The binding of T4 gene 32 protein to MS2 virus RNA and transfer RNA

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ABSTRACT

Fluorescence titrations, absorption spectroscopy and stopped-flow techniques were used to study the interaction of T4 coded 32-protein (P32) with MS2 RNA and total tRNA from E. coli under different ionic conditions. It is shown that the amount of MS2 RNA and tRNA secondary structure melted by P32 varies markedly and reversibly within a range of ionic conditions under which the binding constant of P32 to single-stranded nucleic acids unable to form stable hairpins remains higher than 10^8 M^-1. Kinetic experiments suggest that P32 dissociates from the MS2 RNA rewinding strand with a similar rate constant as calculated for the dissociation from single-stranded regions. Possible in vivo consequences of these findings are discussed.

INTRODUCTION

Gene 32 protein (P32) induced upon infection of E. coli with phage T4 was the first of the group of proteins which bind tightly to single-stranded DNA to be identified. Protein 32 is required for genetic recombination, DNA replication and DNA repair. It stimulates T4 DNA polymerase in vitro. Protein 32 may also protect single-stranded DNA from nucleolytic attack. All these functions depend on the highly cooperative binding of P32 to single-stranded nucleic acids. As a result of cooperativity bound protein monomers are distributed in clusters rather than randomly on the polynucleotide lattice. Binding cooperativity could also be involved in the regulation of P32 biosynthesis. It has been proposed that the repression of the translation of P32 messenger RNA could be due to the cooperative binding of a few molecules of P32 to an unstructured region of its own messenger RNA. The model assumes that P32 is readily displaced from its messenger RNA on to any available single-stranded DNA.
in order to explain the correlation between the expression of P 32 and the levels of intracellular single-stranded DNA.

It is known that some messenger RNAs have tightly folded conformations as a result of a high degree of base-pairing\textsuperscript{12}. Numerous studies have demonstrated that the RNA genomes of the group of bacteriophages f2-MS2-R17 are highly base paired\textsuperscript{13-15}. Furthermore, the translational sequence of their cistrons depends on the conformation of the molecules since secondary and tertiary structures seriously limit the amount of single-stranded nucleic acid available under physiological conditions\textsuperscript{16}. Physical measurements show that ribosomal RNA is also organized in a series of small helical regions\textsuperscript{17,18}.

We were interested in defining the conditions under which P 32 discriminates between single-stranded DNA and RNAs with palindromic sequences capable of folding into hydrogen-bonded intramolecular double-helical regions. This problem is related to the definition of the conditions for the melting of the double-stranded regions of these RNAs by P 32. For these purposes we have used total transfer RNA (tRNA) from \textit{E. coli} and MS2 viral RNA. MS2 RNA serves both as messenger and genome. It has 3569 nucleotides and 52\% G-C content. The knowledge of the complete nucleotide sequence allowed the proposal of secondary structures based on principles of thermodynamic stability\textsuperscript{19}.

**MATERIALS AND METHODS**

_Preparation of gene 32 protein_  

\textit{Escherichia coli} B was used as non permissive host strain for amber mutant (am A 292) phage infection. \textit{E. coli} CR63 was used as permissive host strain. Both strains were gifts from Dr. Brody.

The procedure for the preparation of P 32 was that of Alberts and Frei\textsuperscript{1}, with an additional step of hydroxylapatite chromatography for further purification. Protein fractions were analyzed on sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis according to Laemmli\textsuperscript{20}. Molecular weights were determined using standard proteins of known molecular weight. Protein 32 migrates as a single band with an apparent molecular weight of 35000 daltons. The ratio \(A_{280}/A_{260}\) was found to be 1.8\textsuperscript{1}. 

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The protein concentration was measured using a molar extinction coefficient at 280 nm of 37000$^{21}$.

**Nucleic Acids**

Calf thymus DNA was obtained from Miles. It was further purified by SDS-chloroform extraction. MS2 virus RNA was from Miles, poly(rA) from PL Biochemicals, and E. coli total transfer RNA from Schwarz Mann.

**Fluorescence measurements**

Fluorescence measurements were carried out with a FICA 55 spectrofluorimeter. The signal from the sample was divided by the signal from a reference cell of rhodamine B which has a quantum efficiency that is essentially constant below 580 nm. Data were collected at 4°C. Solutions of P 32 were contained in 3 ml spectrofluorimeter quartz cells. A 5 mm glass-coated stirring bar was used for mixing P 32 with added nucleic acids or salts.

