Self-Association of Bovine Immunoglobulin G1

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The self-association properties of bovine serum immunoglobulin G1 and colostral immunoglobulin G1 (IgG1) in 0.32 M-NaCl/0.01 M-Tris/HCl, pH 8.0, were investigated by analysing sedimentation data according to a monomer–dimer association model. The self-association was characterized by an equilibrium constant of $5.3 \times 10^4 \pm 3.5 \times 10^4 \text{ M}^{-1}$ for serum IgG1 and $1.6 \times 10^3 \pm 0.69 \times 10^3 \text{ M}^{-1}$ for colostral IgG1. The removal of the Fc portion of IgG1 by pepsin digestion abolished its property of self-aggregation. At high total protein concentrations of serum IgG1, low concentrations of the ostensible trimer species were observed. However, no self-aggregation was evident when 0.14 M-NaCl/0.01 M-sodium phosphate, pH 6.0, was used as a solvent, thus confirming results published previously [Tewari & Mukkur (1975) *Immunochemistry* 12, 925–930].

Previous investigations (Tewari & Mukkur, 1975) demonstrated that bovine serum IgG1 and colostral IgG1 underwent self-aggregation under various conditions of ionic strength and pH. Further, the aggregation was found to be concentration-dependent in that an increase in concentration led to the formation of dimers and probably trimers. In the present communication we report on the possible nature and equilibrium constants of this association and the region of the IgG1 molecule that participates in formation of these aggregates.

Experimental

Isolation of bovine serum IgG1 and colostral IgG1

Bovine serum IgG1 and colostral IgG1 were prepared by using methods described previously (Tewari & Mukkur, 1975). The procedure involved precipitation of immunoglobulins with either Na_2SO_4 (for serum) or $(\text{NH}_4)_2\text{SO}_4$ (for colostrum), gel filtration on Bio-Gel P-300, followed by chromatography on triethylaminoethyl (TEAE)-cellulose. The isolated IgG1 was then checked for purity by criteria published previously, the concentration of IgG1 being determined by using an absorption coefficient of $12.1 \text{ M}^{-1} \cdot \text{cm}^{-1}$ (Tewari & Mukkur, 1975).

Peptic digestion of bovine serum IgG1 and colostral IgG1

Peptic digestion was carried out as described by Fang & Mukkur (1976).

Abbreviation used: IgG1, immunoglobulin G1.

Conditions of IgG1 aggregation

IgG1 was dialysed extensively against 0.32 M-NaCl/0.01 M-Tris/HCl, pH 8.0, and subjected to analytical ultracentrifugation (Tewari & Mukkur, 1975).

Determination of sedimentation coefficients ($s_{20,w}$) and weight-average sedimentation coefficients ($\bar{s}_{20,w}$)

A Beckman model E analytical ultracentrifuge equipped with electronic speed control and a schlieren optical system was used. Sedimentation-velocity experiments were performed at either 56000 or 60000 rev./min at 20°C in an An-H rotor.

Double-sector cells were used to allow base-line determination. Patterns were recorded on metallo-graphic plates and measured with a micro-comparator. Sedimentation coefficients of individual species were determined from the rates of movement of the sedimenting boundary as measured by the positions of the maximum ordinates of the schlieren patterns. Weight-average sedimentation coefficients were measured as the rates of movement of the positions of the square roots of the second moments of the refractive-index gradient curves (Goldberg, 1953; Schachman, 1959). Usually 20–40 co-ordinates on each schlieren pattern were measured relative to the solvent base-line in order to calculate this position. Plots of the logarithm of the square root of the second moment versus time were essentially linear. Observed sedimentation coefficients were corrected to values corresponding to a solvent with the viscosity and density of water at 20°C (Schachman, 1957). No corrections were made for concentration-dependence or Johnston–Ogston effects.

Theory

Sedimentation analysis revealed two major boundaries, representing protein species whose relative concentrations changed with total concentration. At higher concentrations a third boundary was evident with serum IgG1, but its concentration was such as to make an insignificant contribution to the weight-average sedimentation coefficient.

For a monomer-dimer association the equilibrium constant (M^{-1}) is given by:

$$K = \frac{c_2}{c_1^2} \cdot \frac{M_1}{2} \quad (1)$$

and

$$c_T = c_1 + c_2 \quad (2)$$

where c_1 , c_2 and c_T are monomer, dimer and total concentrations (mg/ml) respectively and M_1 is the monomer molecular weight. The weight-average sedimentation coefficient for such a system is defined by:

$$\bar{s} = \frac{s_1 c_1 + s_2 c_2}{c_T} \quad (3)$$

where s_1 and s_2 are the sedimentation coefficients of monomer and dimer respectively. Eqns. (1)–(3) can be solved to obtain an explicit function for K :

$$K = \frac{(\bar{s} - s_1)(s_2 - s_1)M_1}{2c_T(s_2 - \bar{s})^2} \quad (4)$$

Results and Discussion

Previous investigations revealed that bovine serum IgG1 and colostral IgG1 underwent self-association in buffers of various values of ionic strength and pH, thus creating problems in the purification of immunoglobulin A (Tewari & Mukkur, 1975). It was further reported that the self-association was reversible and concentration-dependent in that an increase in concentration results in the formation of increased amounts of dimeric and probably trimeric species.

