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CORRELATION BETWEEN BIOLOGICAL ACTIVITY OF 30 S SUBUNITS OF *ESCHERICHIA COLI* RIBOSOMES AND THEIR CONFORMATION CHANGES REVEALED BY OPTICAL MIXING SPECTROSCOPY

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Spectral analysis of light scattered from solutions of 30 S subunits was performed by the method of regularization of the inverse spectral problem. The subunits observed under ionic conditions which preserved their biological activity (200 mM NH_4Cl at 1 mM $MgCl_2$) revealed a monodisperse pattern of scattering with diffusion constant $D = (1.83 \pm 0.10) \times 10^{-7}$ cm^2/s. The polydispersity and compaction of 30 S subunits were observed under inactivation ionic conditions (30 mM NH_4Cl at 1 mM $MgCl_2$). The number of compacted particles correlates with the irreversible loss of biological activity, the ability of 30 S subunits to bind specific tRNA.

1. Introduction

The activity of 30 S subunits in vitro, the capability to bind specifically tRNA, can be abruptly and irreversibly lost during incubation of subunits at low concentrations of monovalent cation and magnesium (100 mM NH_4Cl or lower at 1 mM $MgCl_2$) without visible loss of proteins and degradation of rRNA [1]. It seems that this inactivation is connected with some conformational changes of 30 S subunits. In this paper, we observed by the method of optical mixing spectroscopy and using regularization according to Preobrazensky and Tolpina [2] the difference in conformation of active and partially inactivated 30 S subunits.

2. Materials and methods

Isolation of active 30 S subunits from *Escherichia coli* MRE 600, an enriched preparation of Phe-tRNA^{Phe} (1500 pmol/A_{260} unit) and measurements of activity of 30 S subunits, the association constant of Phe-tRNA^{Phe} and the number of active sites per subunit are described in refs. 3–5. Active 30 S subunits can bind two molecules of Phe-tRNA^{Phe} in the presence of poly(U) with different binding constants [3]. We compared the activity of 30 S subunits according to their capability to bind Phe-tRNA^{Phe} in the presence of poly(U) with the P-site, i.e., with the highest binding constant [3]. So 'active' 30 S subunits at 20 mM $MgCl_2$ and a saturated concentration of Phe-tRNA^{Phe} bind one molecule of Phe-tRNA^{Phe} per P-site of every subunit. 'Inactivated' 30 S subunits under the same conditions can bind only 0.5 mole-

cules of Phe-tRNA^{Phe} per P-site. Inactivation of 30 S subunits was performed by dialysis of active subunits against 20 mM Tris-HCl (pH 7.1) buffer containing 1 mM $MgCl_2$, 0.05 mM Na_2EDTA, 30 mM NH_4Cl and 2 mM 2-mercaptoethanol during 18 h at 5°C [1]. The activity of 30 S subunits before and after optical measurements was the same. Before light scattering measurements the solution of 30 S subunits (1 mg/ml) was centrifuged in a scattering cuvette during 2 h at 18 000 g at 5°C. We used the optical mixing spectrometer described in ref. 6 with a helium-neon laser source ($\lambda = 6328$ Å, 40 mW power) and the 200-channel analyzer S4-73.

In the case of a monodisperse system, for the spectral density of the photocurrent we have a single Lorentzian centered at zero frequency:

$$S(\omega) = \frac{A\Gamma/\pi}{\Gamma^2 + \omega^2} \tag{1}$$

with a half-width (Γ) proportional to the translational diffusion coefficient (D) of the particles in solution:

$$\Gamma = Dq^2 \tag{2}$$

where $q = \frac{4\pi n}{\lambda} \sin \theta/2$ is the absolute value of the scattering vector, θ the scattering angle, n the refractive index of the solution, λ the incident light wavelength, A the Lorentzian area, and ω the circular frequency.

For a polydisperse system the frequency spectrum of the photocurrent is:

$$S(\omega) = \int \frac{A(\Gamma)\Gamma/\pi}{\Gamma^2 + \omega^2} d\Gamma \tag{3}$$

i.e., a superposition of Lorentzians with different widths (Γ). In this case we are interesting in deriving the distribution function $A(\Gamma)$ from the experimental spectrum $S(\omega)$, i.e., in performing a numerical solution of a problem which is not well posed in the sense of Hadamard.