**Absorption spectra**

Absorption spectra were recorded with Cary 15 and Cary 219 spectrophotometers at 24°C.

**Binding constants**

Apparent association constants for the binding of P 32 to nucleic acids were estimated using the formalism described by Kelly et al.\textsuperscript{7}

\[
K_{\text{app}} = \frac{\theta}{(1-\theta) \left[ N_0 \right] - \theta \left[ P_0 \right]} 
\]

At the stoichiometric point this expression can be written as

\[
K_{\text{app}} = \frac{\theta}{(1-\theta)^2 \left[ P_0 \right]} 
\]

where $K_{\text{app}} = K_c \cdot \omega$, $K_c$ is the direct binding constant to a single-stranded nucleic acid site and $\omega$ is the cooperativity constant; $\theta = \Delta F / \Delta F_\infty$, where $\Delta F$ is the protein fluorescence change at a particular ligand concentration and $\Delta F_\infty$ is the protein fluorescence change at saturating ligand concentration. $\left[ N_0 \right]$ and $\left[ P_0 \right]$ are the total concentrations of nu-
cleotide sites and protein, respectively.

**Kinetic measurements**

Kinetic measurements were carried out with a Durrum stopped-flow spectrophotometer.

**RESULTS**

**Fluorescence titrations**

The fluorescence of P32 is partially quenched upon binding a variety of mono-, oligo-, and polynucleotides. At least one tryptophyl residue is located in the nucleic acid binding site. The quenching of tryptophan fluorescence can be exploited to estimate thermodynamic parameters of the P32-nucleic acid interaction.

We have studied the binding of P32 to several polyribo- and polydeoxyribonucleotides in the following buffers. Buffer I: 10 mM NaCl, 10 mM NaCacodylate, 0.2 mM EDTA, pH 7.6. Buffer II: 110 mM NaCl, 10 mM NaCacodylate, 1 mM MgCl₂, pH 7.6. Buffer III: 110 mM NaCl, 10 mM NaCacodylate, 3 mM MgCl₂, pH 7.6.

Figure 1 shows the quenching of the intrinsic tryptophan fluorescence in buffers I and III as a function of increasing concentrations of denatured DNA and poly(rA). The apparent binding constant \( K_{\text{app}} \) and the size of the polynucleotide binding site \( n \) could be estimated from these binding isotherms in the way described under Materials and Methods. The values of \( K_{\text{app}} \) were similar for both polynucleotides and higher than \( 10^8 \text{ M}^{-1} \) (Table I). The extrapolation of the initial part of the curve gave \( n = 5 \pm 1 \) nucleotides.

In the buffer of low ionic strength (buffer I) the binding constant is probably much higher than can be determined from the fluorescence quenching curve. The curvature observed around the stoichiometric point in the quenching curve might be due to imperfections in the investigated system (impurities in the protein preparation, polynucleotides of different chain lengths...). Only lower limit could therefore be estimated for the association constants (see Table 1).

Figure 2 shows the binding of P32 to MS2 RNA. In buffer I the es-
Figure 1. Fluorescence titration curves for the binding of poly(rA) and heat denatured calf thymus DNA to P 32. Curve 1, titration with poly(rA) in buffer III (110 mM NaCl, 10 mM NaCacodylate, 3 mM MgCl₂, pH 7.6); curve 2, titration with poly(rA) in buffer I (10 mM NaCl, 10 mM NaCacodylate, 0.2 mM EDTA, pH 7.6); curve 3, titration with denatured DNA in buffer III; curve 4, titration with denatured DNA in buffer I. Protein concentration 2.4 x 10⁻⁷ M. Temperature, 4°C.

Estimated values of $K_{app}$ and $n$ are $1.2 \times 10^8$ M⁻¹ and 4.5 nucleotides. These values are similar to those found for denatured DNA and poly(rA) in buffer III. The binding of P 32 to MS2 RNA in buffers II and III is markedly weakened. The quenching of P 32 fluorescence at the stoichiometric point (i.e., where $N_o = P_o$) in buffers I, II and III is, respectively, 27 %, 4 % and 2 % (figure 2). It is possible to calculate $K_{app}$ for the binding in buffers II and III under the assumption that the protein fluorescence change at saturating ligand concentration and the site size are the same in all buffers. Taking into account only the beginning of the titration curves.
Figure 2. A, fluorescence titrations curves for the binding of MS2 RNA to P 32. Curve 1, titration in buffer I (10 mM NaCl, 10 mM NaCacodylate, 0.2 mM EDTA, pH 7.6); curve 2, titration in buffer II (110 mM NaCl, 10 mM NaCacodylate, 1 mM MgCl₂, pH 7.6); curve 3, titration in buffer III (110 mM NaCl, 10 mM NaCacodylate, 3 mM MgCl₂, pH 7.6). B, competition experiments with denatured DNA. Protein concentration, 2.4 x 10⁻⁷ M. Temperature, 4°C.

