THE RATE OF BINDING OF INITIATION FACTOR 3 TO THE 30 S RIBOSOMAL SUBUNIT OF *ESCHERICHIA COLI*

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1. Introduction

Various workers have studied the rates of association and dissociation of 30 S and 50 S ribosomal subunits using stopped-flow [1,2] and pressure-jump [3,4] techniques. The association equilibrium between these two is affected by initiation factor 3 (IF3) and an attempt has been made [4] to find out what effect the factor has on the corresponding rates. The method of detection has usually been light-scattering, which is however too insensitive to monitor the reaction between IF3 (mol. wt 20 600) and ribosomal particles (mol. wt $1-3 \times 10^6$). Notwithstanding, the rate of association of IF3 with the 30 S ribosomal subunit is of interest both because this reaction is part of the initiation sequence [5] and because it may yield mechanistic information.

We have prepared the fluorescent derivative dansyl-IF3; its preparation and biochemical characterisation will be detailed [6]. The fluorescence signal of the dansyl group responds to the binding of the labelled factor to the 30 S subunit and was therefore employed to investigate the rate of this association reaction. Here we present evidence that the reaction is diffusion controlled.

2. Experimental and results

Measurements were made on a Durrum stopped-flow apparatus modified for fluorimetry by C.-R. Rabl [7]. The 366 nm line of a mercury lamp was selected by a Schoeffel monochromator and the emitted light was passed through a Schott cut-off filter. The photomultiplier signal was recorded on a Biomation data-logger, the memory of which was

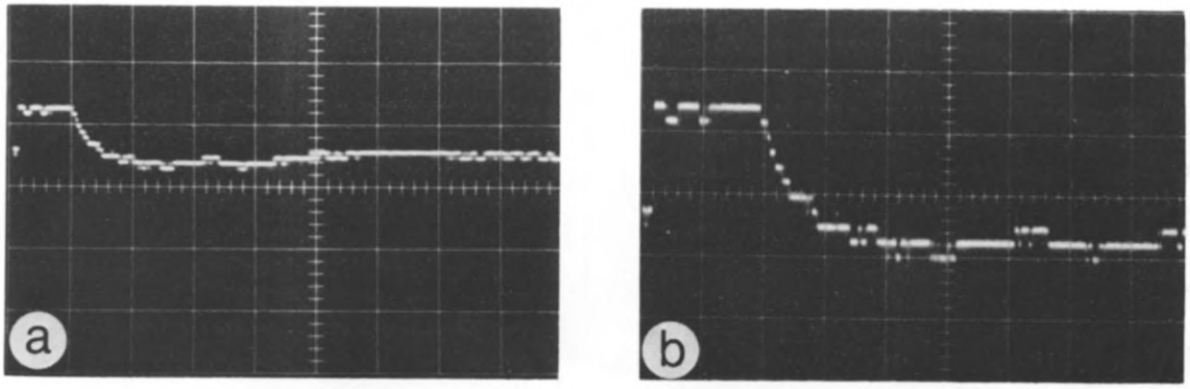

Fig.1. Reaction of dansyl-IF3 with 30 S subunits in the stopped-flow apparatus. Syringe (A): dansyl-IF3 1.0 μM in KCl 100 mM, $Mg(CH_3CO_2)_2$ 10 mM, Tris–HCl 10 mM (pH 7.85). Syringe (B): 30 S subunits 0.7 μM in same buffer. Scale (per large division): (a) vertical 50 mV, horizontal 40 ms; (b) vertical 20 mV, horizontal 20 ms.

continuously displayed on a Tektronix oscilloscope. Kinetic data-points were read off from photographs of the oscilloscope trace.

Second-order rate constants were evaluated by the usual second-order plot [8]; plots showing excessive curvature were rejected. The oscilloscope trace and second-order analysis of a typical shot are shown in fig.1,2.

On account both of the speed of the reaction between dansyl-IF3 and 30 S ribosomal subunits and of the large size of the latter (mol. wt 9×10^5) it was necessary to work with very low reactant concentrations and the signal was correspondingly poor. Nonetheless fairly reliable reaction rate constants were obtained, and an Arrhenius plot could be constructed (fig.3). The second-order rate constant for the association between dansyl-IF3 and the 30 S subunit was found to be 4×10^8 M^{-1} s^{-1} at 20°C, with an activation energy of 6.0 ± 1.5 kcal/mol. Combining the association rate constant with the equilibrium constant of 2×10^7 M^{-1} [5] leads to a dissociation rate constant of 20 s^{-1}.

