PHYLOGENESIS OF THE GENERAL STRUCTURE OF IMMUNOGLOBULINS. RIGIDITY OF 7 S IMMUNOGLOBULINS AND RESTRICTED FLEXIBILITY OF THE 17 S IMMUNOGLOBULINS OF THE TORTOISE, *TESTUDO HORSFIELDI*

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1. Introduction

Previously it has been shown that IgG and IgM of mammals (man, rabbit and rat) are characterized by a flexible structure which facilitates the interaction of the active Fab fragments with antigenic determinants [1–5]. It has also been found that carp macroglobulins have a considerably more compact general structure [6]. Therefore, it was interesting to elucidate whether the differences in the lability of the general structure of immunoglobulins of mammals and carp are connected with the evolution process or may be accounted for by some other phenomena. The purpose of this study is the investigation of the general structure of the 7 S and 17 S immunoglobulins of the tortoise, a representative of the intermediate vertebrate class of reptiles, by the fluorescence polarisation technique previously used for the investigation of the general structure of IgG and IgM of mammals and carp [1–6]. These immunoglobulins were found to have some features similar to those of IgG and IgM of mammals [7].

2. Materials and methods

The immunoglobulins were isolated by the method of Action et al. [8, 9]. Tortoise serum was kept at room temperature and then centrifuged for 15 min at 3000 *g*. The supernatant was applied to a Sephadex G-200 column (3.2 × 100 cm) equilibrated with 0.15 M NaCl in 0.015 M Tris–HCl buffer, pH 8.0. Fractions corresponding to 7 S and 17 S immunoglobulins were subjected to ion-exchange chromatography on DEAE-Sephadex A-50 (2.6 × 34 cm). A linear salt concentration gradient was used for elution (0.1 M–0.7 M NaCl in 0.015 M Tris–HCl, pH 8.0). Fractions corresponding to the maximum of the single peak were pooled and concentrated by ultrafiltration on XM-50 membranes (Amicon). Thereafter the solution was applied either to a Sephadex G-200 column (2.6 × 53 cm) equilibrated with 0.15 M NaCl in 0.015 M Tris–HCl, pH 7.5 (7 S immunoglobulins) or to a Sepharose 6B column (1.9 × 29 cm) equilibrated with the same buffer (17 S immunoglobulins). Immunoelectrophoresis was performed with rabbit antiserum against tortoise whole serum [10].

Fractions concentrated after separation on DEAE-Sephadex were dyed with 1-dimethylaminonaphthalene 5-sulphonyl (DNS) chloride (Fluka), extensively dialysed and passed through columns with Sepharose 6B or Sephadex G-200 as described above. Maximum fractions of a rather symmetrical peak were taken for measurements. The mol wt. of 17 S immunoglobulins was considered to be 900 000, that of 7 S immunoglobulins 150 000. The absorption coefficient was assumed to be 14.0 for both proteins. The molar ratio of DNS to protein was estimated as previously described [1, 6] and found to vary from 35–45 in 17 S immunoglobulins and from 2.3–4.5 in the 7 S. The lifetime of excited DNS molecules (τ) and degree of fluorescence polarisation (p) were measured as described elsewhere [1]; the rotational correlation time (ϕ_h)* was calculated as previously [1, 2, 5, 6].

* ϕ_h is used instead of rotational relaxation time (ρ_h). They are related by $\phi_h = \rho_h/3$.

3. Results and discussion

The ultracentrifugation pattern of 7 S and 17 S immunoglobulin preparations obtained by using the procedure described above is shown in fig. 1. One can see that both preparations are homogeneous and do not contain considerable contamination with low or high molecular weight substances. The sedimentation coefficient of the macroglobulin was found to depend on the concentration as was consistent with the data presented in [9]; that of 7 S immunoglobulin was almost independent of concentration [9]. Extrapolation to zero concentration yielded the values of 17.8 S and 7.2 S, respectively. These constants coincide with earlier values [8, 9]. Immunoelectrophoresis showed that the purified immunoglobulin preparations were devoid of bulk proteins (fig. 2). The precipitation bands corresponding to 7 S immunoglobulin (A) and to macroglobulin (B) were similar to the bands of IgG and IgM of mammals, respectively [8, 11].