of figure 2, the estimated values of $K_{app}$ are 6 x 10⁻⁵ M⁻¹ in buffer II and 1.2 x 10⁻⁵ M⁻¹ in buffer III. In the calculation of $K_{app}$, the concentration of polynucleotide is measured as that of binding sites of 5 nucleotides in length. These values of $K_{app}$ are necessarily very approximate since they assume that every phosphate is the start of a potential binding site. Nevertheless, they serve the purpose of comparing the binding strength of P 32 to MS2 RNA and tRNA molecules in buffers I, II and III.

To assess that the lower efficiency of MS2 RNA in quenching P 32 fluorescence in buffers II and III arises as a consequence of a lower binding strength, competition experiments were carried out between MS2 RNA and denatured DNA for the binding of P 32. Addition of denatured DNA (or poly(A), data not shown) to a complex of P 32 and MS2 RNA in buffers II or III leads to a quenching of P 32 fluorescence down to the values observed in the absence of MS2 RNA (figure 2). On the contrary, addition of MS2 RNA to a saturated complex of P 32 and denatured DNA did not modi-
fy the measured fluorescence change.

Figure 3 shows the binding isotherms of P32 to total tRNA from *E. coli*. In buffer I the estimated values of $K_{\text{app}}$ and $n$ are $1.2 \times 10^8 \text{ M}^{-1}$ and 5.3 nucleotides. In buffer III the value of $K_{\text{app}}$ is lowered by more than 2 orders of magnitude ($K_{\text{app}} \sim 10^6 \text{ M}^{-1}$). Thus, under these ionic conditions tRNA behaves similarly to MS2 RNA. The binding isotherms obtained in buffers II and III for the binding of P32 to MS2 RNA and tRNA can be reproduced starting with systems at equilibrium in buffer I by addition of salts.

The values of $K_{\text{app}}$ for the binding of P32 to MS2 RNA and tRNA are similar to the values for non-cooperative binding determined by Kelly *et al.* They reported values of $4.3 \times 10^5 \text{ M}^{-1}$ for the binding of $d(pA)_4$ (in 50 mM Na$_2$HPO$_4$, 1 mM Na$_2$EDTA, 1 mM $\beta$-mercaptoethanol, pH 7.7) and $0.8 \times 10^4 \text{ M}^{-1}$ for the binding of native calf thymus DNA (in 50 mM NaCl, 1 mM Na$_2$HPO$_4$, 0.1 mM Na$_2$EDTA, pH 7.7). This suggests that under physiological salt concentrations (buffers II and III) the measured value of $K_{\text{app}}$ should be largely dominated by the intrinsic binding constant for an isolated site either single or double-stranded.

![Fluorescence titration curves for the binding of total tRNA from *E. coli* to P32. Curve 1, titration in buffer I (10 mM NaCl, 10 mM NaCacodylate, 0.2 mM EDTA, pH 7.6); curve 2, titration in buffer III (110 mM NaCl, 10 mM NaCacodylate, 3 mM MgCl$_2$, pH 7.6). Protein concentration, $2.4 \times 10^{-7}$ M. Temperature, 4°C.]
Ultraviolet difference spectra

MS2 RNA shows an appreciable hyperchormicity at high temperature relative to its absorbance spectrum at room temperature. The hyperchormicity obtained by heat denaturation at 95°C in the three reference buffers is 26 ± 1% of the initial absorbance. This indicates that, in the absence of P 32, MS2 RNA has roughly the same amount of hydrogen-bonded base pairs in the three buffers. The observed value for the hyperchromicity agrees well with the amount of secondary structure assumed for MS2 RNA (73 % ± 5 base pairs \(^\text{19,33}\)). In the presence of magnesium ions (buffers II and III) a small proportion of tertiary hydrogen bonding could lead to more compact conformations for the molecule which would occlude many potential binding sites without a marked change in the absorption spectrum\(^\text{19}\).

