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Binding of mRNA by an oligopeptide containing an evolutionarily conserved sequence from RNA binding proteins

Martin Schwemmle, Matthias Görlach*, Michael Bader, Thomas F. Sarre and Kurt Hilse

Institut für Biologie III, Universität Freiburg, D-7800 Freiburg, FRG

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Several proteins with an affinity to RNA contain a conserved sequence of 8 amino acids which is postulated as being important for RNA binding. An oligopeptide of 11 amino acids containing this sequence is shown to bind ^{32}P-labelled globin mRNA in a filter binding assay. High concentrations of heparin compete for this binding. 10 other peptides with different sequences do not exhibit affinity to RNA in this assay. These results support the relevance of the conserved peptide sequence in the binding of proteins to RNA.

Ribonucleoprotein; RNA-protein interaction; mRNA

1. INTRODUCTION

RNA is found in eukaryotic cells in association with specific proteins to form ribonucleoprotein (RNP) complexes [1]. The RNA-binding proteins are likely to be involved in RNA biogenesis, metabolism and function [1]. Sequence information obtained from the cloning of RNP proteins revealed a highly conserved octapeptide sequence situated in a domain of roughly 100 amino acids which is reiterated up to four times in single protein species [2]. This conserved octapeptide sequence has been observed in RNA-binding proteins from yeast, plants, insects and mammals and has been named 'RNP1' [12] or 'RNP-CS' [3]. Recent studies on the poly(A)-binding protein of yeast and the mammalian hnRNP protein A1 suggest that this sequence might be essential for RNA binding [2–8].

In this study, we have investigated the binding of globin mRNA to a synthetic oligopeptide of 11 amino acids containing the RNP1 consensus sequence (table 1) from the fourth domain of the yeast poly(A)-binding protein [4], in which cysteine in the highly variable position of the octapeptide sequence was replaced by serine, and tyrosine was added at the COOH-terminus. Our results show that the RNP1 oligopeptide binds to globin mRNA in a heparin-sensitive manner and that oligopeptides with a different amino acid composition, though containing up to four lysine residues, do not exhibit RNA-binding activity.

2. MATERIALS AND METHODS

2.1. *Preparation of peptides*

Peptides were synthesized with a Labortec Peptide Synthesizer by M. Hassauer (Institut für Immunobiologie, Universität Freiburg).

2.2. *Preparation and labelling of purified globin mRNA*

Globin mRNA from rabbit reticulocyte lysate was prepared from salt-washed polysomes by the guanidinium-HCl extraction method and subsequent oligo(dT)-cellulose chromatography according to Aviv and Leder [9]. Further purification was

Correspondence address: K. Hilse, Institut für Biologie III, Schänzlestr. 1, D-7800 Freiburg, FRG

* *Present address:* Department of Biochemistry, Molecular Biology and Cell Biology, Evanston, IL 60208, USA

Abbreviations: NC, nitrocellulose; RNP, ribonucleoprotein; pCp, cytidine 3′,5′-bisphosphate; ssDNA, single-stranded DNA

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performed by gel filtration on a Sephacryl S400 column as described by Safer et al. [10]. 3'-end labelling of mRNA with T4 RNA ligase (Pharmacia) was performed as described by England et al. [11] using 5 μg globin mRNA and 50 μCi [^{32}P]pCp (3000 Ci/mmol; Amersham) in a total reaction volume of 30 μl.

2.3. *Assay for binding of oligopeptides to mRNA*

Peptides were incubated with ^{32}P-labelled globin mRNA (2×10^4 Cerenkov cpm) in binding buffer (10 mM Tris-HCl, pH 7.2, 140 mM KCl, 3 mM $MgCl_2$, 1 mM EDTA) in a total reaction volume of 50 μl for 60 min on ice. For competition experiments the competitor was added after 60 min of incubation and the samples were incubated for a further 20 min. Samples were filtered through a NC membrane (BA 83; Schleicher & Schuell) in a dot-blot apparatus. The NC filter was rinsed immediately twice with 500 μl of binding buffer in the dot-blot apparatus, and subsequently washed three times for 15 min in 50 ml binding buffer at room temperature, air dried and autoradiographed.

