CALORIMETRIC STUDIES ON THE INTERACTION OF RNA AND COAT PROTEIN OF ALFALFA MOSAIC VIRUS

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1. Introduction

Calorimetric analysis of protein nucleic acid interactions provides the most direct approach to quantitatively characterize the energetics involved in these reactions. The lack of any general picture of the nature of the interactions, at least partially, stems from the scarcity of thermodynamic data presently available. However, in view of the general importance of an understanding of the forces governing complex formation between nucleic acids and proteins, it appears to be necessary to furnish thermodynamic parameters, which might lead to a rationalization of the principles pertinent to the process.

Therefore calorimetric studies on the interaction between RNA 1 and the coat protein of alfalfa mosaic virus, have been performed, since the virus is extensively characterized [1–3] with respect to other aspects of physical chemistry and virology and is representative of the large group of viruses with tripartite genomes. The single stranded RNA used in these investigations is RNA 1, the largest part of the genome of the virus. It consists of 3250 nucleotides with mol. wt 1.04×10^6 [3]. The molecular weight of the chemical subunit of the coat protein is 24 280 [4].

2. Materials and methods

Isolation of the alfalfa mosaic virus strain 425 as well as preparation and purification of the protein and the RNA followed published procedures [3,5,6]. The RNA was stored at −20°C in a buffer containing 10 mM NaH_2PO_4, 1 mM EDTA, 1 mM NaN_3, pH 7.0. This buffer was also employed for the calorimetric measurements. The protein was dissolved in water and was stored frozen at −20°C until use. Concentrations of the RNA (anion) and the protein were determined using extinction coefficients of $A^{0.1\%}_{1\,cm}$ 26.3 at 260 nm and $A^{0.1\%}_{1\,cm}$ 0.7 at 280 nm, respectively [3]. Calorimetric measurements were performed with a recently developed flow microcalorimeter [7].

3. Results and discussion

The results of the calorimetric experiments have been compiled in table 1.

The enthalpy, ΔH_B, of binding alfalfa mosaic virus protein to RNA 1, has been calculated per mol nucleic acid rather than per mol protein bound, since within the range of molar ratios of protein to RNA investigated, the enthalpy of interaction does not

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Table 1

Number of measurements	[RNA 1] (μM)	Mol RNA 1/ measurement $\times 10^{10}$	[Protein] / [RNA 1]	ΔQ RNA 1 dilution (mcal)	ΔQ Reaction (mcal)	ΔH_B (kcal (KJ), mol RNA 1^{-1})
3	0.7764	1.16452		−0.395 ± 0.014		
9	0.7764	1.16452	6		−0.346 ± 0.010	408 ± 83 (1707 ± 347)
5	0.7495	1.12424		−0.386 ± 0.019		
7	0.7495	1.12424	25		−0.386 ± 0.019	469 ± 89 (1962 ± 372)

exhibit significant changes. Although the heats of dilution of the RNA, which comprise the heats of dilution of the buffer, and the heats of reaction have been determined with a precision better than ± 5% and ± 3%, respectively, the ΔH_B values corresponding to the difference of these measurements, show a standard deviation of approx. 20%, as given in the last column. Protein dilution resulted in no detectable heat effect. The results of these studies are the positive enthalpies of 408 ± 83 kcal/mol RNA 1, when protein associates with RNA 1 in a molar ratio of 6 : 1, and of 469 ± 89 kcal/mol RNA 1, when 25 mol protein were added per mol RNA 1. It is reasonable to assume that the degree of complex formation between protein and RNA increases with increasing numbers of protein molecules since virus like particles are formed at neutral pH and low ionic strength when a sufficient amount of protein has been added [8]. It has been shown that after addition of about 15 molecules of radioactively labeled protein per molecule of RNA 1 virtually all label can be found in a fast sedimenting complex [9]. Furthermore it is well known that RNAs of alfalfa mosaic virus are able to withdraw protein subunits from the regularly built coat of particles [5]. A few coat protein molecules, however, attached to the RNA abolish its ability to uncoat intact particles [9,10]. These experimental results are suggestive of the idea that a few high affinity sites on the RNA are responsible for the specific interaction between RNA and protein. These sites supposedly are involved in the function of the protein to initiate infection, since without coat protein the genome RNAs of alfalfa mosaic virus though having messenger polarity are not infectious [9,10].

The close similarity of the enthalpies determined for binding of 6 or 25 protein molecules per RNA molecule is also consistent with the idea of the saturation of a few specific sites for protein on the RNA. However, the ΔH-values cannot serve yet to derive the enthalpy/mol protein, due to the indeterminacy with respect to the exact number of proteins sufficient to absorb the saturation enthalpy. An extension of the experiments to molar ratios of protein/RNA 1 below 6 : 1 will enable us to determine the stoichiometry of the reaction and thus to identify the enthalpy of interaction per mol protein.

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