MONITORING THE KINETICS OF THE ANTIGEN—ANTIBODY REACTION BY A LIGHT SCATTERING METHOD

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Measurement of the characteristics of light scattered in a space where a serological reaction is taking place can be used to study the process of formation of antigen—antibody complexes. The kinetics of the antigen—antibody reaction has so far been studied only by measuring the integral intensity of scattered light [1, 5].

In the present investigation a technique of photon-correlation spectroscopy together with a method developed previously [2] were used to study the kinetics of the antigen—antibody reaction simultaneously by measuring changes in the integral intensity and correlation function of scattered light.

EXPERIMENTAL METHOD

A polysaccharide from group A streptococcus (polysaccharide A), obtained by Coligan's method [6], was used. Antibodies against polysaccharide A were isolated from the serum of rabbits immunized with a pepsinized culture of group A streptococcus on a Sepharose 4B—polysaccharide A immunosorbent by the method described previously [3]. Solutions of the polysaccharide in a concentration of 12.5 μg/ml and of antibodies in a concentration of 700 μg/ml were filtered through Millipore filters (450 nm). A mixture of 0.25 ml of the polysaccharide solution and 0.25 ml of the solution of antibodies was tested. Measurements were made on a correlation spectrometer (Malvern Instruments). The source of exciting light was a helium-neon laser with emission wavelength of 633 nm and a power of about 10 mW. The angle of scatter was 90°. Measurements of integral intensity (the number of counts in the zero channel of the correlator) and the correlation function of intensity of scattered light were measured every 10 sec for 70 min after pooling the solutions of polysaccharide and antibodies. The correlation function was processed by computer in an approximation to one exponential function and values of the parameter $\Gamma = 1/\tau = Dq^2$ were obtained (τ denotes correlation time, D the coefficient of translation diffusion, q the wave vector of scatter, $q = 1.9 \times 10^5$ cm^{-2}). Measurements were made at 21°C.

EXPERIMENTAL RESULTS

The results of two series of measurement are shown in Fig. 1. Values of the parameters I and Γ for the original components were: for anitbodies $I \sim 8I_1$ ($I_1 = 10^4$ Hz), $\Gamma \sim 3.5 \cdot 10^3$ sec^{-1}; for antigen (without additional dilution) $I \sim 0.18\ I_1$ with a background intensity of $\sim 0.15\ I_1$. Because of the very weak scatter of the polysaccharide, the correlation function was determined in this case by scattering on larger, extrinsic scatterers. Immediately after pooling of the component (t = 0) the integral intensity ($\sim 4.5\ I_1$) within the limits of error of measurement ($\sim$10%) coincided with the additive intensity ($\sim 4.1\ I_1$) and was entirely determined by scatter on antibody molecules.

It will be clear from Fig. 1 that the curve of integral intensity as a function of time has a maximum. This type of dependence is characteristic of systems with an excess of antigen [5]. If the concentration of antigen was reduced by half (Fig. 1, bottom graph) the

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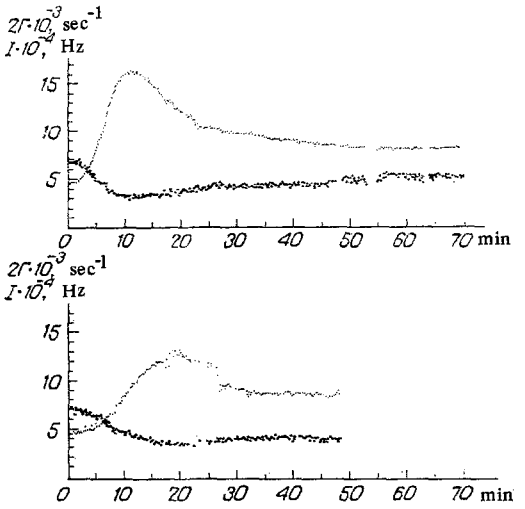

Fig. 1. Spectrum width 2Γ and integral intensity I of laser beam, scattered in a system of polysaccharide A—antibodies against polysaccharide A. In bottom graph concentration of polysaccharide A was reduced by half.

maximum became less marked and it was observed after about twice as long a time.

The maximum of integral intensity corresponds to the minimum on the curve Γ(T). Since the parameter Γ is proportional to the coefficient of diffusion, which in turn is inversely proportional to particle size, it will be evident that the extrema of the curves correspond to antigen—antibody complexes of maximal size.

The mean diameter of the complex d can be estimated from the coefficient of diffusion by the known Stokes—Einstein equation:

$$D = kT/(3\pi\eta d),$$

where k is Boltzmann's constant, T the absolute temperature, and η the viscosity of the solvent.