Recently, a method of regularization was suggested for the solution of such a problem [2] (see Appendix) which we used here to find the distribution $A(\Gamma)$ from $S(\omega)$. We are interested in the distribution of the number of particles according to their dimensions $N(R)$ which can be obtained using the Stokes-Einstein relation:

$$D = \frac{kT}{6\pi\eta R} \tag{4}$$

where k is Boltzmann's constant, T the absolute temperature, η the viscosity of the solution and R the macromolecular radius. We assume equal density for all particles with $R \ll \lambda$ and scattering proportional to the square of their mass. Then we find:

$$N(R) = \frac{1}{R^8} A\left(\frac{kT}{6\pi\eta R} q^2\right) \tag{5}$$

3. Results and discussion

Fig. 1 shows the dependence of the half-width of the spectrum (Γ) vs. $\sin^2\theta/2$ for active (a) and inactivated (b) 30 S subunits. This dependence yields for the active particles a diffusion constant $D_{20,w} = (1.83 \pm 0.10) \times 10^{-7}$ cm^2/s and a hydrodynamical diameter 235 ± 12 Å. The analysis of polydispersity according to the above-mentioned method [2] for both active and inactivated particles is shown in fig. 2. We can see the distribution functions of the line width $A(\Gamma)$ for active (a) and partially inactivated (b) 30 S subunits, the

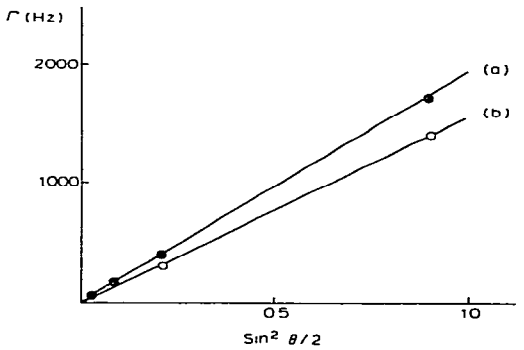

Fig. 1. The dependence of the half-width (Γ) vs. $\sin^2\theta/2$ (heterodyne detection). (a) Active subunits in 20 mM Tris-HCl (pH 7.1) buffer containing 200 mM NH_4Cl, 1 mM $MgCl_2$, 0.05 mM Na_2EDTA and 2 mM 2-merceptoethanol; (b) inactivated subunits in 20 mM Tris-HCl (pH 7.1) buffer containing 30 mM NH_4Cl, 1 mM $MgCl_2$, 0.05 mM Na_2EDTA and 2 mM 2-mercaptoethanol.

