Volume 275, number 1,2, 221-225 FEBS 09162 November 1990

Electron microscopy study of human myeloma immunoglobulin G_1

S.N. Ryazantsev[a], V.M. Abramov[1], V.P. Zav'yalov[1] and V.D. Vasiliev[2]

[1]*Institute of Immunology, 142380 Lyubuchany, Moscow Region, Chekhov District, USSR* and [2]*Institute of Protein Research, Academy of Sciences of the USSR, 142292 Puschchino, Moscow Region, USSR*

Received 25 September 1990

Human immunoglobulin G_1 Van was studied by negative staining, freeze drying and high resolution shadow casting. The Fab and Fc subunits of an intact IgG_1 molecule were shown to possess limited mobility. It was found that about 70% of molecules in the IgG_1 Van specimen are not flat but have a tripod-like shape.

Immunoglobulin G_1; Electron microscopy; Tripod-like conformation

1. INTRODUCTION

Human IgG_s are divided into four subclasses significantly differing in their biological properties. The main differences in the primary structure of the subclasses are concentrated in the hinge region [1,2]. It is reasonable to assume that the structure of hinge region can determine an extent of flexibility of IgG of different subclasses, affecting their biological properties. The method of NMR did not reveal, however, any correlation between an extent of flexibility and biological properties of human IgG subclasses [3,4]. For instance, IgG_1 displaying good reactivity with the complement and IgG_4 practically not binding the complement [4] show equal correlation times (about 60 ns) at physiological temperature (37°C) [5]. Besides, the late or non-precipitating pig antibodies of class G possessing the most rigid structure among all the known IgG (correlation time is about 200 ns) nevertheless bind the complement rather well [6]. These data lead to the assumption that the hinge region structure influences the effector functions not only through the generation of a definite extent of the molecule flexibility but also by affecting the other parameters. One of these parameters can be a predominant shape of the molecule characterized by a certain relative location of the Fab and Fc subunits [7].

In the present work our aim was to continue the investigation of conformation of human IgG subclasses by means of electron microscopy and to study the myeloma IgG_1 Van which was described previously by the other physical methods [3,8–10].

Correspondence address: V.P. Zav'yalov, Institute of Immunology, 142380 Lyubuchany, Moscow Region, Chekhov District, USSR

2. EXPERIMENTAL

Immunoglobulins were obtained from myeloma serum by 3-fold reprecipitation with ammonium sulphate followed by ion-exchange chromatography using DEAE-cellulose. Then they were subjected to gel filtration in a AcA-34 Ultragel (LKB, Sweden) or on Toyoparl-HW55F (Toyo, Soda, Japan) to remove degradation products. Chromatography was carried out in buffer containing 0.5–0.1 M ammonium acetate, pH 7.8. According to the data of immunoelectrophoresis and SDS-PAGE, the immunoglobulin preparations were homogeneous.

The immunoglobulin preparation was dialyzed against 0.01 M ammonium acetate, pH 7.8. Just before use, the sample was centrifuged at 12 000 × *g* for 15 min to remove aggregates and diluted with ammonium acetate to a concentration of $A_{280} \approx 0.01$ U/ml. Negative staining was carried out according to Valentine et al. [11] with 1% aqueous uranyl acetate. Freeze-drying and high resolution shadow casting were carried out according to the procedure described in [12].

The preparations were analysed with a JEM-100C electron microscope (JEOL, Japan) at 80 kV, 25 μM objective aperture and magnification of 80 000. An anticontamination liquid N_2 trap was used. Magnification was calibrated with a cross-grating replica (Balzers Union, Liechtenstein).

3. RESULTS

Figs 1 and 2 show micrographs of the negatively stained and shadowed preparations of human IgG_1. In most images, the Fab and Fc subunits can be distinguished by the two-fold axis. The molecules are located on the supporting film so that the Y-like view is the only one. Therefore, the method of negative staining used alone provides no information on the orthogonal ('lateral') view of the molecule. Shadowing, in contrast to negative staining, allows one to estimate dimensions and shape of the molecule in the direction perpendicular to the supporting film using the shape and length of the shadow.