Many shots gave an 'infinity' reading which

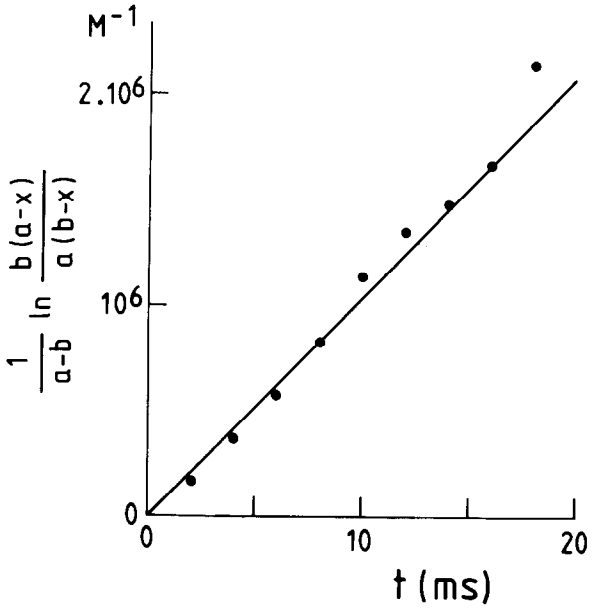

Fig.2. Second-order plot of fig.1b: a and b are initial concentrations of free 30 S subunits and dansyl-IF3; x is the time-dependent concentration of [30 S dansyl-IF3]. The gradient is equal to the rate constant.

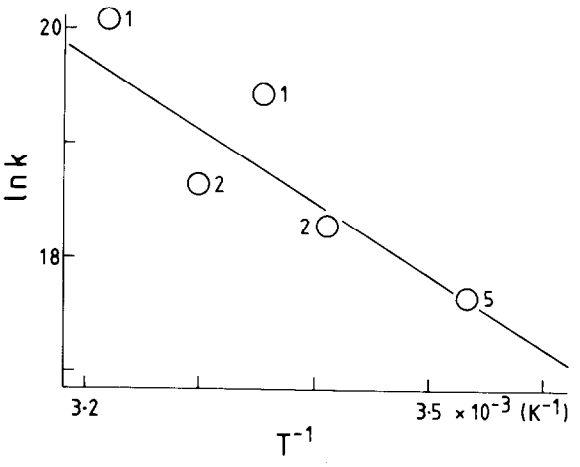

Fig.3. Arrhenius plot of the second-order rate constant for the association reaction. The numbers next to the points give the weighting (no. expt).

decreased slowly over 100–200 ms (compare fig.1a,b). The decrease was always very small, having < 20% of the amplitude of the initial reaction, and was sometimes not seen at all. Further work will be devoted to finding out whether this effect is genuine.

3. Discussion

Evidence for diffusion control of reaction rate in aqueous solution is usually:

(i) A high rate of reaction, giving reasonable agreement with the von Smoluchowski theory of collision rates [9];

(ii) An apparent activation energy of 5–6 kcal/mol, resulting from the temperature dependence of the viscosity of water [10];

(iii) A dependence of reaction rate upon viscosity [10–12].

The observed rate constant for 30 S–IF3 association is indeed as high as many rate constants observed for those enzyme–substrate binding steps which are taken to be diffusion-controlled [13]. Application of the von Smoluchowski eq. (1) leads to a collision radius* of ~ 4 Å, which is the kind of distance over which a recognition interaction might be expected to

* It is assumed that $D_{30\,S} << D_{IF3} = 10^{-6}$ cm . s^{-1} [14]

take place, although the application of this equation to a reaction between two macromolecules certainly oversimplifies the issue since rotational diffusion and the size of the area of interaction are neglected:

$$K = \frac{4\pi N}{1000}(D_A + D_B)(r_A + r_B) \quad (1)$$

The apparent activation energy fulfils the requirements of diffusion control, although the accuracy of the determination is rather low.

We therefore regard the association as diffusion-controlled. In other words, the equilibrium between free IF3, free 30 S subunits and their association complex is established at least in vitro as fast as physically possible. This is consistent with the postulated anti-association function of IF3 (e.g., [15,16]). A substantial conformational change of the 30 S subunit (e.g., [17,18]) in this time-scale is not very likely, but could occur after binding and might possibly be correlated with the slow fluorescence change which we observed following the binding.

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