IDENTICAL CHARACTER OF DIFFERENCES BETWEEN MURINE AND HUMAN NORMAL AND MYELOMA IgG STUDIED BY THE MONOLAYER METHOD

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Several different views are currently held on the nature of myeloma immunoglobulins. Some workers consider that myeloma proteins differ from normal only in their individuality and homogeneity [9, 11]. Others put forward the idea that immunoglobulins of myeloma origin are pathological [10, 12].

It has been shown by the method of monomolecular layers [7] that human myeloma IgG differ from normal IgG isolated from serum obtained from healthy blood donors in their orientation on the water—air and water—oil interface and their lower resistance to surface denaturation. The reason for this difference is evidently not that myeloma IgG is homogeneous, but that normal IgG is heterogeneous. The significantly less heterogeneous fraction of pig antihapten IgG antibodies did not differ in its behavior in monolayers from the total IgG fraction [6]. It has also been established that normal human, bovine, equine, and rabbit IgG have identical orientation at phase boundaries and equally high conformational stability. It was considered interesting to determine whether differences between human normal and myeloma IgG correspond to differences between these IgG in other species of animals.

The aim of this investigation was to study the properties of murine normal and myeloma IgG and to compare them with the properties of human normal and myeloma IgG.

EXPERIMENTAL METHOD

IgG were isolated from serum of noninbred mice by a combination of methods of fractionation with caprylic acid followed by ion-exchange chromatography on DE-32 cellulose [1]. Myeloma IgG 1 was isolated from ascites fluid of mice with plasmacytoma MOPC-21. Myeloma IgG 1 was obtained by two-stage chromatography on DE-32 cellulose and protein-A-sepharose (from Pharmacia, Sweden). The NaCl used to prepare solutions was of the highly pure grade.

Surface tension was measured by Wilhelmy's method based on equilibrium of a glass plate [4]. The accuracy of measurement of surface tension was ±0.15 mN/m. Monolayers were obtained by careful layering of solutions of proteins in 0.15M NaCl solution as described previously [13].

EXPERIMENTAL RESULTS

The rate of surface denaturation of proteins, a phenomenon to which they are exposed at the water—air interface, can be determined by the monolayer method. In the course of surface denaturation the native structure of the protein molecules is disturbed and all polypeptide chains tend to become oriented on the interface. The speed of this process depends both on the nature of the protein and on the pH and ionic strength of the underlying solution. For some conformationally stable proteins, this rate under certain conditions is so small that the orientation of native molecules on the interface can be established [3].

A comparative study of the stability of the structure of normal murine IgG and myeloma IgG 1 $\varkappa$ molecules was carried out in monomolecular layers on the interface between aqueous so-

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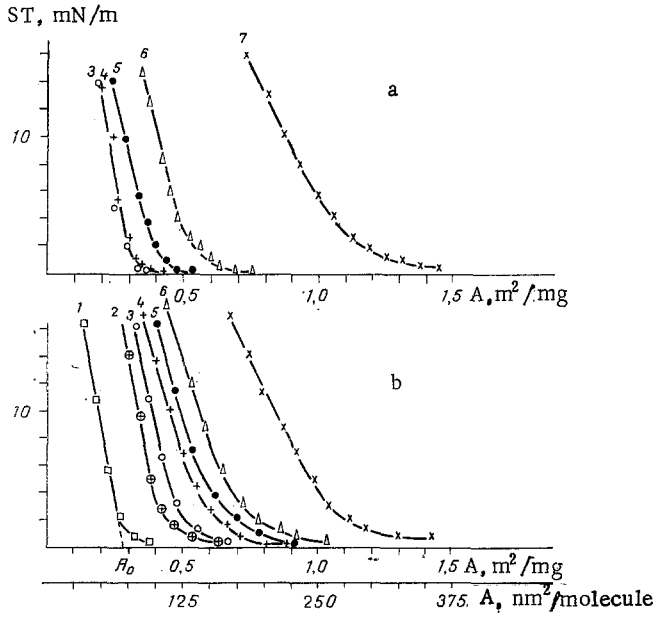

Fig. 1

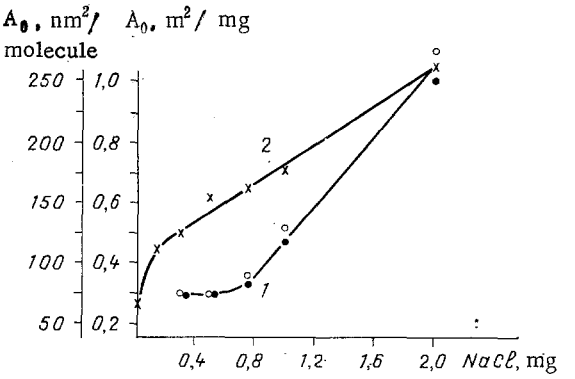

Fig. 2.