MS2 RNA also shows an hyperchromic effect upon interaction with P 32. Figure 4 shows the difference spectra for complexes of P 32 and MS2 RNA, that is the spectra of P 32 - MS2 RNA complexes minus those of the separate components under the same conditions. In buffer I the hyperchromic effect shown by MS2 RNA upon binding at a ratio of protein

\[ \frac{P}{RNA} = 0.015 \]

\[ \frac{P}{RNA} = 0.010 \]

\[ \frac{P}{RNA} = 0.005 \]

\[ \lambda (\text{nm}) \]

\[ \Delta OD \]

Figure 4. Difference spectra of MS2 RNA - P 32 complexes. Input MS2 RNA, 7.16 \(\times\) 10\(^{-6}\) M; P 32, 1.47 \(\times\) 10\(^{-6}\) M. Curve 1, difference spectrum in buffer I (10 mM NaCl, 10 mM NaCacodylate, 0.2 mM EDTA, pH 7.6); curve II, difference spectrum in buffer II (110 mM NaCl, 10 mM NaCacodylate, 1 mM MgCl\(_2\), pH 7.6); curve 3, difference spectrum in buffer III (110 mM NaCl, 10 mM NaCacodylate, 3 mM MgCl\(_2\), pH 7.6). Each difference spectrum was obtained with tandem cells at 24°C.
Table I. Apparent binding constants and stoichiometry for the association of polynucleotides with P 32. Binding was measured in the buffers I, II and III (see RESULTS).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Buffer</th>
<th>$K_{app}$ M$^{-1}$</th>
<th>Stoichiometry (nucleotides/protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>poly (rA)</td>
<td>I</td>
<td>&gt; 1.8x10$^8$</td>
<td>4.5</td>
</tr>
<tr>
<td>denatured DNA</td>
<td>I</td>
<td>&gt; 2.8x10$^8$</td>
<td>6</td>
</tr>
<tr>
<td>MS2 RNA</td>
<td>I</td>
<td>~1.8.10$^8$</td>
<td>4.5</td>
</tr>
<tr>
<td>t-RNA</td>
<td>I</td>
<td>~1.2x10$^8$</td>
<td>5.3</td>
</tr>
<tr>
<td>MS2 RNA</td>
<td>II</td>
<td>6.0x10$^5$</td>
<td>-</td>
</tr>
<tr>
<td>poly(rA)</td>
<td>III</td>
<td>1.2x10$^8$</td>
<td>4.5</td>
</tr>
<tr>
<td>denatured DNA</td>
<td>III</td>
<td>1.4x10$^8$</td>
<td>5.7</td>
</tr>
<tr>
<td>MS2 RNA</td>
<td>III</td>
<td>~1.2x10$^5$</td>
<td>-</td>
</tr>
<tr>
<td>t-RNA</td>
<td>III</td>
<td>~1.0x10$^6$</td>
<td>-</td>
</tr>
</tbody>
</table>

to nucleic acid binding sites of 1.03 (equivalent to a fractional saturation of nucleic acid binding sites of 0.95) is 23 % of the initial absorbance. This effect is about 89 % of the hyperchromicity obtained by heat denaturation at 95 °C (26 % of the initial absorbance). In buffer II the hyperchromicity decreases down to 12 % whereas in buffer III it is only 3 %. In agreement with fluorescence quenching data, addition of salts to a complex at equilibrium in buffer I restores the percentages for hyperchromicity observed in buffers II and III.

The correlation between low values for the hyperchromicity and low values of $K_{app}$ in buffers II and III supports the idea that under physiological salt concentrations, sites for cooperative binding on MS2 RNA represent a very small proportion of the total number of sites.

Kinetics of MS2 RNA renaturation in presence of P 32

The hypochromic effect observed upon increasing the ionic strength of a complex of MS2 RNA with P 32 in buffer I has been exploited to follow the renaturation of MS2 RNA in the presence of P 32 under physiological salt conditions. We have used a stopped-flow apparatus for the purpose of this experiment. One syringe contained a complex at equilibrium in buffer...
I, \( \bar{\theta} = 0.89 \) (where \( \bar{\theta} \) is the fraction of polymer binding sites occupied by the ligand at equilibrium). The other syringe contained buffer III at twice its concentration. Upon mixing, the transmission at 260 nm increased as a result of base stacking. A logarithmic plot of the signal change versus time yielded a straight line, indicating that this process could be described by a single relaxation time (Figure 5). We have found a value of \( 1/\tau = 0.14 \) sec\(^{-1} \). A four-fold change in the concentration of the solution containing the complex did not change the value of \( 1/\tau \). This result excludes any significant contribution from intermolecular hybridization to the hypochromic change.