3. RESULTS AND DISCUSSION

When ^{32}P-labelled globin mRNA is incubated under physiological ionic conditions, i.e. 140 mM KCl and 2 mM $MgCl_2$, with increasing amounts of the oligopeptide containing the RNP1 consensus sequence (table 1) and subsequently applied to a NC filter, the mRNA is retained on the filter in the presence of a 30–300-fold molar excess of the oligopeptide (fig.1). This result shows that an RNP1 containing peptide has RNA-binding activity and, therefore, might be responsible for the binding of protein fragments of RNP proteins to ssDNA-agarose or poly(ethenoadenylate) [5,6]. In order to test the specificity of the amino acid sequence of the RNP1 oligopeptide for mRNA binding, we performed the binding assay with 10 oligopeptides of different amino acid compositions (table 1) derived from the SV40 large T antigen [12]. As fig.2 shows that none of them is capable of binding mRNA in the range of 30–200-fold molar excess, despite the fact that two of them (peptide 1 and 2) are reported to have affinity to DNA [12]. The retention of RNA on NC filters in the presence of the RNP1 oligopeptide is also not due to its greater affinity to NC compared to the other oligopeptides since amidoblack staining indicates that all peptides are retained in comparable amounts on the filter (data not shown). The reversibility of the binding of RNP1 oligopeptide to labelled globin mRNA is indicated by the ability of unlabelled globin mRNA to compete for this binding when it is added after one hour of incubation (data not shown).

Table 1

Sequences of the peptides used in this study

Peptide	Sequence
1	Ser-Glu-Glu-Met-Pro-Ser-Ser-Asp-Tyr
2	Gln-His-Ser-Thr-Pro-Pro-Lys-Tyr
3	Gln-His-Ser-Thr-Pro-Pro-Lys-Lys-Lys-Arg-Lys-Val
4	Thr-Thr-Lys-Glu-Lys-Ala-Ala-Leu-Leu-Tyr-Lys-Lys-Ile-Met-Glu-Tyr
5	Ile-Ser-Arg-His-Asn-Ser-Tyr
6	Ser-His-Tyr-Lys-Tyr-His-Glu
7	Asp-Val-Lys-Gly-Thr-Gly-Gly-Glu-Ser-Tyr
8	Lys-His-Leu-Asn-Lys-Arg-Thr-Gln-Ile-Tyr
9	Ala-Pro-Gln-Ser-Ser-Gln-Ser-Val-Tyr
10	Lys-Pro-Pro-Thr-Pro-Pro-Glu-Pro-Thr
P	Ser-Lys-Gly-Phe-Gly-Phe-Val-Ser-Phe-Ser-Tyr
	Lys Phe Gly Phe Phe
Consensus	Arg-Gly-Tyr- Val-Tyr-Val- X -Tyr

Peptides 1–10 are derived from the SV40 large T antigen. P indicates the RNP1 oligopeptide derived from the fourth domain of the yeast poly(A)-binding protein and modified as described in the text. Consensus indicates the consensus sequence RNP1. Variable positions are indicated by the two possible amino acids or with 'X' for any amino acid

The minimal size, however, has a certain impact on the RNA-binding capability of the RNP1 oligopeptide. Obviously, more than one molecule of oligopeptide is necessary for binding and/or retention of one molecule of labelled mRNA on the NC filter (fig.1). Furthermore, heparin competes with the oligopeptide in its interaction with mRNA. High concentrations (125-fold molar excess over mRNA) of this polyanion drastically reduce the amount of filter-bound labelled mRNA in the presence of the RNP1 oligopeptide (fig.3). The heparin sensitivity of the binding of the oligopeptide observed here is somewhat in contrast to the data obtaind for hnRNP proteins [13] or the yeast poly(A)-binding protein [14] which has been reported to bind to ssDNA or ribonucleotide homopolymers in a heparin-resistant manner. The lack of surrounding amino acid sequences in the RNP1 oligopeptide is likely to account for this fact.

