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A Kinetic Study of the Binding between Ribosomal Subunits in E. coli

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Biofisica. — *A Kinetic Study of the Binding between Ribosomal Subunits in* E. coli (*). Nota di LUCIANA DANUSSO (**) e ANNA REALE SCAFATI (**), presentata (***) dal Corrisp. M. AGENO.

RIASSUNTO. — La cinetica della dissociazione dei ribosomi di *E.coli* in soluzione, a bassa (10^{-4}M) concentrazione di ioni Mg^{++}, è stata studiata, analizzando in funzione del tempo la concentrazione delle componenti da 70 S, 50 S, 30 S rispettivamente, mediante la tecnica della sedimentazione a zone in ultracentrifuga analitica. Si è potuto dimostrare che il processo

$$70\,S \rightleftarrows 30\,S + 50\,S$$

è una reazione di equilibrio, per la quale sono state determinate le costanti di associazione e dissociazione, e la loro dipendenza dalla temperatura. I valori che sono stati ricavati per le energie di attivazione (12 Kcal/mole per l'associazione e 9 Kcal/mole per la dissociazione) confermano che i legami tra le subunità ribosomiche sono dei legami di tipo debole, le cui energie sono confrontabili con quelle dei legami idrogeno.

This report concerns an investigation of the bonds between the ribosomal subunits, currently defined as 30 S and 50 S, which constitute the 70 S particle found in ribosomes extracted from *E. coli* cells, using the classical technique described by Tissières et al. [1].

It is well known that the configuration of the ribosomes in solution depends strongly upon the magnesium concentration. Ultracentrifuge analyses have shown that in high magnesium concentrations (10^{-2}M) two types of particles are present, corresponding to 70 S and 100 S sedimentation coefficients, whilst in low concentrations (10^{-4} M) one finds sedimentation coefficents corresponding to 30 S and 50 S, together with a small amount of 70 S.

Some information about the nature of these associations and of the bindings, in which the magnesium ions must somehow participate, can be obtained by studying the kinetics of the dissociation-association process:

$$(1) \qquad 70\,S \underset{\text{high}[Mg^{++}]}{\overset{\text{low}[Mg^{++}]}{\rightleftarrows}} 30\,S + 50\,S$$

The aim of this study was to ascertain whether the reaction (1), at a given magnesium concentration, represents a true chemical equilibrium.

An affirmative answer would imply that for a given magnesium concentration the characteristic kinetics of the process—as defined by the dependence of the concentration of the various components upon time—can be

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predicted, on the basis of simple hypotheses, and that it is possible to determine the equilibrium constant. In addition, by following the reaction at different temperatures, one should also be able to determine the activation energies, which can be related to the binding energy between the particles.

In writing equation (1) no account was taken of the fact that at high [Mg^{++}] two sequential processes exist, so that one should write:

$$100\,S \rightleftarrows 2\,(70\,S) \rightleftarrows 2\,(30\,S + 50\,S)\,.$$

If however the experiment is limited to the study of the dissociation process, by transferring the ribosomes at $t = 0$ from a high to a low Mg^{++} concentration, one finds that the velocity of the first stage

$$100\,S \longrightarrow 2\,(70\,S)$$

is much higher than that of the second one. Consequently, at low [Mg^{++}] one need only take into account the reaction (1) considering as the initial state the 70 S configuration of the particles.

In this instance, introducing the following notation

$$[70\,S] = c\,(t)$$

$$[30\,S] = [50\,S] = a\,(t)$$

and defining

k_1 as the association constant, i.e. the probability per unit time that one 30 S and one 50 S particle associate to form one 70 S particle;

k_2 as the dissociation constant, i.e. the probability per unit time that one 70 S particle dissociates into one 30 S and one 50 S particle;

one can write, if c_0 , a_0 are the initial values for $c\,(t)$, $a\,(t)$:

$$c\,(t) = c_0 - x\,(t)$$

$$a\,(t) = a_0 + x\,(t).$$

Consequently for the variations of c and a, in the infinitesimal time interval dt, one can write

$$dc = -\,da = k_1\,a^2\,dt - k_2\,c\,dt$$

and the differential equation

$$\frac{dc}{dt} = -\,\frac{da}{dt} = k_1\,a^2 - k_2\,c \tag{2}$$

together with the initial conditions

$$c\,(0) = c_0$$

$$a\,(0) = a_0$$

completely describe the process. The analytical solutions, for $a(t)$ and $c(t)$ are:

$$(3)\qquad \begin{cases} a(t) = -\dfrac{k_2}{2k_1} + \dfrac{\sqrt{A/2}}{k_2}\,\mathrm{tgh}\left[\sqrt{\dfrac{A}{2}}(t-B)\right] \\ c(t) = \dfrac{A + \dfrac{k_2^2}{2}}{2k_1k_2} - \dfrac{\sqrt{A/2}}{k_2}\,\mathrm{tgh}\left[\sqrt{\dfrac{A}{2}}(t-B)\right] \end{cases}$$

where

$$(4)\qquad \begin{cases} A = 2k_1k_2\left(c_0 + a_0 + \dfrac{1}{4}\dfrac{k_2}{k_1}\right) \\ B = \dfrac{k_1}{\sqrt{A/2}}\,\mathrm{arctgh}\left[\dfrac{k_1}{\sqrt{A/2}}\left(a_0 + \dfrac{k_2}{2k_1}\right)\right]. \end{cases}$$

From (3) one immediately sees that $c(t)$ and $a(t)$ must approach constant equilibrium values, a_∞ and c_∞, as $t \to \infty$, given by

$$(5)\qquad \begin{cases} a_\infty = \dfrac{1}{k_1}\left[\sqrt{\dfrac{A}{2}} - \dfrac{k_2}{2}\right] \\ c_\infty = \dfrac{1}{k_1k_2}\left[\sqrt{\dfrac{A}{2}} - \dfrac{k_2}{2}\right]^2. \end{cases}$$

From (5) one may express the equilibrium constant, K, in terms of the concentrations at the equilibrium, and one finds, is agreement with the law of mass action, that

$$K = \frac{k_1}{k_2} = \frac{a_\infty^2}{c_\infty}.$$

Going further, once k_1, k_2 are known for at least two temperatures, one can obtain the activation energy of the association and dissociation processes respectively by assuming that the Arrhenius equation holds true; so that

$$(6)\qquad \begin{cases} k_1 = A_1\, e^{-E_1/RT} \\ k_2 = A_2\, e^{-E_2/RT} \end{cases}$$

where R is the gas constant, T the absolute temperature, E_1, E_2 the activation energies, and A_1, A_2 are the so called frequency factors.

The data on the concentrations were obtained from a series of experiments extensively described elsewhere [3], in which the ribosomes extracted from a RNAase$^-$ strain of *E. coli* kindly supplied by R. F. Gesteland [2], and suspended in 10^{-3}M tris buffer with 10^{-2}M Mg^{++}, where afterwards transferred by dilution at $t = 0$ in the same buffer with 10^{-4}M Mg^{++}.

The ribosomal suspension, brought after dilution at a concentration of 160 μg/ml, was kept at a predetermined temperature in a water bath, using a magnetic stirrer to avoid sedimentation. At convenient time intervals a 0.015 ml sample was taken, and analysed in a Spinco model E ultracentrifuge,

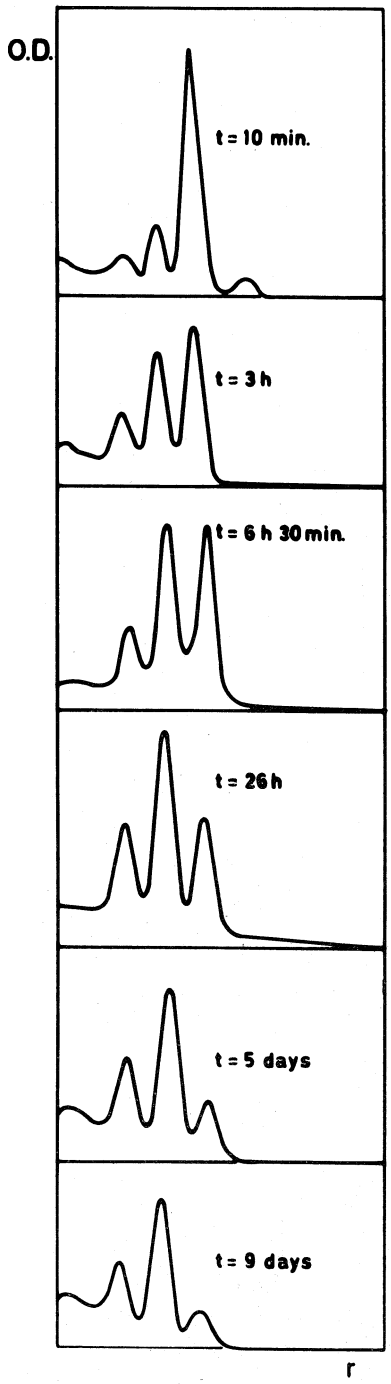

Fig. 1.