We analysed about 1000 images of the shadowed IgG molecules. The outlines of the molecule shadow depend

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/90/$3.50

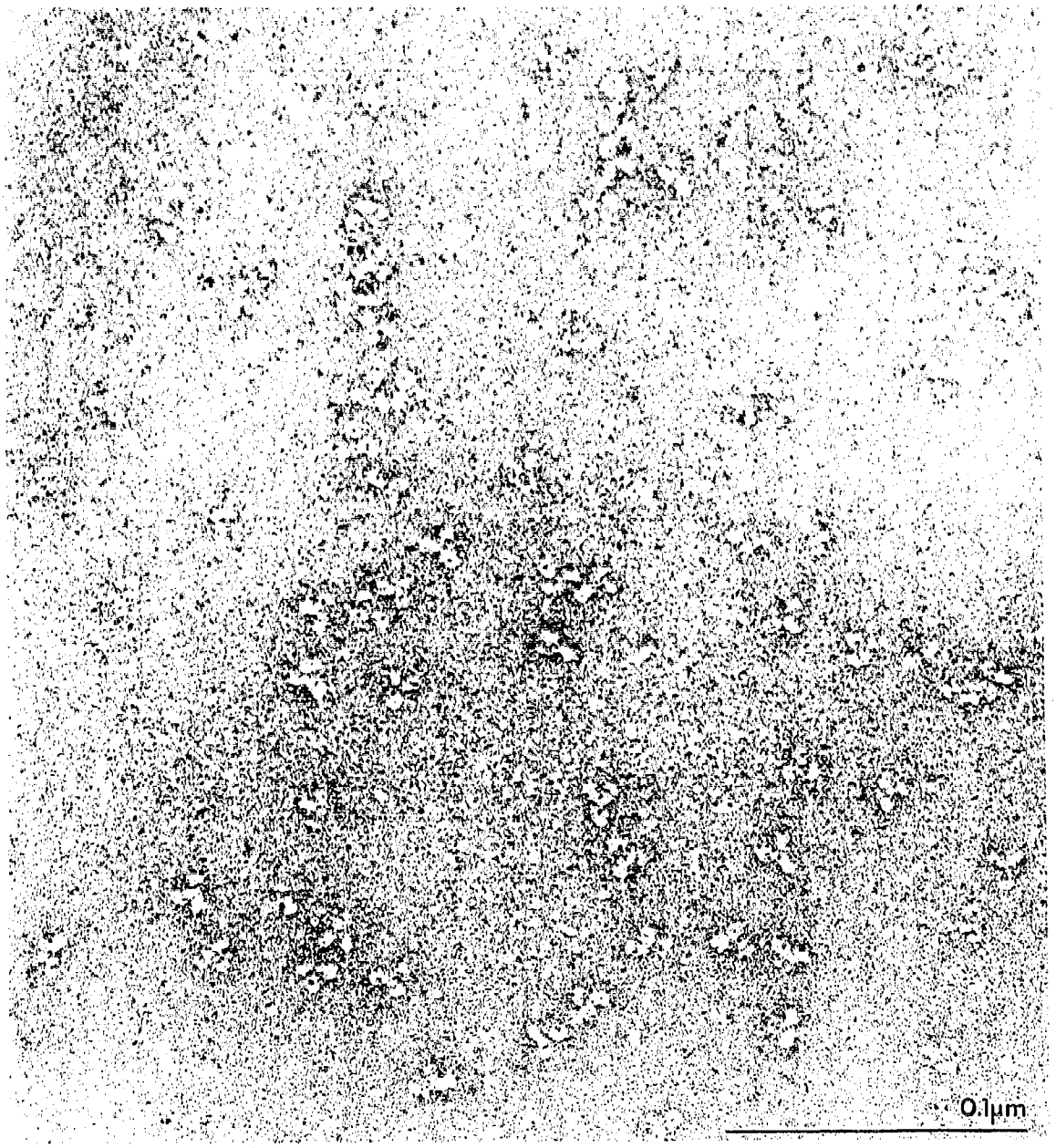

Fig. 1. Human IgG_1 negatively stained with uranyl acetate.

not only on the shape of the molecule but also on its orientation relative to the direction of shadowing. According to the shape of shadows all images can be divided into 2 groups (Fig. 3): (i) shadows with an obtuse apex when the width of the tip approximately equals that of the base; (ii) triangle-shaped shadows with a pointed tip.