Calculation of the data for Fig. 1 (top graph) gives $d \sim 43$ nm (diameter of antibodies) for the beginning of the reaction, $d \sim 94$ nm for the maximum of I (the minimum of Γ), and $d \sim 67$ nm for the end of the reaction.

Comparison of the measured mean value of the size of the antibodies with the characteristic dimensions of the isolated immunoglobulin molecule ($\sim 24 \times 4$ nm) shows that dimer, and possibly trimer, associated are present in a suspension of antibodies [7].

The values obtained for d indicate that over the whole time range the mean diameter of the scattering particles was significantly smaller than the wavelength of the exciting light (633 nm) or, in other words, over the whole range the Rayleigh model of scatter is satisfied. In the Rayleigh model for spherical particles $I \sim d^6$. The values of I obtained experimentally point to the existence of a weaker dependence of I on d, more probably $I \sim d^2$. This means that antibody complexes and the antigen—antibody complexes formed are essentially nonspherical in shape. The dependence $I \sim d^2$ is characteristic of scattering on long cylinders [4]. Although a detailed description of the mechanism of complex formation in these experiments is difficult because of the polydispersed nature of the test samples, it may be noted in conclusion that measurement of the spectrum and intensity of the polarized and depolarized components of scattered light in sufficiently monodispersed specimens makes it possinle, in principle, to describe the kinetics of antigen—antibody interaction fully.

LITERATURE CITED

1. I. M. Aref'ev, L. O. Barsegyants, M. F. Vereshchaka, et al., Lab. Delo, No. 12, 716 (1979).

2. I. M. Aref'ev, A. P. Es'kov, M. N. Korn, et al., Byull. Éksp. Biol. Med., No. 3, 376 (1978).
3. V. Yu. Kolesnikova, É. V. Gnezditskaya, and I. M. Lyampert, Byull. Éksp. Biol. Med., No. 12, 708 (1980).
4. G. Van de Hulst, Light Scattering by Small Particles [Russian translation], Moscow (1961).
5. G. F. Buffone, J. Savory, and J. Hermans, Clin. Chem., 2, 1735 (1975).
6. J. E. Coligan, W. C. Shnufe, and T. J. Kundt, J. Immunol., 114, 1654 (1975).
7. T. Mukkur and G. D. Smith, Biochem. J., 183, 463 (1979).

TECHNICAL FACTORS IN VARIABILITY OF SILVER STAINING OF THE NUCLEOLUS ORGANIZER OF HUMAN CHROMOSOMES

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In recent years after development of the technique of selective staining of the nucleolar organizers (NO) of chromosomes in the metaphase of mitosis by means of silver nitrate interest in the study of these regions, containing ribosomal genes, has increased considerably. It has been shown that the number of NO stained with silver and the degree of staining are stable characteristics of the karyotype of a given individual. Nevertheless, many workers have noted variability of silver staining of some usually palely stained NO within the same tissue [1, 2]. The reasons for this phenomenon, which is of considerable theoretical and practical interest, have been studied, and attention has been concentrated on factors of a technical character.

The aim of the present investigation was to study the effect of the time of silver staining and of some other conditions on intercellular variability of NO in human chromosomes, to determine the optimal conditions for silver staining, and to select for analysis metaphase plates (MP) with maximally stained NO.

EXPERIMENTAL METHOD

Peripheral blood lymphocyte cultures from four persons conventionally identified by the numbers 1, 2, 3, and 4, were used. Preparations of metaphase chromosomes were obtained by the standard method and kept for 1 week in an incubator at 37°C. Silver staining was carried out by two methods. The first method was the Ag-1 method suggested by Bloom and Goodpasture [3]: two drops of a 50% aqueous solution of $AgNO_3$ were dropped on the preparation, a coverslip was applied, and it was introduced into a wet chamber and incubated at 37°C for 12, 24, 48, or 72 h. The second method was the writer's own modification of the Ag-1 method: preparations placed in $AgNO_3$ solution in the wet chamber were kept under a DRT-230 UV lamp for 20, 40, 60, or 80 min. After removal of the coverslip and washing of the preparations in water they were incubated in 0.2 N NaCl solution for 6-7 min at 65°C, taken through a series of alcohols, and stained with a 2% phosphate buffer solution of Giesma stain to obtain longitudinal differential staining of the chromosomes. With the first method of staining 50 MP were analyzed in each case, with the second method from 10 to 50 MP. The state of NO was estimated from the intensity of staining, expressed in points: 0) no staining, 1 point) pale staining, 2 points) average, 3 points) intense staining. The mean ability of MP to take up the dye in a given sample was determined as the sum of the points divided by the number of MP.

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