in order to obtain the concentration of the various ribosomal components at that time. The run was made at the same temperature as that of the water bath, precooling the rotor if necessary. The band centrifugation technique was used, pouring the sample into the well of a synthetic boundary cell filled with heavy water, to which 10^{-4}M Mg^{++} ions were added. The advantages of this technique have been discussed in a previous paper [4].

The experiments were continued until the concentrations had reached constant values, or up to the degradation of the sample, which happened after a number of days or after a shorter time, depending on the temperature.

The experiments were carried out a 3° C, 7° C, 15° C and 20° C. The concentrations of the various components were determined with a planimeter from microdensitometer traces (made with a Joyce instrument) of photographs of the ultracentrifuge cell, taken at convenient times during the run, when the components were well resolved. The components were identified by determining their sedimentation coefficients.

Fig. 1 shows some of the microdensitometric patterns relating to the 3° C kinetics. The samples were taken at the times indicated on each curve. In fact a much greater number of tests were made, as is shown on the graph of the complete kinetics plotted in fig. 2. Here the experimental points are reported with their errors, as evaluated by repeated analysis of a given sample under constant conditions. The continuous line represents the best fit of the experimental data, made with the help of an IBM 7040 computer, using the solutions (3) of the differential equation (2), and a modified Montecarlo method [5] of interpolation.

Fig. 3 reports the experimental data and the best fitting curve for the 7° C experiment, and similarly fig. 4 and 5 show the 15° C and 20° C data respectively. Only the concentration of the 50 S particle is reported for $a(t)$,

because of a possible contamination of the 30 S peak with the continuous distribution corresponding to the slower degraded material, the amount of which is difficult to evaluate.

For each value of the temperature, a set of optimizing parameters was determined, together with the χ^2 of the best fit and Table I shows these results. The statistical method used for the interpolation of the curves does not give a direct evaluation of the errors of the above parameters. Information

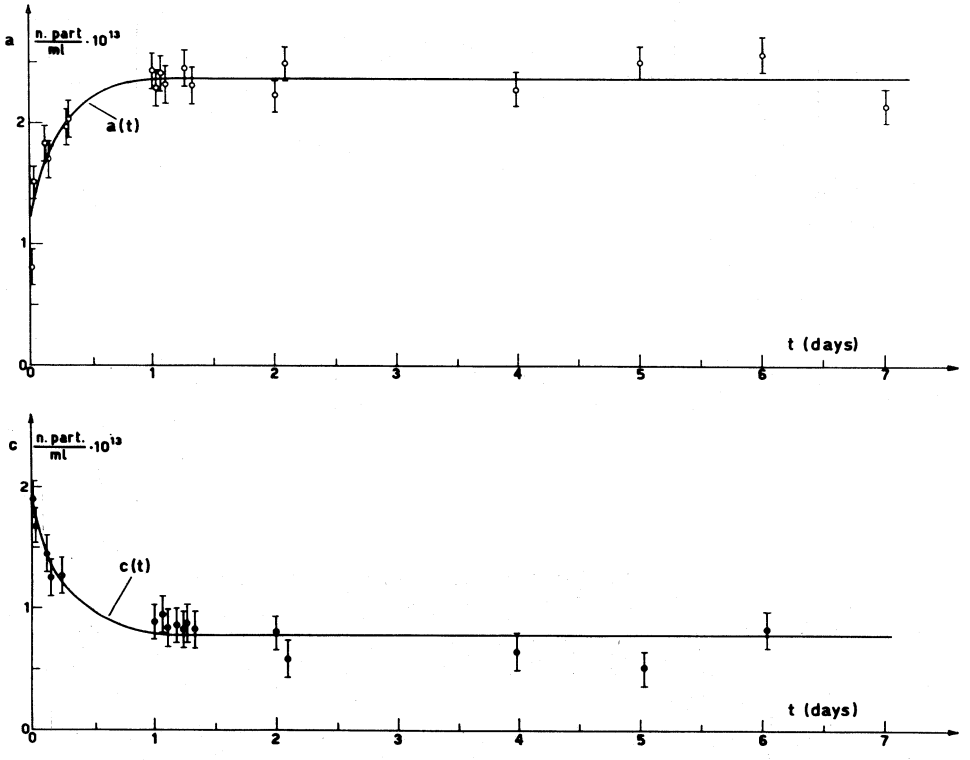

Fig. 2.

about these errors can be obtained by repeating the procedure many times and considering the half value of the dispersion of the parameter values which correspond to comparable values of the χ^2. The errors reported in Table I were obtained in this way.