The first variant can be interpreted as the case when all the components of the IgG molecule are equally elevated over the supporting film.

In the second case (Fig. 3B) the pointed shadow shape can indicate that, at least, a part of the molecule is elevated over the other parts, i.e. the molecule is not flat.

We chose about 150 IgG_1 images with the molecule orientation excluding possible occurrence of a triangle-shaped shadow of the flat molecule, namely: the two subunits lie in the straight perpendicular to the direction of shadowing, and the third subunit is located at the triangle apex facing an electron source. Fig. 4 shows the histogram of molecule distribution according to the shadow lengths. A molecule height can easily be estimated from a magnitude of the shadow length and an angle of shadowing. However, since the supporting

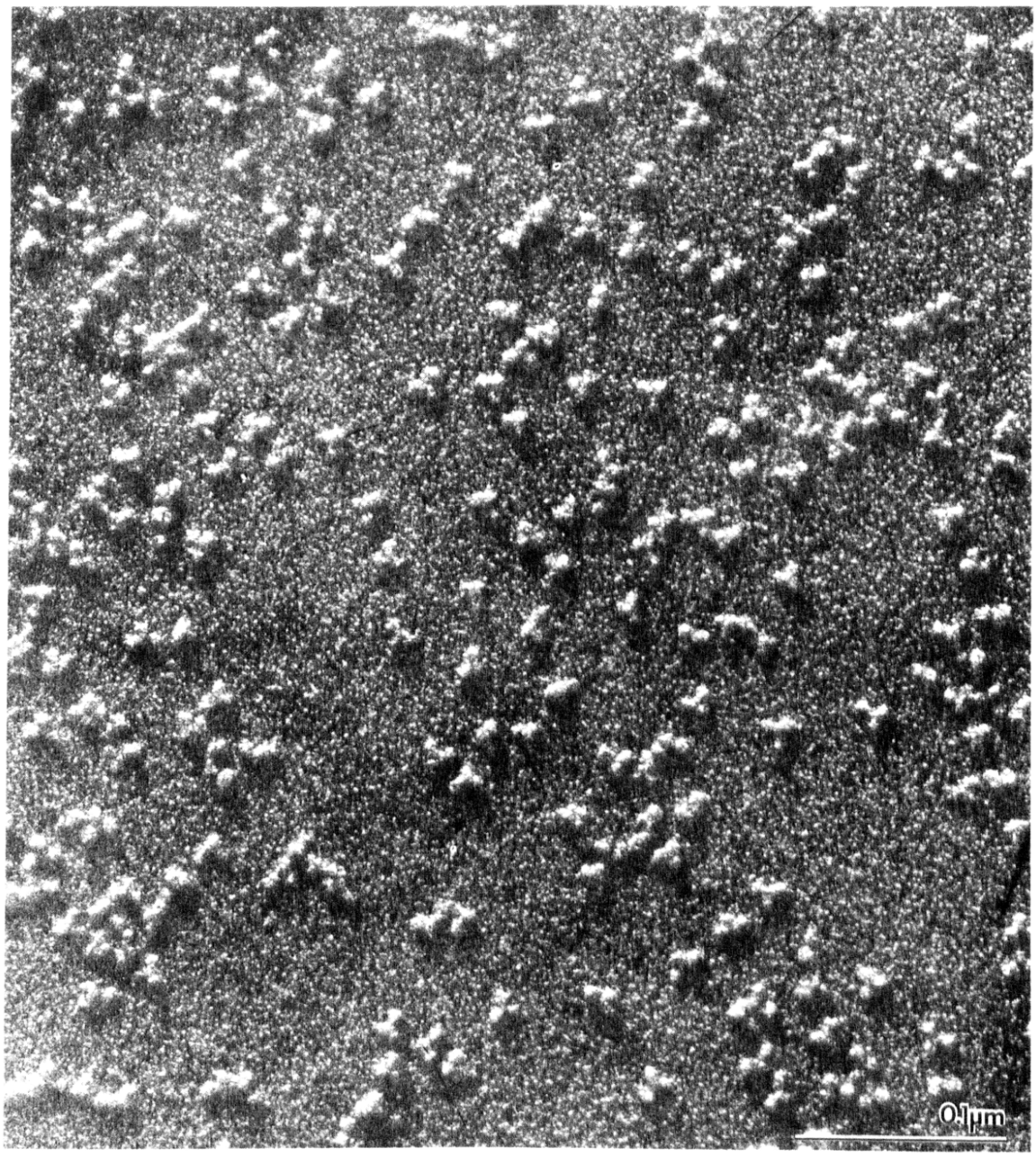

Fig. 2. Freeze-dried and high-resolution shadowed human IgG_1.

film can be inclined, the angle of shadowing cannot be determined accurately. Therefore, as a parameter characterizing the molecule height, we used the ratio of the shadow length of the whole molecule (L) to that of the individual Fab subunit (L_o) lying nearby. Thus, the L/L_o ratio indicates how many times the whole molecule is as high as the Fab subunit. It turned out that molecules with an L/L_o ratio close to 1 have an obtuse shadow apex. The molecules with the triangle-shaped shadows had the L/L_o always exceeding 1. Histogram in Fig. 4 clearly demonstrates that in the IgG_1 preparation under study about 70% of molecules are convex. The L/L_o ratio greatly varies thereby: from 1.2 (for almost flat molecules) to 1.7 (for convex molecules). Thus, in our IgG preparation about 30% of molecules have a flat conformation. Transformation of conformation from the flat to convex one is likely to occur due to molecule flexibility in the 'hinge region', the long axis of the Fc subunit being inclined to the Fab. A wide range of the L/L_o values indicates that in solution the Fab and Fc molecules seem to possess a rather high extent of mobility. The conformation can be considered as the predominant one when $L/L_o = 1.5$ (Fig. 4). We defined the molecules of this kind as tripod-like.