The data from polarisation experiments are presented in fig. 3 as Perrin–Weber plots of $1/p$ versus temperature–viscosity ratio. The values of τ = 10.2–11.8 nsec were estimated for tortoise macroglobulin preparations with different binding ratios with a phase fluorometer. Using the equation which takes into

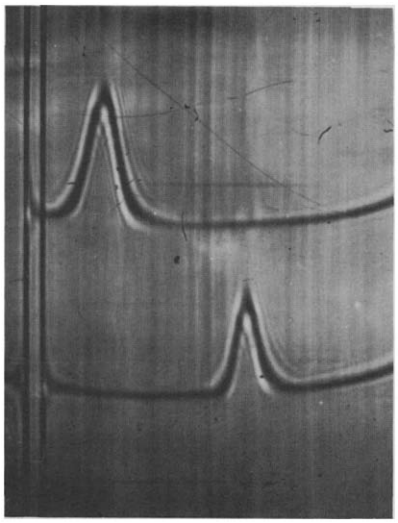

Fig. 1. Sedimentation pattern of tortoise purified immunoglobulins. A): 7 S immunoglobulin at concentration 2.0 mg/ml; B): macroglobulin, 0.8 mg/ml. Photographs were taken 30 min after reaching 50740 rpm, ANE-rotor, 20°, 0.015 M Tris–HCl pH 7.5. in 0.15 M NaCl.

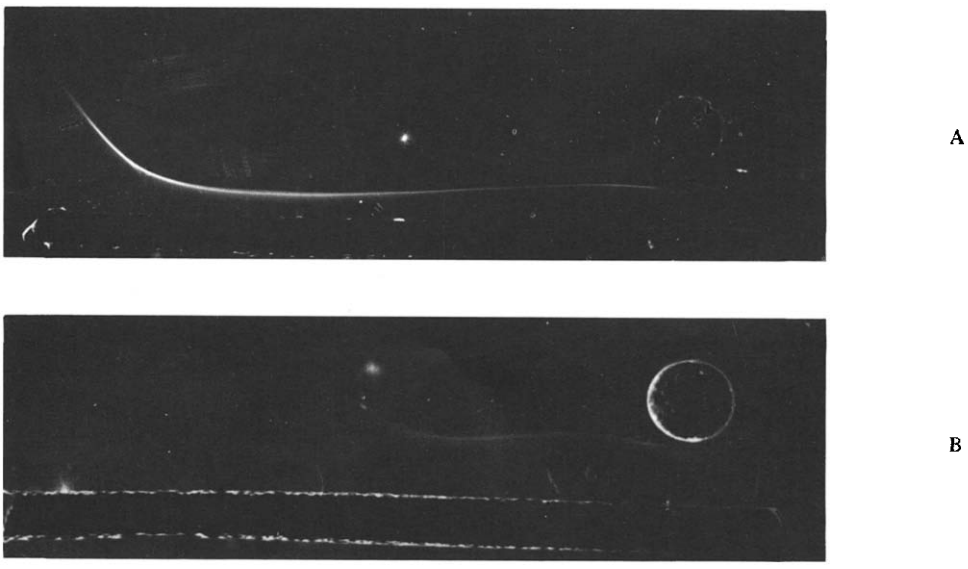

Fig. 2. Immunoelectrophoretic analysis. A): 7 S immunoglobulins of the tortoise, 7.0 mg/ml; B): tortoise macroglogulin, 4.1 mg/ml. Precipitation was obtained by using rabbit antiserum against the tortoise whole serum. Cathode is on the left.

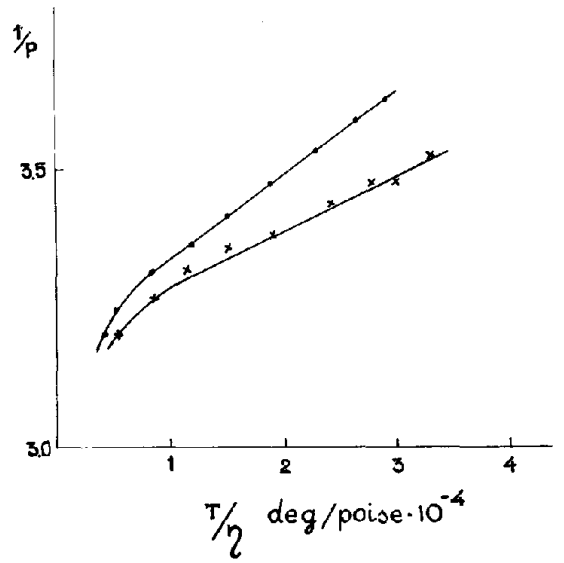

Fig. 3. Depedence of reciprocal of the fluorescence polarisation ($1/p$) upon temperature divided by viscosity (T/η) for DNS-conjugates of 7 S immunoglobulins (•—•—•) and macroglobulin (×—×—×) (25°C). Wavelength of excitation by polarised light 365 nm.

consideration the thermally activated dye rotation [12], ϕ_h has been found to be 103 nsec. This value was almost independent of the molar ratio of DNA to protein. For the preparations of 7 S immunoglobulins with different binding coefficients τ was equal to 11.3–13 nsec; in this case ϕ_h was found to be 68 nsec and to depend on the binding ratio rather inconsiderably, too.

