compound. This material is, however, lost completely during ethanol precipitation. Because of the bleeding we have used the columns for only 5 to 6 subsequent runs.

Preparation of RNA. Cellular RNA was prepared by the proteinase K method (9) after lysis of whole cells (bovine lymphocytes or
HeLa cells) with SDS buffer. RNA had been labeled in vivo for 16 h with \(^{3}H\)-uridine (5 μCi/ml) in some cases. DNA was removed after ethanol addition by spooling on a glass rod. Polyadenylated RNA from bovine lymphocytes was prepared via oligo (dT)-cellulose (10). Ribosomal RNA was isolated from the 28S peak from a sucrose gradient of RNA which did not bind to oligo (dT)-cellulose. The decapping of RNA was performed with tobacco acid pyrophosphatase (11). Poly(A)⁺RNA from chick oviduct cells was a generous gift of Dr. H. Hauser, Stockheim.

**Electrophoretic analyses of RNA.** RNA was analyzed on polyacrylamide gels (10% PAA) according to Maxwell (12). The ratio of polyacrylamide to methylene-bis-acrylamide was 30 : 1.5 to increase crosslinking which gave a better resolution of the snRNA range (4 - 8S). Gels were run for 640 V • h and fluorographed (13).

**In Vitro translation.** In vitro translation was performed in the mRNA-dependent rabbit reticulocyte lysate (14). 25 μl assays contained 1 μg of polyadenylated RNA and 20 μCi of \(^{35}S\)-methionine. Translation in the presence of 5' mGTP required an increase of the K-acetate concentration to 140 mM (15).

**RESULTS**

**Experimental rationale.** The use of matrix-coupled boronate groups is a well-established technique in RNA methodology. This method has been used for the separation of aminoacylated from uncharged tRNAs (3), for the isolation of tRNA containing the modified base Q (4), and for the isolation of 3'-terminal regions of other RNA molecules (5). All these procedures relied on the reaction of the 2', 3' terminal cis-diol group in RNA with the boronate group to form a diester linkage under appropriate conditions. Since eukaryotic mRNA by presence of the 5' cap possesses a cis-diol group at its 5' end as well, 5' terminal fragments from mRNA have also been purified on boronate-containing matrices (6). The basic difficulty frequently encountered in these cases was that efficient binding of long RNA chains was only achieved under conditions where a relatively high background of unspecific RNA binding had to be tolerated. The specificity should be improved, however, under conditions
where the presence of a cis-diol group was still necessary but not sufficient for efficient binding. A feature unique to the 5' cap structure is its positive charge. We therefore searched for binding conditions where the presence of a positive charge was required for binding. We found that such 5' cap-specific binding was possible by the combination of (i) the inert agarose matrix which eliminates background problems, (ii) higher temperature (room temperature as compared to 4°C) which reduces binding of RNAs with a single cis-diol group, and (iii) a high-salt buffer which probably is helpful in reducing background as well as single cis-diol group binding. A number of model compounds representing analogs of the cap were used to demonstrate specificity and to probe the contribution of various parts of the cap structure in the binding process (table 1).

Parameters of the binding mechanism. The coenzyme NAD$^+$ has a structure which is very similar to the cap: it carries a posi-