One of the most striking features of the RNP1 oligopeptide is the positively charged amino acid lysine followed by an alternating sequence of

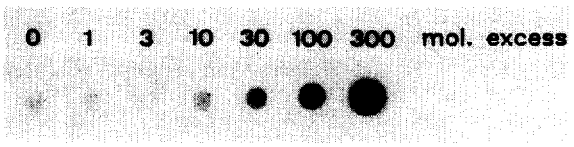

Fig.1. Concentration-dependent binding of RNP1 oligopeptide to globin mRNA. 2×10^4 Cerenkov cpm ^{32}P-labelled globin mRNA (0.025 pmol) were incubated with RNP1 oligopeptide in the indicated molar excess over mRNA in binding buffer for 60 min on ice. The samples were filtered through NC membranes. The autoradiograph of the NC filter is shown.

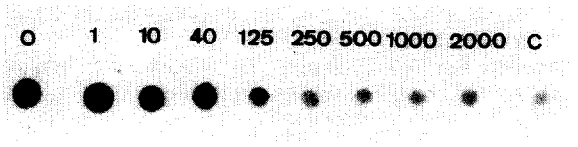

Fig.3. Competition of the binding of RNP1 oligopeptide to globin mRNA by heparin. 2×10^4 Cerenkov cpm ^{32}P-labelled globin mRNA (0.025 pmol) were incubated with 5 pmol (200-fold molar excess) RNP1 oligopeptide in binding buffer for 60 min on ice. Then heparin in the indicated molar excess over mRNA was added and incubation was continued for 20 min. The samples were filtered through NC membranes. The autoradiograph of the NC filter is shown. C, no peptide, no heparin.

aromatic and small amino acids (table 1). It was postulated that this amino acid sequence is capable of RNA binding by two cooperating mechanisms: (i) the ionic interaction between the lysine residue and the phosphate backbone of the RNA; (ii) the intercalation of the aromatic residues between the nucleotide bases which is probably facilitated by the neighbouring small amino acids allowing freedom of movement [15–18]. Basic or aromatic residues which are not in the suitable context, as is the case for the peptides 1–10 (table 1), do not promote RNA binding in contrast to those in the RNP1 oligopeptide (fig.2).

The data presented here support the relevance of the RNP1 consensus sequence for the RNA-binding activity of proteins. It is, however, probably not the only RNA-binding motif of these proteins, since a recent study has shown that its deletion or mutation in the yeast poly(A)-binding protein results only in a retardation but not in an arrest of growth [19], though an in vivo complementation by other proteins for the poly(A)-binding protein deficiency has not been ruled out

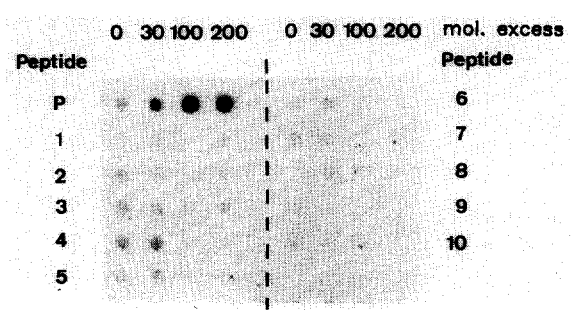

Fig.2. Concentration-dependent binding of 11 oligopeptides to globin mRNA. Symbols correspond to table 1, conditions to fig.1.

in this case. Further experimental work is in progress to elucidate the role of the RNP1 sequence in naturally occurring RNP proteins, e.g. by means of antibodies raised to carrier protein-linked RNP1 oligopeptide.

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