PHYSICAL PARAMETERS OF *ESCHERICHIA COLI* TRANSLATIONAL INITIATION FACTOR 3 BINDING TO POLY(A)

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Received 4 January 1981; revised version received 16 March 1981

1. Introduction

In procaryotes, the binding of mRNA to 30 S ribosomal subunits requires initiation factor 3 (IF3) protein [1–3]. Both IF3 and the ribosome binding regions of mRNA appear to bind to the same domain of the 30 S ribosomal subunit, in the cleft between the head and the platform [4–7]. IF3 is thought to function either indirectly, by altering 30 S subunit conformation so as to favor mRNA:rRNA base pairing [8], or directly, by denaturing mRNA ribosome binding sites to single strands [9], which could catalyze mRNA:rRNA base pairing.

Study of the protein–nucleic acid interactions of IF3 may be of value in elucidating its function. We have reported strong IF3 binding to single-stranded polynucleotides, along with AUG-specific binding to oligonucleotides [9]. IF3 binds to single-stranded polynucleotides with a stoichiometry of 14 ± 1 nucleotides/IF3 [10], and IF3 binding reduces the circular dichroism (CD) of single-stranded polynucleotides similarly to thermal melting [11]. Here, IF3 titration of poly(A) CD at 25°C in a pH 7.5 buffer with 17 mM Na^+ is reported, and analyzed according to equation for ligands binding cooperatively to overlapping sites on a lattice in [12]. An intrinsic binding constant, K, of $(1.3 \pm 0.8) \times 10^6$ M^{-1}, and a cooperativity constant, ω, of 25 ± 7 were calculated by a nonlinear least-squares fit to Scatchard plot data. IF3 titration of poly(A) CD showed a strong salt dependence.

2. Materials and methods

IF3 was prepared as in [10,11], then purified to apparent homogeneity by an additional phosphocellulose column purification with a 0.1–1.0 M KCl gradient in buffer A (10 mM Na_2HPO_4, pH 7.5, 1 mM dithiothreitol, 0.1 mM EDTA, 5% glycerol). Poly(A) was purchased from Sigma. CD measurements were carried out on a Jasco J-500C spectropolarimeter in a 1.0 ml, 1.0 cm pathlength thermostatted cell. CD measurements were made on 4.85 μM poly(A) in buffer A at 25°C, to which was added 1.0 μl aliquots of 32.8 μM IF3. For NaCl back-titration, 1.0 μl aliquots of 5.0 M NaCl were added to the above solution after the addition of 20 μl IF3. All measurements were corrected for dilution. No evidence of light scattering was observed. To prepare a Scatchard plot [13] of the CD titration data, binding densities, ν, and free IF3 concentrations, L, were calculated for each point by assuming a site size of 14 nucleotides/IF3 [10,11]. Binding densities were calculated in terms of IF3 molecules/nucleotide. Scatchard plot data were then fit to eq. (15) in [12] by means of a nonlinear least-squares multiparameter curve fitting program, LINCVX, which was purchased from Scientific Programmers (Bethlehem, PA).

3. Results

Titration of the CD of poly(A) at its peak at 263 nm by IF3 at 25°C is shown in fig.1. The titration converges to the same endpoint, 7.5 $M^{-1} \cdot cm^{-1}$, as seen at 6°C in [11], and corresponds in magnitude and spectral shape to that seen at 57°C in the absence of IF3 [14]. Extrapolation of the downward slope of the initial portion of the titration plot to this endpoint value yields a stoichiometry of 14 nucleotides/IF3, in agreement with filter assay measurements at 0°C [10] and CD measurements at 6°C [11].

If one assumes that reduction of CD from the

Elsevier/North-Holland Biomedical Press

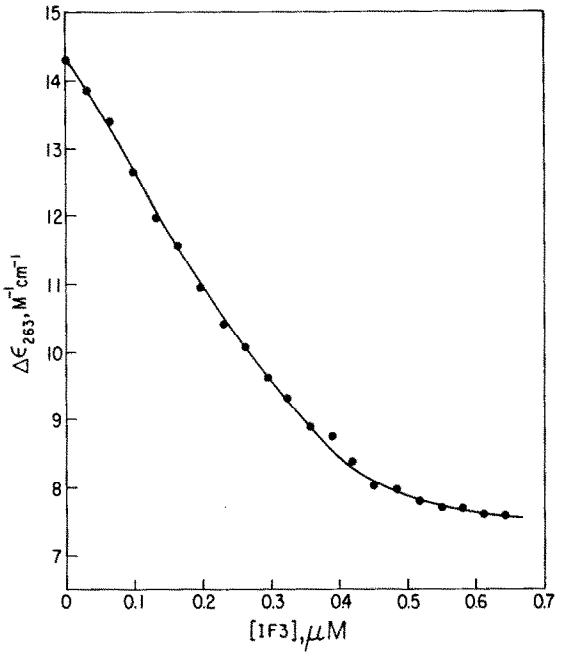

Fig.1. Titration of poly(A) CD by IF3. Poly(A), 4.85 μM at 25°C, was titrated with IF3, as in section 2, and the CD at 263 nm was measured following each addition.