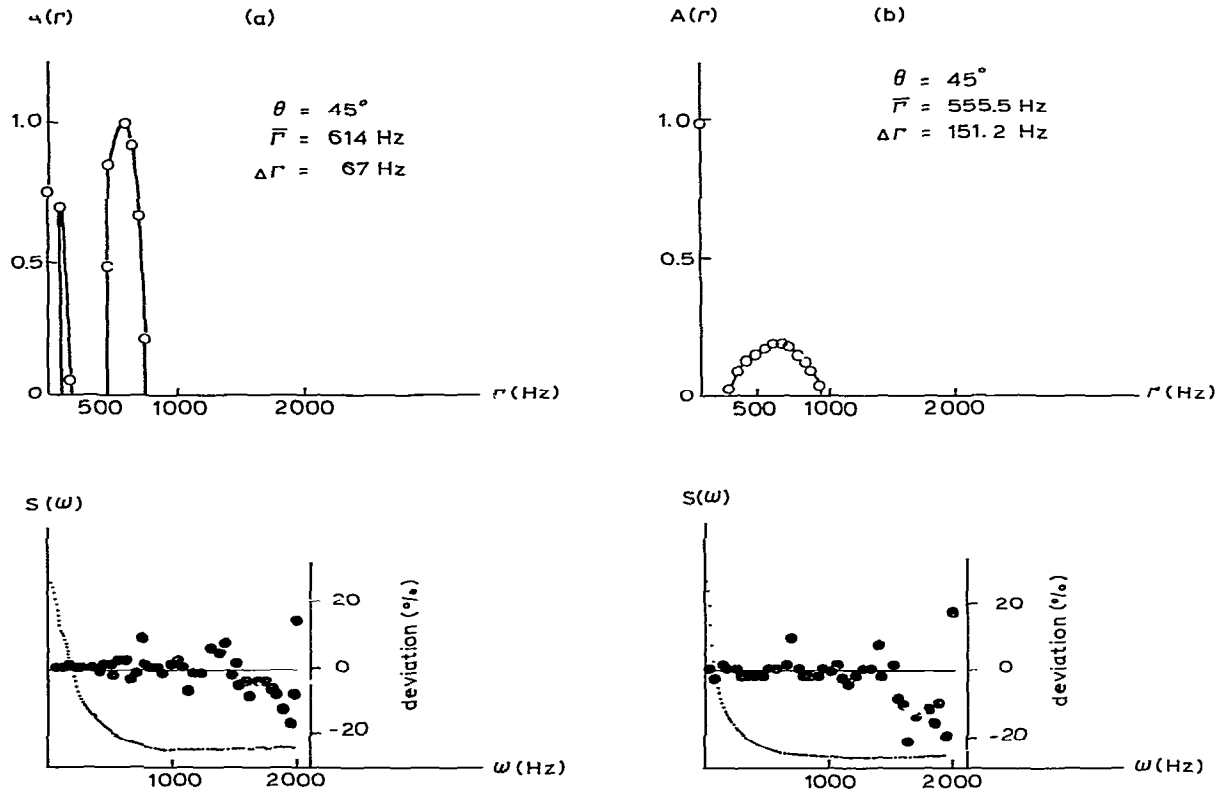

Fig. 2. The constructed functions $A(\Gamma)$ (upper). the experimental spectrum and relative deviation between the experimental and calculated spectrum (see section 2) (lower) for active (a) and inactivated (b) 30 S subunits at $\theta = 45°$.

experimental spectra $S(\omega)$ at $\theta = 45°$ and the relative deviations of the spectra calculated according to eq. 3 using $A(\Gamma)$ obtained according to ref. 2. For active 30 S subunits we found a narrow distribution $A(\Gamma)$ (fig. 2a). The 'very narrow' line at small Γ does not arise from ribosomal subunits but is due to the presence of a small quantity of 'dust' with radius larger than 800 Å.

Under inactivating conditions the distribution function is broader (fig. 2b). Now using eq. 5 and taking into account that $\int_0^\infty A(\Gamma)\mathrm{d}\Gamma = 1$, we found the distribution function $N(R)$ (see section 2) as shown in fig. 3. We can see that under inactivating conditions (low NH_4^+) about 50% of 30 S subunits are more compact. This primary effect of compacting correlates with the irreversible loss of biological activity of 30 S subunits (about 50%) measured at high Mg^{2+} concentration and after heat reactivation [7]. The scattering by a small number of aggregates which appeared even at low magnesium concentration, during the preparation (fig. 3) could lead, without using a proper analysis of polydispersity, to quite the opposite conclusion; i.e., that the mean radius of particles increases (D decreases) under inactivating conditions (fig. 1, cf. a and b). Such an analysis has not yet been performed in the study of scattering by ribosomal particles [8,9].

Thus, the incubation of active 30 S subunits at low monovalent cation concentration (30 mM

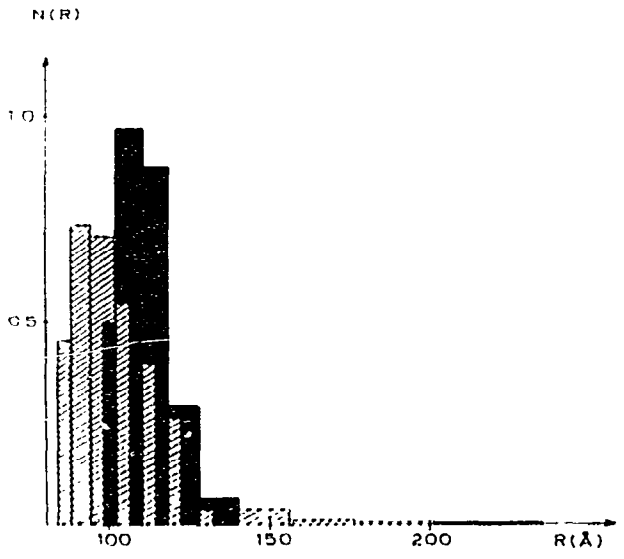

Fig. 3. The distribution function in R-space. (solid bars) Active and (hatched bars) inactivated particles.