The units used in the derivation of this table are 10^{13} particles/ml for the concentrations and days for the time.

The values of k_1 and k_2 in Table I, and the corresponding absolute temperatures, were used to find, with the least square approximation method, the dependence of the two probabilities upon the temperature, according to the relations (6), which, in a semilogarithmic plot versus 1/T, correspond to straight lines. Each value of k_1 and k_2 were given weights, expressed by the confidence level associated with the χ^2 of the corresponding best fit.

TABLE I.

TEMPERATURE	k_1	k_2	A	B	χ^2	Number of degrees of freedom
3°C	0.35±0.08	2.54±0.5	8.82±4.5	0.534±0.09	31	32
7°C	0.75±0.05	4.71±0.25	32.4±3.7	0.270±0.02	30.8	38
15°C	0.88±0.1	9.1 ±1.1	109 ±25	0.147±0.003	10.6	6
20°C	1.31±0.15	9.05±0.6	138 ±25	0.112±0.01	14.5	9

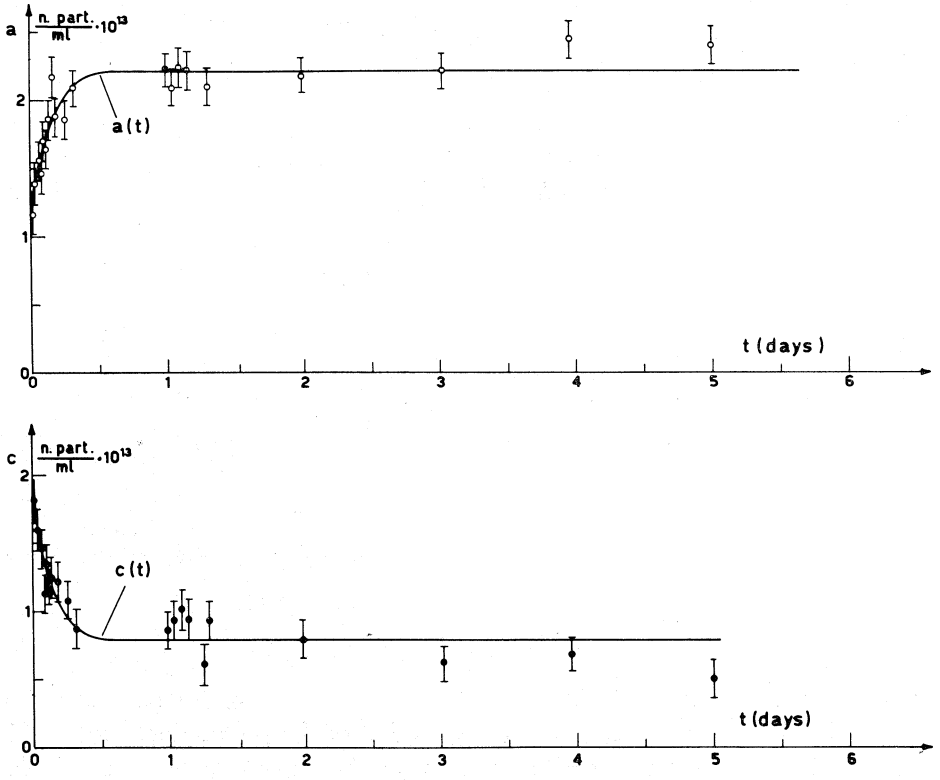

Fig. 3.

The activation energies which were deduced in these low [Mg^{++}] conditions, were the following.

$$E_1 = 11.25 \pm 3.5 \text{ Kcal/mole}$$

for the association process

$$E_2 = 9.2 \pm 1.8 \text{ Kcal/mole}$$

for the dissociation process.

These results, although affected by relatively high experimental errors, allow one to draw some conclusions about the binding of the two ribosomal subunits.