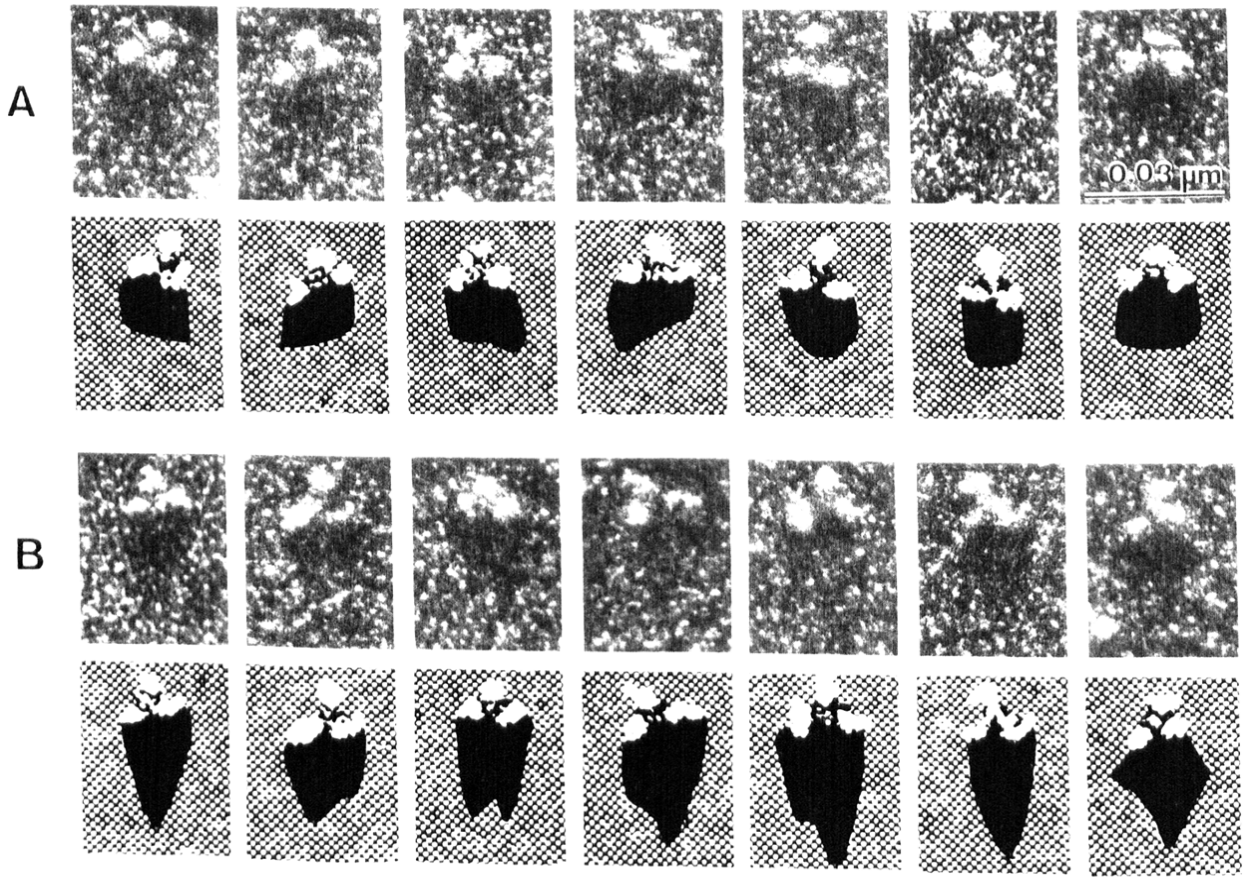

Fig. 3. Separate images of human IgG_1. (A) Convex molecules, triangle-shaped shadow. (B) Flat molecules, trapezium-like shadow.

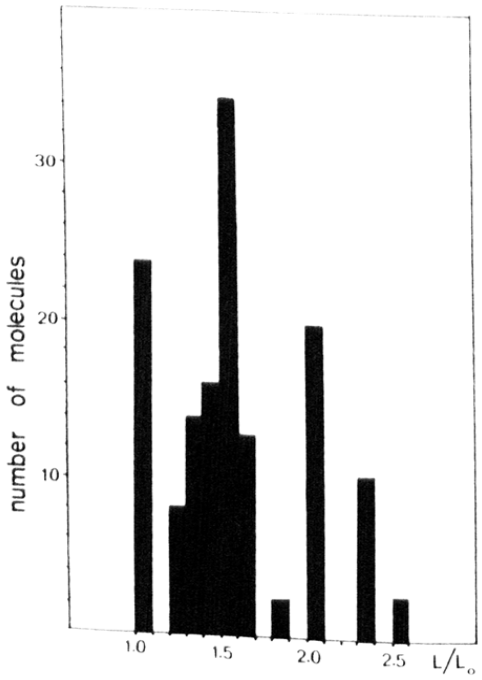

Fig. 4. Height distribution of the shadowed IgG_1 Van molecules. The ratio of the shadow length of the whole molecule (L) to that of the individual Fab subunit (L_0) lying nearby was used as a parameter.