<table>
<thead>
<tr>
<th>Compound</th>
<th>Binding</th>
<th>Retardation till fraction</th>
<th>Number of cis-diol groups</th>
<th>Positive charge</th>
<th>Polyanion</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAD$^+$</td>
<td>&gt; 98%</td>
<td>-</td>
<td>2</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>NADH</td>
<td>&gt; 98%</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>m$^7$G</td>
<td>&gt; 98%</td>
<td>-</td>
<td>1</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>NADP$^+$</td>
<td>-</td>
<td>13 - 21</td>
<td>1</td>
<td>+</td>
<td>(+)</td>
</tr>
<tr>
<td>m$^7$GpppAm</td>
<td>-</td>
<td>13 - 21</td>
<td>1</td>
<td>+</td>
<td>(+)</td>
</tr>
<tr>
<td>m$^6$A</td>
<td>-</td>
<td>9 - 12</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>pm$^7$G</td>
<td>-</td>
<td>9 - 12</td>
<td>1</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>pA</td>
<td>-</td>
<td>4 - 5</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>NADPH</td>
<td>-</td>
<td>3</td>
<td>1</td>
<td>-</td>
<td>(+)</td>
</tr>
<tr>
<td>GpppAm</td>
<td>-</td>
<td>3</td>
<td>1</td>
<td>-</td>
<td>(+)</td>
</tr>
<tr>
<td>poly(U)</td>
<td>-</td>
<td>2</td>
<td>1</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>28S rRNA</td>
<td>-</td>
<td>2</td>
<td>1</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

1 - 2.5 $A_{260}$ units (or 10$^6$ cpm in the case of $^3$H-28S rRNA) were applied to the column under standard conditions. Fractions 2 and 3 represent the flow-through, whereas substances eluting between fractions 4 - 25 were classified as retarded. Bound substances only left the column after application of elution buffer. Substances containing more than 2 negative charges are operationally defined as polyanions.
tive charge on the nicotinamide residue, has a 5'-5' diphosphate bridge and two cis-diol groups. We may consider this substance as an analog of the shortest possible capped RNA chain. As expected, NAD$^+$ is fully retained on the column; loss of the positive charge as in the reduced form NADH still allows complete binding probably because both cis-diol groups are available and - as should be stressed - are in close proximity in this small molecule. Even if we lose the second cis-diol group but maintain the positive charge as in m$^7$G we obtain full binding. The presence of additional anionic phosphate groups, however, as in NADP$^+$ prevents binding but allows strong retardation. A single cis-diol group as in m$^6$A is not sufficient for binding but again enough for retardation. This example also shows that it is not the presence of a methyl group but rather the positive charge which induces binding. This is also indicated by the retardation behaviour of 5'pm$^7$G. Here, the positive charge is partially neutralized intramolecularily by the 5' phosphate group. Total loss of the positive charge in 5'AMP (5'pA) further reduces retardation. A single cis-diol group close to negatively charged phosphate as in NADPH practically abolishes retardation. Finally, homopolymeric poly(U) or ribosomal RNA appear in the flow-through and are not retained at all. As expected, the cap analogs m$^7$GpppAm and GpppAm fit into this pattern by showing the same binding properties as their counterparts NADP$^+$ and NADPH, respectively. The agarose used in this study contains boronate groups which are coupled to the matrix via an aromatic linker (I) (4):

\[
\text{Agarose-O-CH}_2\text{-C-}
\begin{array}{c}
\text{NH-} \\
\text{B(OH)$_2$}
\end{array}
\rightleftharpoons \text{R-B(OH)$_2$}
\]

(I)

At alkaline pH the planary boronate group picks up a hydroxyl ion to form a tetrahedral structure according to (II) (8, 16):

\[
\text{R-B(OH)$_2$} \overset{+\text{OH}^-}{\text{pH}>8} \rightleftharpoons \text{[R-OH$^\Theta$]}(X)
\]

(II)

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This compound (X) has a pK of 9.1. It is this tetrahedral structure which is required for efficient cis-diol binding (III):

\[
\begin{align*}
\text{OH} & \quad \text{OH} \\
\text{R} & \quad \text{OH} \\
\end{align*}
\]

\[
\begin{align*}
\text{O} & \quad \text{P} \\
\end{align*}
\]

\[
\begin{align*}
\text{H}_2 & \quad \text{O} \\
\text{C} & \quad \text{O} \\
\text{Base} & \quad \text{OH} \\
\end{align*}
\]

\[
\begin{align*}
\text{OH} & \quad \text{OH} \\
\text{R} & \quad \text{OH} \\
\end{align*}
\]