In the present investigation, the buffer used to ensure maximum polymerization was 0.32M-NaCl/0.01M-Tris/HCl, pH8.0. This buffer has been employed frequently during the process of purification of bovine serum immunoglobulins and colostral immunoglobulins (Mukkur & Froese, 1971; Mukkur, 1972; Tewari & Mukkur, 1975).

When either serum IgG1 or colostral IgG1 was subjected to analytical velocity ultracentrifugation at various concentrations, two sedimentation boundaries were observed, a small third peak appearing at higher concentrations with serum IgG1 (Fig. 1). The amount of material represented by the third peak was only 2% of the total at 7.34 mg/ml and this has been ignored in the calculations reported below. With

Fig. 1. Schlieren patterns of serum IgG1 (a and b) and colostral IgG1 (c and d) at various concentrations either in 0.32M-NaCl/0.01M-Tris/HCl, pH8.0 (a and d), or in 0.14M-NaCl/0.01M-sodium phosphate buffer, pH6.0 (b and c).

Experimental details are given in the text. The sedimentation velocity runs were performed either at 60000 rev./min with pictures being taken at 14 min after reaching speed (a and b) or at 56000 rev./min with pictures being taken at 23 min after reaching speed (c and d).

both samples the relative proportion of the slowest boundary decreased as the concentration was increased. In contrast, no aggregation of either serum IgG1 or colostral IgG1 was observed when the solvent used was 0.14M-NaCl/0.01M-sodium phosphate buffer, pH6.0 (Fig. 1b and 1c). The sedimentation coefficients of the individual boundaries observed at pH8.0 were measured (Tables 1 and 2) and compared with values expected if the faster-sedimenting species were assumed to be aggregates

Table 1. *Sedimentation coefficients of bovine serum IgG1 at various concentrations*
Experimental details are given in the text. N.D., Not detected.

Concn. (mg/ml)	$s_{20,w}$ of individual peaks* (S)			$s_{20,w}$ in phosphate buffer, pH 6.0† (S)	$\bar{s}_{20,w}$ * (S)	$10^{-4} \times K$ (M ⁻¹)
	1	2	3			
7.34	6.66	10.60	13.6	7.0	9.01	3.5
3.43	6.84	10.80	N.D.	7.05	8.50	9.4
2.87	6.87	10.84	N.D.	7.08	8.35	3.0

* The buffer used was 0.32M-NaCl/0.01 M-Tris/HCl, pH 8.0.

† The buffer used was 0.14M-NaCl/0.01 M-sodium phosphate, pH 6.0.

Table 2. *Sedimentation coefficients of bovine colostral IgG1 at various concentrations*
Experimental details are given in the text.

Concn. (mg/ml)	$s_{20,w}$ of individual peaks* (S)		$s_{20,w}$ in phosphate buffer, pH 6.0* (S)	$\bar{s}_{20,w}$ * (S)	$10^{-3} \times K$ (M ⁻¹)
	1	2			
12	6.46	10.26	6.74	7.41	2.6
10	6.55	10.40	6.78	7.24	1.9
8.5	6.62	10.50	6.84	7.08	1.3
5.9	6.74	10.72	6.95	6.90	0.50

* Buffers were the same as those given in Table 1.

of the monomeric species. For these calculations it was assumed that the particles sedimented as anhydrous spheres, in which case their sedimentation coefficients would be proportional to their molecular weights raised to the $\frac{2}{3}$ power (Schachman, 1959). This proportionality was observed in every case. Clearly IgG1 from both sources conforms to a monomer-dimer slow equilibrium system, with trimer becoming apparent at high concentrations.

Because of the equilibrium nature of the species, it was not valid to calculate equilibrium constants from the apparent relative amounts of the species represented by the peaks. Rather, the total boundary between solvent and solution of original composition was treated as a reaction boundary from which equilibrium constants could be calculated once the weight-average sedimentation coefficient was known. The monomer-dimer equilibrium constants calculated in this way are shown in Tables 1 and 2. Average values are $5.3 \times 10^4 \pm 3.5 \times 10^4 \text{ M}^{-1}$ and $1.6 \times 10^3 \pm 0.69 \times 10^3 \text{ M}^{-1}$ for serum IgG1 and colostral IgG1 respectively. The association constant for serum IgG1 is 1–2 orders of magnitude greater than that for colostral IgG1, this presumably reflecting the known physicochemical differences between the two immunoglobulins (Tewari & Mukkur, 1975).

The next objective was to determine the region of IgG1 that was predominantly responsible for self-association. The IgG1 was dialysed against 0.2M-sodium acetate buffer, pH 4.5, and subjected to peptic digestion. Ultracentrifugation of both serum IgG1

and colostral IgG1 treated in this way failed to reveal any self-association. However, non-digested control samples treated in the same way also showed no self-association at pH 4.5. When digested and control samples were subsequently redialysed against 0.32M-NaCl/0.01 M-Tris/HCl buffer, pH 8.0, only the non-digested sample retained the ability to self-associate. The digested sample showed only one sedimenting boundary with a sedimentation coefficient of 5.35S, which is characteristic of fragment F(ab')₂ (Fang & Mukkur, 1976). Since pepsin digestion results in the breakdown of the Fc region of IgG1 to diffusible peptides, it was concluded that this region is essential for self-association. Such interactions may be between Fc regions of different IgG1 molecules or between the Fc and other regions of the same IgG1 molecule.

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