Previously we described in detail the electron microscope images of pig IgG molecules [7]. The IgG_1 molecules shown in Figs 1, 2 and 3 have similar structural features. Some of them are shown in the model presented in Fig. 5. The model does not pretend to be a detailed description of the IgG_1 structure but is

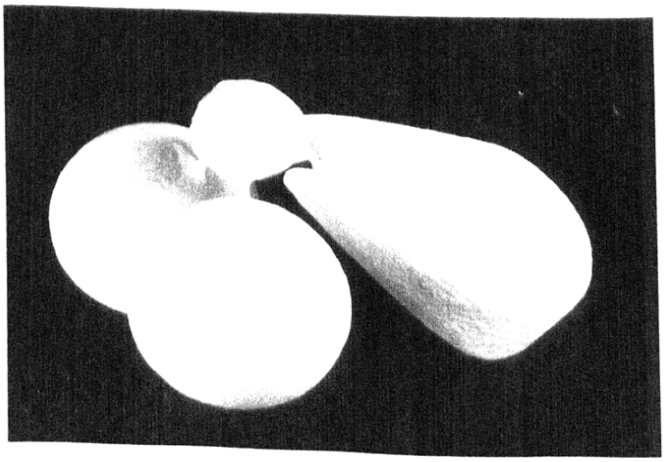

Fig. 5. Schematic presentation of the IgG model illustrating the tripod-like conformation of human IgG_1 Van.

presented only to illustrate the tripod-like shape of human IgG_1 molecule.