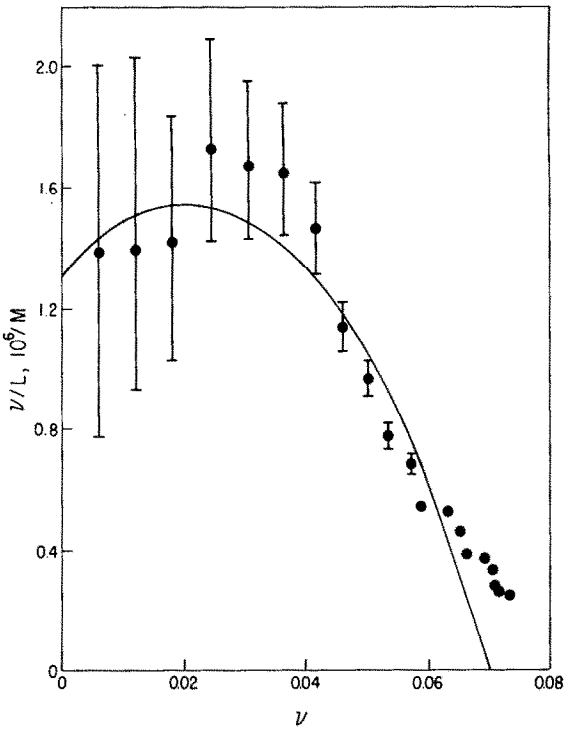

Fig.2. Scatchard plot of IF3 binding to poly(A). Data points from fig.1 were used to calculate binding densities and free IF3 concentrations as in section 2. The solid line is the theoretical curve derived from a nonlinear least squares fit of the data to eq. (15) in [12].

maximum value in the absence of IF3 down to the endpoint value is proportional to IF3 binding to poly(A), and that IF3 binding stoichiometry, n, is 14 nucleotides/IF3, one may calculate the binding density, ν, the free IF3 concentration, L, and the ratio ν/L for each experimental point in fig.1. These data are shown in fig.2 as a Scatchard plot [13]. The points in fig.2 show significant scatter for low values of ν, but do not appear to lie on a straight line. Since such a result suggests cooperativity in the binding of IF3 to poly(A), an attempt was made to fit the experimental data to the equation for cooperatively interacting ligands binding to overlapping sites on a linear lattice [12]. The latter theory assumes an infinite lattice; in the case of a real finite lattice, such as poly(A), ν/L values at high ν curve away from the theoretical plot [15], as may be seen in fig.2. Iterative variation of values of the intrinsic binding constant, K, and the cooperativity constant, ω, by use of a nonlinear least squares multiparameter curve-fitting program, converged to a solution where $K = (1.3 \pm 0.8) \times 10^6\ M^{-1}$, and $\omega = 25 \pm 7$.

The salt dependence of IF3 binding to poly(A) was briefly examined by adding aliquots of NaCl to a solution of poly(A) saturated to excess with IF3, as

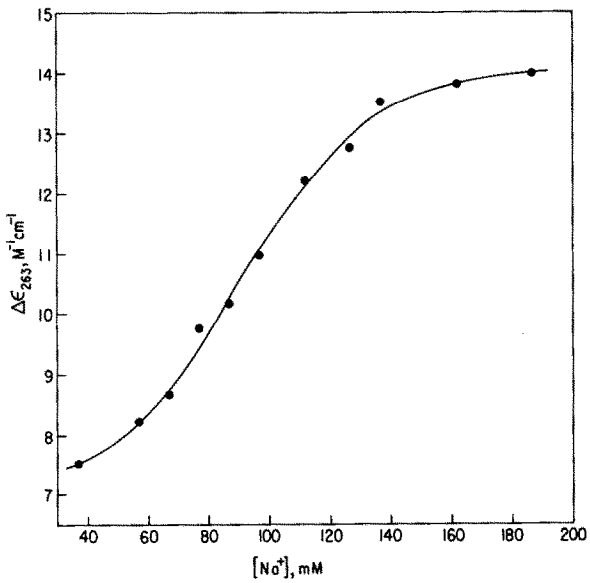

Fig.3. Back-titration of IF3-saturated poly(A) by addition of NaCl. CD of the endpoint solution in fig.1 was measured for successive additions of NaCl as in section 2.

shown in fig.3. It is clear that the ability of IF3 to reduce the CD of poly(A) is much less at physiological ionic strengths than in the low ionic strength conditions of fig.1.

4. Discussion

It would appear that IF3 binding to poly(A) is similar to S1 site II binding to poly(C) at 0.12 M Na^+ [16], *Artemia salina* HD40 protein binding to poly(A) or poly(C) at 50 mM Na^+ [17], and calf thymus UP1 protein binding to poly(A) at 1 mM Na^+ [18].

Multiple binding of IF3 to a single-stranded polynucleotide is not a physiological reaction, but it does provide some insight for the physiological case. The relatively low cooperativity constant, close to that of S1 [16], probably reflects some of the protein–protein interactions which occur when IF3 binds to the 30 S ribosomal subunit. By contrast, the bacteriophage T4 gene 32 protein, which is observed to function by multiple, cooperative binding to single-stranded polynucleotides, has a cooperativity constant of 10^3 [19].

The intrinsic binding constant of IF3 is reasonable in comparison with analogous proteins. However, the strong inhibition of IF3 denaturation of poly(A) at higher ionic strengths suggests that the binding to poly(A) is primarily ionic, and probably not sequence-specific, in agreement with [10,11]. While the product $K\omega = 3 \times 10^7\ M^{-1}$ observed at 17 mM Na^+ is in the same range as that for IF3 binding to 30 S ribosomal subunits at physiological ionic strengths [20–22], $K\omega$ at 0.1–0.2 M Na^+ would certainly be much lower. Similar experiments with naturally occurring sequences from 16 S rRNA and mRNA are the logical next step.

Acknowledgements

I thank R. Weslie Tyson for his aid in preparing IF3. This work was supported by US National Institutes of Health grants GM 23248, GM 24128, and GM 27462.

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