NH_4^+ at 1 mM Mg^{2+}) results in compacting of about 50% of particles and this compacting correlates with the irreversible loss of activity of 30 S subunits in binding of specific tRNA. This paper demonstrates the usefulness of the regularization method [2] in the numerical solution of inverse spectral problems for the analysis of polydispersity of scattering particles in solution.

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Appendix by S. Tolpina

The idea of the proposed method for the solution of inversion problems in optical mixing spectroscopy is to impose a priori restrictions, making the problem correct according to Tikhonov, before the algeoraic formulation and then to solve the problem using non-linear programming methods.

The physical formulation of optical mixing spectroscopy problems makes it possible to formulate a problem correctly according to Tikhonov as a problem of minimization of the mean squared deviation

$$\rho_g = \int_a^b \left\{ f(\nu) - \int_a^b K(\nu,\Delta) g(\nu) \mathrm{d}\Delta \right\}^2 \mathrm{d}\nu \qquad \text{(A1)}$$

over the distribution $g(\Delta)$ with the additional restrictions

(1) $g(\Delta) \geq 0$

(2) $\int_a^b g(\Delta)\mathrm{d}\delta \leq M, \quad M = (2/\pi)\int_a^b f(\nu)\mathrm{d}\nu$ (A1′)

(3) $g(\Delta) = \int_a^b \tilde{g}(\Delta')\,\mathrm{rect}_d(\Delta - \Delta')\mathrm{d}\Delta'.$

where $\tilde{g}(\Delta')$ is the solution of eq. A1 for the unperturbed function $F(\nu)$ and $\mathrm{rect}_d(\Delta - \Delta')$ is defined by

$$\mathrm{rect}_d(\Delta - \Delta') = \begin{cases} 1, & |\Delta - \Delta'| \leq (d/2) \\ 0, & |\Delta - \Delta'| > (d/2) \end{cases}$$

where d is the resolution interval.

In the uniform discretization of the problem (eqs. A1–A1′) the discretization step h, which depends on the dispersion of the errors in the measured spectrum $f(\nu)$, is usually much larger than d. When $h > 3d$ the problem, eqs. A1–A1′, reduces to that of the minimization of the deviation on a set of an N-dimensional vector $\vec{g}$.

$$W(\vec{g}) = h \sum_{n=1}^{N} \left(f_n - \sum_{m=1}^{N} a_{nm} g_m \right)^2 \qquad \text{(A2)}$$

with the restrictions

(1) $g_m \geq 0, \quad m = 1, 2, \ldots, N$

(2) $\sum_{m=1}^{N} g_m \leq M$ (A2′)

Considering the problem (eqs. A2–A2′) as a non-linear programming problem with perturbations, due to the discretization error and the errors of the $f(\nu)$ measurements, and analyzing the influence of these two perturbations on the accuracy of the solution, one can easily show that the estimate of the optimal (from the point of view of

minimal solution error) resolution-to-band ratio, N_{opt}, is

$$N_{opt} \approx k\frac{f(a)}{\sigma}, \qquad k < 0.4.$$

The relative error of the solution is given by

$$\|e\| \approx k_1\sqrt{\frac{\|\Sigma\|}{f(a)}}$$

which differs from the linear dependence of the solution error on the measurement error common for conditionally correct problems.

The algorithm of solving the problem (eqs. A2–A2′) for $N \equiv N_{opt}$ is based on the gradient projection method.

The advantages of the proposed method are: (i) the absence of a priori assumptions concerning the smoothness of the solution which makes it possible to find not only smooth but also δ-shaped distributions; (ii) there is no need to find the regularization parameter α and the stabilizing functional; (iii) the simplicity of the calculations.

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