The energies involved appear to be definitely smaller than the ones generally found for a chemical bond e.g. of covalent character. In fact, the

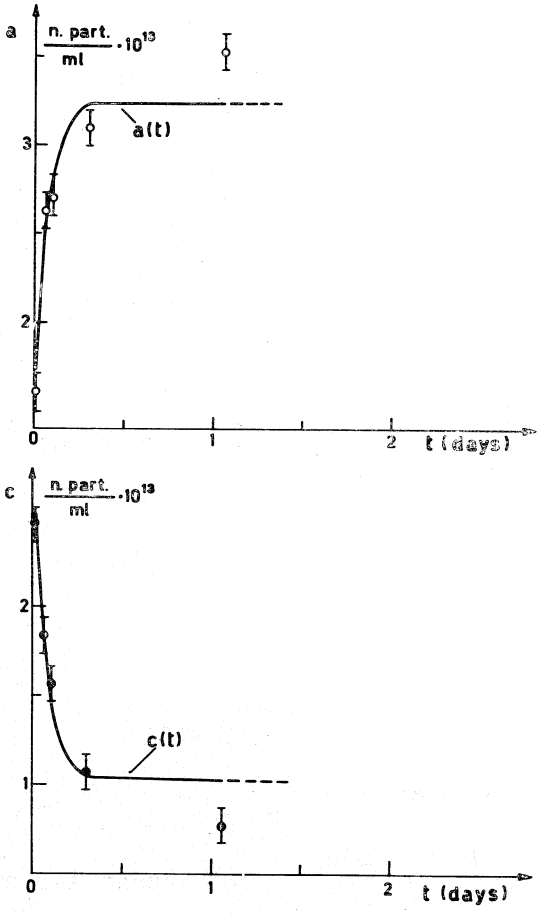

Fig. 4.

values obtained are in the energy range of typical hydrogen bonds, which, as reported in the literature, have an activation energy of about 6 Kcal/mole per bond. Consequently the binding energy for the ribosomes could correspond to the existence of one or two hydrogen bridges between the subunits.

In this case however, the binding could also be due to a different mechanism, directly involving the Mg^{++} ion. The alternative hypothesis that the Mg^{++} ion only has the function of neutralizing the negative charges of the phosphate groups of the ribosomal RNA, thus allowing the establishment of hydrogen bridges between the RNA molecules of the two subunits, seems unacceptable. The role of Mg^{++} seems to be too specific, for it can be replaced

only by Ca^{++} ions, and some complex organic molecules such as polyamines. No other positively charged ion makes the association between the subunits possible.

Other studies are necessary to understand how the Mg^{++} ion is responsible for the establishment of such weak bonds. Our next aim will be to obtain

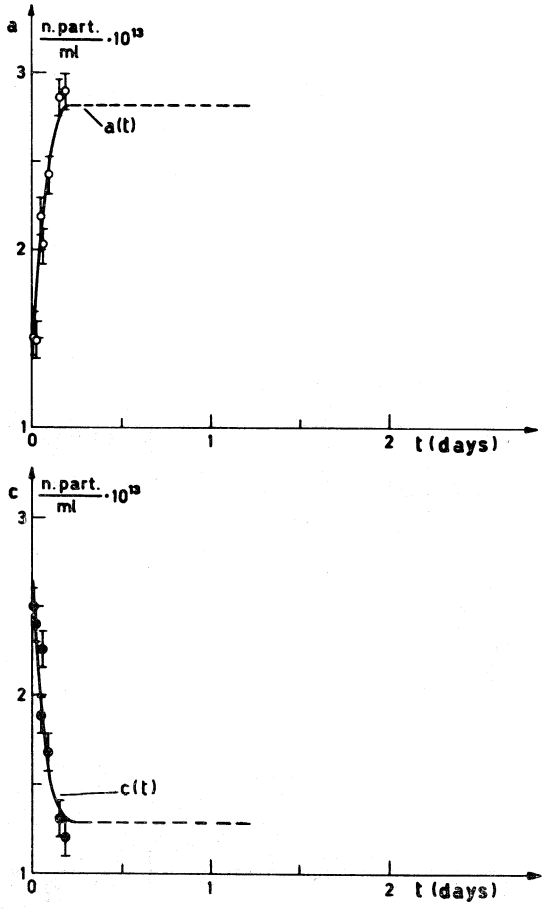

Fig. 5.

greater precision in the determination of the binding energies, starting from the present results, which must be considered as a significant but preliminary indication.

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