4. DISCUSSION

Electron microscopy studies provide evidence that the Fab and Fc subunits involved in the intact human IgG_1 Van molecule possess mobility. This result is in agreement with the data obtained for the same myeloma IgG_1 Van sample by polarization of fluorescence [5], spin label method [8], impulse NMR method [3].

Comparison of the results obtained for IgG_1 Van with the data previously obtained for the late non-precipitating pig antibodies and human IgG_3 Kue [7,13] allows one to conclude that probability of formation of the tripod-like conformation is not equal for different IgGs. It is highest for the non-precipitating pig antibodies (above 85%), somewhat lower for human IgG_1 Van (about 70%) and practically equiprobable to all possible conformations for human IgG_3 Kue.

The predominant conformation of IgG molecules in solution seems to be associated with the realization of their biological properties. The late pig antibodies have a rigid tripod-like structure [7]. The Fab and Fc subunits are fixed relative to each other, this probably induces steric hindrances for the formation of precipitates. It should also be emphasized that in case of the tripod-like conformation the Fab subunits do not produce steric hindrances for the interaction of the C_H2 domains of the Fc subunit with globular parts of C1q. In our opinion, a high complement-binding activity may be due to two different causes: (i) a high molecule flexibility when there exists a significant probability of occurrence of 'opened' (exposed to C1q) state of the C_H2 domains. This is examplified by human IgG_3 Kue; (ii) a low molecule flexibility when the C_H2 domains are accessible for the interaction with C1q. The late pig antibodies serve as an example. IgG_1 Van seems to stand midway between these two cases.

REFERENCES

[1] Dayhoff, M. (1972) Atlas of Protein Sequence and Structure, Nat. Biomed. Res. Found., Silverspring, USA.
[2] Michaelsen, T.E., Frangione, B. and Franklin, E.C. (1977) J. Biol. Chem. 252, 883–889.
[3] Ivannikov, A.I., Abramov, V.M., Volkov, V.Ya. and Zav'yalov, V.P. (1983) Mol. Biol. (Moscow) 17, 734–740.
[4] Calvanico, N.J. and Tomasi, T.B. (1978) in: Immunochemistry of Proteins, vol. 3 (Atassi, M.Z. ed) pp. 1–86, Plenum Press, New York.
[5] Dudich, E.L., Nezlin, R.S. and Franek, F. (1978) FEBS Lett. 89, 89–92.
[6] Fust, G., Medgyesi, G.A. and Franek, F. (1977) Immunochemistry 14, 259–262.
[7] Ryazantsev, S.N., Vasiliev, V.D., Abramov, V.M., Zav'yalov, V.P. and Franek, F. (1989) FEBS Lett. 244, 291–295.
[8] Zav'yalov, V.P., Abramov, V.M., Ivannikov, A.I., Loseva, O.I., Dudich, I.V., Dudich, E.L., Tishchenko, V.M., Khechinashvili, N.N., Franek, F., Medgyesi, G., Zavodszky, P. and Jaton, J.-C. (1981) Haematologia (Hungary) 14, 85–94.
[9] Denesyuk, A.I., Tishchenko, V.M., Abramov, V.M. and Zav'yalov, V.P. (1983) Mol. Biol. (Moscow) 17, 1262–1271.
[10] Abramov, V.M., Arkhangelskaya, Z.A. and Zav'yalov, V.P. (1983) Biochim. Biophys. Acta 742, 295–302.
[11] Valentine, R.C., Shapiro, B.M. and Stadtman, E.R. (1968) Biochemistry 7, 2143–2152.
[12] Vasiliev, V.D. and Koteliansky, V.E. (1979) Methods Enzymol. 59, 612–629.
[13] Ryazantsev, S.N. and Vasiliev, V.D. (1984) in: Abstr. 8th Eur. Congr. on Electron Microscopy, Budapest, Pub. Progr. Com., vol. 2, p. 1487.