Table 1 shows value of ϕ_h for tortoise immunoglobulins found experimentally. In the same table the values are given (ϕ_0) calculated for a rigid spherical protein particle with similar molecular weight, hydration and partial specific volume [8, 9, 14, 15]. In these calculations the value 0.3 g/g was taken for hydration of immunoglobulins of lower vertebrates. Table 1 includes also the values of ratio ϕ_h/ϕ_0 which decrease with appearance of freedom of intramolecular rotation. In the case of tortoise 7 S immunoglobulins this ratio is equal to 1.26. This is a direct indication of a compact general structure of these proteins. Contrary to mammalian IgG, tortoise 7 S immunoglobulins do not possess pronounced intramolecular freedom of rotation of high molecular weight fragments. It is rather likely that these distinctive features of the general structure may be responsible for the differences in behavior of IgG of mammals and 7 S immunoglobulins of tortoise in a number of experiments (despite of the similarity of molecular weights). For example, upon gel filtration through a Sephadex G-200 column (V = 800 ml) human IgG is eluted much later than tortoise 7 S immunoglobulins (36 ml diference); IgG of mammals are characterized by a more pronounced dependence of sedimentation coefficient on the concentration. The ratio ϕ_h/ϕ_0 for tortoise macroglobulin is 0.32. This shows that in this case freedom of intramolecular rotation of flexibly linked small subunits does exist. However, this freedom is much more restricted as compared to IgM of mammals.

Table 1

Rotational correlation time of DNS-conjugates of immunoglobulins of different vertebrate classes ($T/\eta = 3.34 \times 10^4$ deg/poise, water 25°C).

DNS conjugate	Mol wt.	$\phi_h$exp (nsec)	$\phi_0$calc (nsec)	ϕ_h/ϕ_0
Carp macroglobulin*	7×10^5	128	251	0.51
Frog macroglobulin**	9×10^5	135	326	0.42
Tortoise macroglobulin	9×10^5	103	326	0.32
Human macroglobulin (IgM)***	9×10^5	27–47	350	0.08–0.13
7 S frog immunoglobulin**	15×10^4	67	54	1.24
7 S tortoise immunoglobulin	15×10^4	68	54	1.26
7 S human and rat immunoglobulin (IgG)****	15×10^4	20–21	59	0.35

* Taken from [6].
** Taken from [13].
*** Taken from [5].
**** Taken from [1, 2].

Table 1 shows the regularity of changes of the ratio ϕ_h/ϕ_0 in the course of phylogenesis: from 0.51 for macroglobulin of the carp to 0.08–0.13 for that of man and from ~1.25 for 7 S immunoglobulins of the frog and tortoise to 0.35 for IgG (7 S) of mammals. Since the hydrodynamic parameters used for calculation of the axial ratio of a molecule approximated to an ellipsoid are rather similar for the immunoglobulins of lower and higher vertebrates [8, 9, 14, 16] and cannot considerably alter the value of the ratio ϕ_h/ϕ_0 [14, 17] **, it is clear that the regularity noted is due to the fact that the general structure of immunoglobulins becomes more labile in the process of evolution of the vertebrate type.

Therefore, one can think that the evolution of the antibody structure is directed towards greater flexibility which facilitated interaction of a polyvalent antibody with antigens and thus favoured survival. One can expect that the other representatives of the classes under study are characterized with the same features.

A suggestion may be put forward that the structural changes mentioned above which improve the functional properties in the evolution process may be accounted for by the fact that immunoglobulins are rather young proteins existing only in vertebrates [7]. Many enzymatic or other systems which had appeared at early stages of development of animals underwent such selection much earlier.

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** For example, change of the axial ratio of molecule approximated to an elongation rotation ellipsoid from 1 to ∞ results in the increase of ϕ_h/ϕ_0 by 90% only [17].

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