Fig. 1. ST—A curves for monolayers of mouse normal IgG (a) and myeloma IgG 1 κ (b) on NaCl solutions of different concentration. 1) Distilled water, 2) 0.15 N NaCl, 3) 0.3 N NaCl, 4) 0.5 N NaCl, 5) 0.75 N NaCl, 6) 1.0 N NaCl, 7) 2.0 N NaCl. Here and in Fig. 2, monolayer formation time was 15 min.

Fig. 2. Dependence of limiting area A_0 on concentration of NaCl solution for monolayers of murine normal IgG (1) and myeloma IgG 1 κ (2). Empty circles denote normal murine IgG, filled circles — normal human IgG, crosses — murine myeloma IgG 1 κ.

lutions of NaCl, with different salt concentrations, and air. Curves of surface tension (ST) as a function of area A pertaining to one protein molecule in the monolayer (ST—A curves) are given in Fig. 1 (monolayer formation time 15 min). By extrapolating the steeply climbing regions of the ST—A curves to ST = 0, the areas A_0 occupied by one protein molecule in a densely packed monolayer can be determined for solutions of different ionic strength. The relationship between A_0 and NaCl concentration for murine normal and myeloma IgG is illustrated in Fig. 2. For comparison, values of A_0 for normal human IgG taken from [6] are also given in Fig. 2. It will be clear from Fig. 2 that A_0—NaCl concentration curves for normal murine and human IgG coincide (curve 1). The horizontal part of these curves is evidence that within the NaCl concentration range from 0.3 to 0.6 M the surface denaturation rate is minimal and is independent of the salt concentration in solution. The constancy of A_0 within this interval is due to the stability of normal IgG molecules, which tend to preserve the native conformation on the surface. The value of A_0, namely 75 nm^2/molecule, is evidence of the horizontal orientation of the IgG molecules, in which the area of the protein molecule in the monolayer is only very little more than the area of greatest cross-section of the IgG molecule. The area of greatest cross-section of the IgG molecule, calculated as the sum of the greatest cross-sections of three IgG fragments using the data from [8], is 66 nm^2. (The area of least cross-section, equal to twice the area of cross-section of the Fab-fragment, is 19 nm^2.) It will be noted that the true value of the area occupied by a native protein molecule in the monolayer can be determined only by studying surface denaturation kinetics. However, as will be shown below, because of the slow rate of this process in the zone of stability, the values of A_0 for normal IgG, determined for a monolayer formation time of 15 min, differ only very little from the true value. The increase in A_0 for normal murine IgG within the NaCl concentration range from 0.75 to 2.0 M, from the value corresponding to the size of the native molecule up to a value of about 1 m^2/mg, evidence of total surface denaturation of the protein [2], indicates an increase in the rate of unrolling of the protein molecules, proportional to the salt concentration in solution.

For murine myeloma IgG (Fig, 2, curve 2) within the NaCl concentration range from 0.3 to 0.6 M, corresponding to the zone of stability of normal IgG, the observed values of A_0 were significantly higher than the area of the native molecule. The high rate of the surface de-

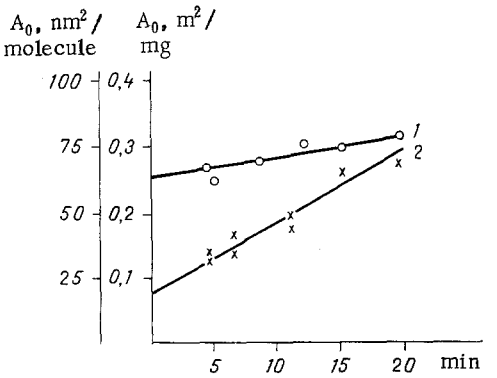

Fig. 3. Dependence of limiting area A_o on monolayer formation time for murine normal IgG (1) and myeloma IgG 1 κ (2).

naturation process under these conditions did not permit the orientation of the molecules in the monolayer to be determined. A similar decrease in conformational stability compared with normal has been observed for several human myeloma IgG [7]. Yet another difference between the murine myeloma protein studied and normal is that it formed monolayers on 0.15M NaCl and distilled water. A value of A_o equal to 70 nm²/molecule (Fig. 2, curve 2) corresponded to the monomolecular layers on distilled water. This value was apparently evidence of the horizontal orientation of molecules of this protein on the water surface. However, for human myeloma IgG, in every case when their orientation in the monolayers could be established, it was vertical [7]. Considering the high rate of surface denaturation of murine myeloma IgG and a considerable monolayer formation time (15 min), it might be supposed that the value obtained for A_o is not evidence of the horizontal orientation of the molecules, but characterizes molecules which have largely undergone surface denaturation.