It is not possible to propose a pathway for the renaturation of MS2 RNA in the presence of P 32 based solely on the present kinetic data. Nevertheless, the hypochromic effect which reflects base stacking should be

![Figure 5](image)

**Figure 5.** A, change of transmission of MS2 RNA complexed with P 32 after an ionic strength perturbation. Initial concentration of MS2 RNA, 1.11 x 10\(^{-5}\) M; P32, 2.02 x 10\(^{-6}\) M. Initial ionic conditions: 10 mM NaCl, 10 mM Na cacodylate, 0.2 mM EDTA, pH 7.6. Final ionic conditions: 115 mM NaCl, 15 mM Na cacodylate, 3 mM MgCl\(_2\), 0.1 mM EDTA, pH 7.6. Temperature 20°C. B, logarithm of the transmission change (arbitrary units) versus time.
limited by the protein dissociation since it has been shown that cooperatively bound P 32 fully unstacks the bases. If we assume that the rate limiting step of MS2 RNA renaturation is the dissociation of P 32, 1/\( \phi \) can be related to the kinetic dissociation constant, \( k_D \), of the protein by means of the relationship derived by Peterman and Wu from Schwarz's theory of cooperative binding,

\[
1/\phi = \frac{c_{\text{uaa}} 2k_D}{c_a} 
\]

where \( c_{\text{uaa}} \) is the equilibrium concentration of segments of bound ligands with nearest neighbor interaction and \( c_a \) is the concentration of bound ligand. The value of \( c_{\text{uaa}} \) can be calculated using statistical mechanics theory. According to Schwarz, in the case of strong cooperativity

\[
\bar{c}_{\text{uaa}} = \left[ \frac{\omega (1 - \theta)}{\omega} \right]^{1/2} c^o_B 
\]

where \( c^o_B \) is the total concentration of binding sites on the polynucleotide.

Inserting equation (4) into equation (3), the expression for the relaxation time becomes

\[
1/\phi = 2 k_D \sqrt{(1 - \bar{\theta}) / \bar{\theta} \omega} 
\]

where \( \omega \) is the cooperativity constant determined by Kelly et al (\( \omega = 10^3 \)). \( \bar{\theta} \) is calculated from the equilibrium constant (table I). Equation (4) assumes that all binding sites on the polynucleotide are equivalent. In our case the polynucleotide chain folds back on itself to form a series of double helical regions with different conformational free energies. Even if in buffer I most of the secondary structure of MS2 RNA is melted by excess P32, at low and medium saturation we do not know to what extent the distribution of conformational free energies could effect the number of protein clusters and hence the rate of protein dissociation. This effect should be very small for nearly saturated complexes.

We have found a value of \( k_D = 6.2 \text{ sec}^{-1} \). This value coincides, within the limits of experimental precision, with that obtained by Suau et al for the cooperative dissociation of P 32 from its complex with single-stran-
ded nucleic acids. Peterman and Wu reported a value of 27 sec\(^{-1}\) for the dissociation of P 32 from single-stranded DNA in 0.6 M NaCl. Thus, it seems that bound P 32 is displaced from the RNA rewinding strand with a rate constant which does not essentially differ from its dissociation rate constant from single-stranded nucleic acids unable to form stable hairpins.

DISCUSSION

We have shown that at relatively low ionic strength (20 mM) P 32 is capable of melting most of the secondary structure of MS2 RNA and tRNA. Under such ionic conditions the binding of P 32 seems to be nonspecific with respect to base composition and sequence and also with respect to whether the nucleic acid contains ribo- or deoxyribonucleotides. The apparent association constant for the binding of P 32 to denatured DNA, poly(rA), MS2 RNA and tRNA is higher than 10\(^8\) M\(^{-1}\). Under physiological salt concentrations the affinity of P 32 for denatured DNA and poly(rA) still remains higher than 10\(^8\) M\(^{-1}\). On the contrary, the binding of P 32 to MS2 RNA is lowered by a factor of 1.5x10\(^3\) and to tRNA by a factor of 1.2x10\(^2\) when going from buffer I to buffer III. At the same time the hyperchromic effect obtained upon binding becomes smaller. The low value of \(K_{\text{app}}\) and of the hyperchromicity under physiological salt concentrations indicates that the cooperative binding of P 32 is hindered by the presence of stable double helical regions. This must be a consequence of the characteristic nucleotide sequence of natural RNAs. Because of the large palindromic complementarity along the single strand, the RNA chain is capable of folding back on itself and form stable secondary structures which do not bind P 32 cooperatively.