The observed specificity of binding, however, is not just a simple function of the concentration of cis-diol groups per molecule. The formation of the cyclic diester between the boronate group and the ribose probably is not a one-step reaction. We think that other parameters affect binding. Stacking interaction between the aromatic linker and the 3' terminal nucleotide may help to stabilize the product. But of greater importance in this respect is the presence of a positive charge on the 3' terminal nucleotide as in the case of all mature caps which contain a positively charged m^7G residue. As exemplified by the very strong retention of NADP^+, the presence of this positive charge together with one cis-diol group is sufficient for binding.

Diester formation shifts the pK of the compound (XX) to pH 5.1 (16) thereby drastically stabilizing the tetrahedral boronate structure. This mechanism permits elution of the bound RNAs by a simple pH shift (4).

**Specificity of the binding.** To be useful for the purification of capped RNA two conditions must be fulfilled:

(i) non-capped RNA should not be retained on the column thus eliminating the danger of contamination of the bound RNA, and

(ii) the binding must be dependent on the presence of the 5' cap.

The first point was checked by applying the homopolymer poly(U) as well as ribosomal RNA to the column. Both RNAs did not bind (>98% of A_260 units (poly(U)) or cpm (^3H-rRNA) in flow-through) (table 1).
To demonstrate the second point we used RNA preparations from three different sources, namely from human HeLa cells, from lectin-stimulated bovine lymphocytes, and from chick oviduct cells. When \(^{3}H\)-uridine-labeled RNA from HeLa cells (5-15S gradient cut) was run on the column, 17% (as cpm) were found in the binding fraction. Treatment of the RNA with tobacco acid pyrophosphatase which cleaves the triphosphate bridge of the cap (11) practically abolished the binding (fig. 1). The residual binding (approx. 5% of previously bound cpm) may be due to the presence of tRNA for reasons discussed below. To show that the binding does include all mRNA, we applied poly(A)\(^{+}\)RNA fractions from HeLa cells or oviduct cells to the column. Over 80% were bound. All of the bound material was retained by the matrix when re-applied in a second run. After pyrophosphatase treatment, however, less than 1% was retained (these data not shown). This demonstrates the dependence of the binding on the presence of a 5' cap structure.

Analysis of bound RNA. As already pointed out we expected to find mainly the capped snRNAs and mRNAs being retained on the column.

a. Small nuclear RNA. Total cellular RNA from HeLa cells or lectin-stimulated bovine lymphocytes (16 h labeling with \(^{3}H\)-uridine) was either applied directly or a 5 to 15S cut was taken from a sucrose gradient (fig. 2A). The analysis of the RNA species in the flow-through and eluate reveals the high selec-
Fig. 2. Gel electrophoretic analysis of flow-through and eluate RNA fractions.

a) HeLa 5-15S RNA: Chromatography was performed under standard conditions as described under Methods and shown in fig. 1. Flow-through fractions 2 and 3, 4 and 5, 6-8, and 9-13 were pooled to give the respective lanes FT 1-4; eluate fractions 2 and 3, 4 and 5, 6 and 7 were pooled to give lanes E 1-3, respectively. Lane M shows a marker RNA from HeLa cells.

b) Total bovine lymphocyte RNA: Chromatography was performed using a modified binding buffer (MBB: 50 mM HEPES, pH 8.5; 200 mM NaCl; 10 mM MgCl₂; 20% (v/v) ethanol (7)). Flow-through fractions 2-4 and 5-13 gave lanes FT 1-2; lanes E 1-4 were obtained from eluate fractions 2-5. Elution buffer was identical in a) and b).