The correct conclusion regarding orientation on the surface or conformationally unstable molecules can be drawn from kinetic data. The surface denaturation kinetics of murine myeloma IgG is illustrated in Fig. 3. For comparison the corresponding relationship for normal protein is shown in the same figure. It was found previously [5] that the true value of the area of the native molecule, determined from kinetic curves, is independent of the rate of the surface denaturation process. Since measurement of A_o—time curves is most conveniently done at the minimal rate of this process, measurements for myeloma IgG were made on distilled water, and for normal IgG in the zone of stability of the native structure on 0.5 M NaCl.

It will be clear from Fig. 3 that dependence of A_o on time is linear and, consequently, the rate of denaturation remained constant at the beginning of the process. The straight line of A_o as a function of time intercepts on the ordinate a segment corresponding to the area occupied on the interface by an IgG molecule immediately after application of the monolayer. For normal IgG this is 64 nm²/molecule, which confirms the conclusion that the orientation of these molecules is horizontal. For murine myeloma IgG this area was 20 nm²/molecule, unambiguous evidence of the vertical orientation of the molecules on the interface. Differences between murine myeloma and normal IgG are thus analogous to differences found previously for human myeloma IgG [7]. These differences are, first, that myeloma proteins have a vertical orientation on the water—air interface, by contrast to normal, which are oriented horizontally, and second, that their conformational stability is less.

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REGRESSION OF PLISS LYMPHOSARCOMA IN RATS WITH HYPOVITAMINOSIS A AND SOME BIOCHEMICAL PARAMETERS

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Many investigations showing that vitamin A and its derivatives have anticarcinogenic activity [10] and that retinoids inhibit growth of some transplantable tumors [6, 13, 14] have been published in recent years. Meanwhile the effect of vitamin A deficiency on growth of experimental tumors has not been adequately studied. Early investigations showed [8] that in rats kept on a diet deficient in vitamin A the rate of growth of several sarcomas and carcinomas is reduced, but the vitamin status of the animal was not evaluated in these experiments. The results of our own experiments in which, under strictly controlled conditions, it was shown that Pliss lymphosarcoma (PLS) in rats in a state of hypovitaminosis A undergoes complete regression, and that retinoic acid (RA), unlike retinol, does not support tumor growth, are described below.

EXPERIMENTAL METHOD

Male Wistar rats weighing initially 150-160 g were used. The animals were given food and water ad lib. The main experiments were carried out on 60 rats divided into three equal groups. The rats were kept on an artificial diet, including 20% casein, heated to 120°C for 48 h to destroy vitamin A [2], 67% potato starch, 5% refined sunflower oil, 3% of mixed salt [5], 4.6% of autoclaved bakers' yeast, 0.1% inositol, 0.2% choline chloride, and essential vitamins [11] except vitamin A. Rats of group 1 (control) additionally received 500 μg of retinyl acetate with 0.2 ml of sunflower oil once a week perorally. Rats of group 2 did not receive retinyl acetate. Rats of group 3 received 200 μg of the methyl ester of RA on alternate days with 0.2 ml sunflower oil. After 2 months half the rats of each group were inoculated subcutaneously with PLS by the standard method [1], using material obtained after a single passage of the tumor in rats in a state of hypovitaminosis A. On the 8th day after inoculation of the tumor these rats were killed for biochemical tests. During the first 7 days after inoculation of PLS, instead of the methyl ester of RA the rats of group 3 received 100 μg of [11-^{14}C]-RA (specific radioactivity 160 μCi/mmole) daily, perorally, with 0.2 ml of sunflower oil.

Amino acids extracted from blood plasma with 70% methanol were determined on the Liquimat III amino-acid analyzer (Labotron, West Germany), under standard conditions recommended by the firm (the authors are grateful for technical help to G. S. Kaloshina, Junior Scientific Assistant at the A. I. Bakh Institute of Biochemistry, Academy of Sciences of the USSR).

The concentrations of retinyl palmitate (RP) and retinol in liver and PLS tissue (calculated per gram wet weight) were determined spectrophotometrically after preliminary fractionation of the extract by thin-layer chromatography on aluminum hydroxide [3], and the identity of the corresponding fractions and their quantitative proportions were confirmed by radioactive labeling (preliminary injection of [11-^{14}C]retinol into the animals). Labeled RA and its derivatives, extracted from the tissues with a mixture of chloroform and methanol (1:1), were

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