The intrinsic constant for the binding of P 32 to an isolated single-stranded binding site (\(K_{c}\)) is only one or two orders of magnitude higher than the binding constant for an isolated double-stranded binding site (\(K_{h}\)). On the basis of such a difference in the intrinsic binding constants, P 32 would not be very active as a melting protein for RNA structures\(^{22}\). However, P 32 binding to double-stranded regions is not cooperative whereas the binding to single-stranded nucleic acids is highly cooperative (\(\omega = 10^3\)).
Thus, the factor that controls the binding to single stranded regions is the product $K_c \omega$. It should be noted that P32 does not force open a double stranded structure but rather traps transiently opened single strands which appear as a result of structural fluctuations. For cooperative binding, with $\omega = 10^3$, and under conditions of tight binding to single strands, i.e., in the range of ionic conditions defined by buffers I and III, we have $(\Delta G^*)_{\text{coop}} = -RT \ln(\omega)$, which defines the free energy change involved in the transfer of a protein molecule from an independent binding site to a contiguous binding site. Thus, double-stranded regions with net thermodynamic stability greater than $\sim -4$ kcal/mole will be left intact whereas less stable regions will be melted by the growing clusters of P32. A very small change of stability of the double-stranded regions around $-4$ kcal/mole (induced, e.g., by a change of the ionic environment) can bring about a large change in $K_{\text{app}}$ through providing or removing sites for cooperative binding. The situation can be more complicated if the melting of a hairpin structure creates more than one binding site for P32.

The conformational free energy of a RNA can be subdivided into two terms: a favorable term from helix regions and an unfavorable term from the loop regions (hairpins, internal loops and bulges). Considering that the free energies are added over the size of the binding site (5 base pairs), the unfavorable contribution from loops can be very important (see Gralla and Crothers). Even in the absence of loops, P32 is capable of melting A-T rich regions that are relatively less stable. We have shown that in buffer I most of the secondary structure of MS2 RNA and tRNA is melted by P32. On the other hand, in buffer III the secondary structure of MS2 RNA and tRNA remains essentially unaltered in presence of P32. Inside the interval of ionic conditions defined by buffers I and III, the secondary structure is melted in part. Thus, buffers I and III roughly define a set of ionic conditions for either minimal or maximal structuration of MS2 RNA and tRNA in the presence of P32.

The denaturation of MS2 RNA and tRNA by P32 in buffer I is reversible for the original absorbance at 260 nm and the low values of $K_{\text{app}}$ can...
be restored by addition of NaCl and MgCl₂ up to the concentrations of buffers II and III. Thus, once the bases are in register, P 32 does not interfere with base-stacking and helix formation provided the stability of the newly formed double-helical regions is higher than approximately -4 kcal/mole. The increase of fluorescence intensity indicates that P 32 has been displaced from the renaturing regions.

We have exploited the hypochromic change at 260 nm accompanying base-stacking to follow the kinetics of the renaturation of MS2 RNA in presence of P 32. Under these conditions the formation of hairpin helices is characterized by a rate constant of 6.2 sec⁻¹, whereas Porschke ²⁹ reported 2x10⁴ sec⁻¹ as rate constant for the formation of a hairpin helix from (Ap)₆(Cp)₆(Up)₅U. Our observed rate of helix formation is rather low. However, it is compatible with a process limited by the rate of cooperative protein dissociation ²⁶. The dissociation of P 32 from rewinding DNA strands could be characterized by the same value of the kinetic dissociation constant as found for the dissociation of P 32 from single-stranded polynucleotides unable to form stable secondary structures.

Our results are compatible with the translational model for the regulation of P 32 synthesis proposed by Russel et al ¹⁹. The fact that cellular RNAs under physiological conditions have a fraction of their secondary structure with a net thermodynamic stability not much greater than -4 kcal/mole points towards the convenience of a precise regulation of the synthesis of P 32 in order to prevent excess levels that could be potentially dangerous.

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