Activity of the boronate matrix. The non-capped 5S RNA and most of the tRNA is in the flow-through. Retained by the column are a number of bands which can be correlated to the known positions of snRNAs (17). SnRNAs U₁, U₂ and U₃ are completely retained and snRNA U₅ appears enriched in the eluate. The non-capped cytoplasmic species L₁ and L₂ as well as snRNA U₆ (structure of 5' end not exactly known (18)) are not bound. Close inspection of the gel shows that bands which show up in the flow-through in the region of U₁ and U₂/U₃ migrate just below the bands in the eluate. They may, therefore, represent snRNAs which have lost their capped 5' ends (e.g. U₁*) (17). In addition, a comparison of the fainter bands in fig. 2A (the gel was overloaded to show...
these bands) reveals that bands in the flow-through and the eluate are at different positions in most cases. We may be looking at families of degradation products which have either lost fragments from the 5' (flow-through) or the 3' region (eluate), respectively.

There is some material in the eluate in the tRNA region of the gel. Although part of this material may represent 5' degradation products of capped RNAs, most of it is probably tRNA. It is known that tRNAs carrying the modified base Q (which contains a cis-diol as part of the modification (19)) are bound by boronate matrices (4). Yet another reason, however, seems to be responsible for tRNA binding: we have argued above that the presence of a positive charge (carried by the m^7G) in the vicinity of a cis-diol group causes binding to the matrix. Many tRNAs have a m^7G in position 46 which is relatively close to the 2', 3' cis-diol group due to the compact tertiary structure of tRNA. To test the assumption that the presence of a m^7G causes tRNA binding, we compared the following three tRNA species (a kind gift of Prof. M. Sprinzl, Bayreuth): tRNA^Phe (m^7G, cis-diol) from yeast, 2'deoxy-tRNA^Phe (m^7G only) from yeast, and tRNA^Tyr (no m^7G, cis-diol) from E.coli. 2 A_260 units of the respective tRNAs were applied to the column and the A_260 monitored. No binding was observed in the absence of either a m^7G (tRNA^Tyr) or a cis-diol (2'deoxy-tRNA^Phe). Only the presence of a m^7G together with a cis-diol group (tRNA^Phe) leads to the, however, incomplete (~5%) binding of the tRNA (data not shown). If desired, the contamination of the bound snRNAs by tRNA can be totally avoided by using nuclear RNA as starting material. The binding of tRNA can also be largely eliminated by increasing the stringency of the binding with respect to the presence of caps. This can be achieved by changing the buffer used during application of the sample (7). The use of a buffer containing low salt and 20% ethanol leads to a reduction of material in the tRNA range but also lowers the amount of snRNAs U_2 and U_3 bound (fig. 2B). The most plausible explanation for the latter fact seems to be a conformational inaccessibility of the 5' cis-diol group in U_2/U_3, whereas U_1 is still retained to a high extent probably because its 5' end remains accessible.
We have done all binding reactions at room temperature. It can be expected that at lower temperatures the stringency of the binding reaction will also be lower (7). A few other conditions that we have tried should be mentioned:

(i) denaturing agents: although the matrix material tolerates solvents like DMSO or formamide no useful separations were obtained with these agents. The presence of urea prevented binding of RNA at all;

(ii) sorbitol: the use of sorbitol as a cis-diol competitor was unsuccessful since only 30% of the bound material could be recovered.

b. mRNA. Poly(A)$^+$RNA from HeLa cells, lectin-stimulated bovine lymphocytes, and chick oviduct cells was used in these assays. As pointed out above, we first showed that the binding of these mRNAs was dependent on the 5' cap: material that was bound in a first run could be completely rebound in a second run but lost all binding ability after pyrophosphatase treatment (fig. 1) (11). The RNA which was bound as well as the fraction which was not were then translated in vitro in the rabbit reticulocyte lysate. Although this system will also accept non-capped mRNA it does so with less efficiency and, in addition, both types of mRNA can be distinguished through the inhibition of their translation by cap analoga (15). The translation of capped mRNA, for instance, can be strongly inhibited by the inclusion of 5' m$^7$GTP in the assay, whereas non-capped mRNA is almost insensitive against the competitive effect of this substance (20).

The results of the in vitro translation experiments are given in fig. 3. It was consistently found that the non-bound RNA was a poorer template (1.5 - 2 fold) for protein synthesis but, on the other hand, could not be inhibited by 5' m$^7$GTP (an enzymatically decapped RNA showed an identical behaviour). The translation of the bound fraction under the same conditions was reduced to 40% (fig. 3A). A high precision liquid chromatography analysis (by M. Sprinzl, Bayreuth) showed no caps in the flow-through RNA (after RNAase P1 and bacterial alkaline phosphatase treatment). All these data indicate that capped mRNA is bound by the matrix. It is interesting to note that the highest degree of flow-
through mRNA is found in RNA from lymphocytes. mRNA from these cells has been known to be notoriously less well translatable than e.g. HeLa mRNA (10). The reason may be a higher percentage of non-capped or decapped RNA in lymphocytes. Polyacrylamide gels of translation products did not reveal any difference between bound and non-bound mRNA products (fig. 3B).

COMMENTS

Borionate groups coupled to a matrix support have been used in a number of studies to isolate RNA chains which have a cis-diol configuration in which both OH groups are unmodified. Ordinarily this is the 3' end of the RNA but it may also be a cis-diol located somewhere else in the molecule as in the case of the modified base Q or in a 5' cap structure. The binding is effected by formation of a cyclic diester between the boronate group and the cis-diol. However, these methods did not gain wide acceptance because of the rather low specificity (i.e. high background of other RNA) and the restriction to relatively short RNA chains like tRNA (4) or 5' RNase T1 fragments of mRNA (6). We have eliminated these drawbacks by the combined use of (i) the inert agarose matrix (7), (ii) higher temperature and (iii) a high-salt binding buffer (4, 21). The binding of RNA with only a single cis-diol group is prevented under such conditions. Retention becomes dependent on either the presence of a second cis-diol in close proximity to the first one (as in the small through mRNA is found in RNA from lymphocytes. mRNA from these cells has been known to be notoriously less well translatable than e.g. HeLa mRNA (10). The reason may be a higher percentage of non-capped or decapped RNA in lymphocytes. Polyacrylamide gels of translation products did not reveal any difference between bound and non-bound mRNA products (fig. 3B).

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cap analogs like NADH and possibly also Q-modified tRNA) or on the presence of a positive charge which keeps the cis-diol in the vicinity of the negatively charged boronate group thereby stabilizing RNA binding. The binding therefore seems to be highly specific for fully methylated and hence biologically mature caps. We have shown that the cap-bearing snRNAs as well as mRNA can be successfully purified on the boronate matrix. Although there are limitations to this method (e.g. the high ionic strength of the binding buffer may be problematic with some ligands) it still seems advantageous to the use of matrix-coupled antibodies where the low efficiency of the coupling reaction still limits the capacity of the columns (22).

A number of applications for the boronate matrix are immediately evident: it should be very helpful for various aspects of work with snRNAs and the respective ribonucleoprotein particles. With respect to mRNA it should be possible to determine the kinetics of cap degradation and its role in cytoplasmic mRNA metabolism. For any in vitro translation work it will be useful to select maturely capped mRNA before its use in in vitro protein synthesis. Finally, it should be mentioned that the matrix has also been useful in the isolation of proteins carrying a poly(ADP) ribose modification because of the presence of several closely spaced cis-diol groups (23).

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We are grateful to Profs. Paul Agris and Mathias Sprinzl for helpful discussions and suggestions. We also thank Martin Feyerabend and Klaus Melchers for their help with some of the experiments and Judith Naurath and Klaus Grabert for the preparation of the manuscript. This work was supported by a grant from the Deutsche Forschungsgemeinschaft to Klaus P. Schäfer.

REFERENCES