THE CONFORMATION OF HUMAN IMMUNOGLOBULIN D

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1. Introduction

Human immunoglobulin D (IgD) is present in low concentrations in normal human serum, usually less than 1% of the total immunoglobulin population [1]. Little is known of the physiological role of this immunoglobulin class since IgD molecules appear to lack the structural requirements for many effector functions, such as skin sensitisation and complement activation [2]. There have also been few reports of antibody activity associated with IgD. However, recent work suggests that IgD may be particularly active as a lymphocyte membrane antigen receptor [3]. Chemically-derived data, such as the lack of convincing homology of the 'hinge' region with other human immunoglobulin heavy chain sequences [4], the comparatively low proline content [5], the distinctive susceptibility to proteolysis and high carbohydrate content [6], all suggest a unique structure of IgD within the general immunoglobulin structural pattern of two heavy and two light polypeptide chains.

We have studied two isolated IgD myeloma proteins using circular dichroism (CD) and isoelectric focusing (IEF) in order to try to clarify any unusual structural features of the molecule that could be responsible for the distinctive physiological behaviour of this immunoglobulin class. Neither the IEF pattern nor the CD spectra of IgD have been reported previously.

2. Materials and methods

Serum from two patients (A and B) suffering from multiple myeloma (IgD) were generous gifts from Dr I. C. M. MacLennan and Dr O. Cromwell, respectively. Following precipitation with 19.3% ammonium sulphate containing 4% NaCl, 30 mg of each immunoglobulin fraction were electrofocused on a preparative LKB Uniphor sucrose gradient at 4°C as previously described [7]. Fractions containing IgD eluted between pI 5.4 to 6.1 for IgD 'A' and 4.7 to 5.8 for IgD 'B'. These fractions were pooled and concentrated by ultrafiltration.

Analysis of the purified proteins by immunoelectrophoresis showed no reaction against antisera specific for human γ, α or μ heavy chains and a single precipitin line against rabbit antiserum specific for δ heavy chains (fig. 1). The rabbit antiserum to human δ heavy chain was a kind gift from the Department of Experimental Pathology, Birmingham University. A single precipitin line against antisera specific for L-type light chains was also obtained with both IgD preparations.

Analytical isoelectric focusing of the purified IgD myeloma proteins in 7.5% polyacrylamide rods was carried out as described previously [8]. Ampholine carrier ampholytes (pH 4–9) were used.

Circular dichroic spectra were recorded on a Cary 61 recording spectropolarimeter as described previously [9]. All samples had been dialysed against 0.01M Na_2HPO_4-NaH_2PO_4 buffer, pH 7.4, and passed through a sterile Millipore filter (0.45 μ) immediately prior to CD analysis. A mean residue weight of 109 was assumed and data are expressed as mean residue ellipticity (θ') in degrees. cm^2/decimole. Protein concentrations were determined spectrophotometrically using an extinction coefficient as 280 nm of $E^{1\%}_{1\ cm} = 14.5$ for IgD [6].

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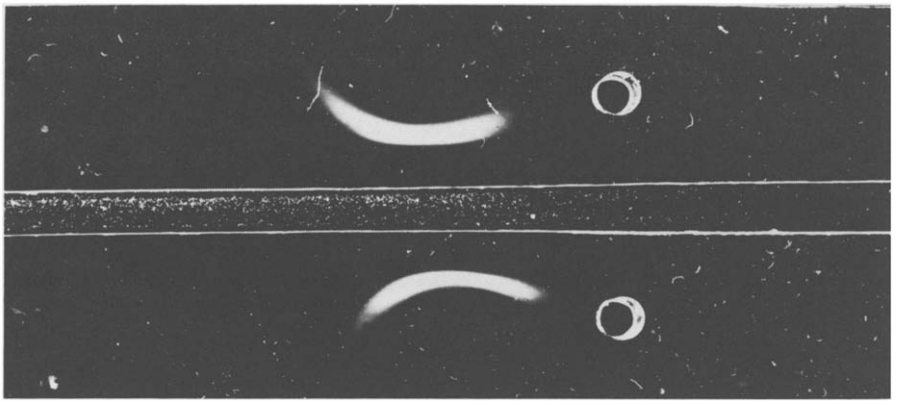

Fig. 1. Immunoelectrophoresis of IgD 'A' (bottom well) and IgD 'B' (top well) against rabbit antiserum to human δ chains. Anode on right.

3. Results and discussions

Both IgD myeloma proteins showed a high degree of microheterogeneity when analysed by isoelectric focusing (fig. 2). They focused between pI values of 5.4–6.1 for IgD 'A' and 4.7–5.8 for IgD 'B'. Both proteins exhibited similar multi-line patterns of greater than 10 lines. Williamson et al. [10] have proposed that the microheterogeneity of IgG myeloma proteins on IEF is due to deamidation of the polypeptide chains. However the IgD myeloma proteins exhibit a higher degree of heterogeneity than IgG myeloma proteins [11] and their IEF pattern is similar to that shown by IgA myeloma proteins, for which the microheterogeneity has been ascribed to the carbohydrate content and the acidity of the isoelectric points

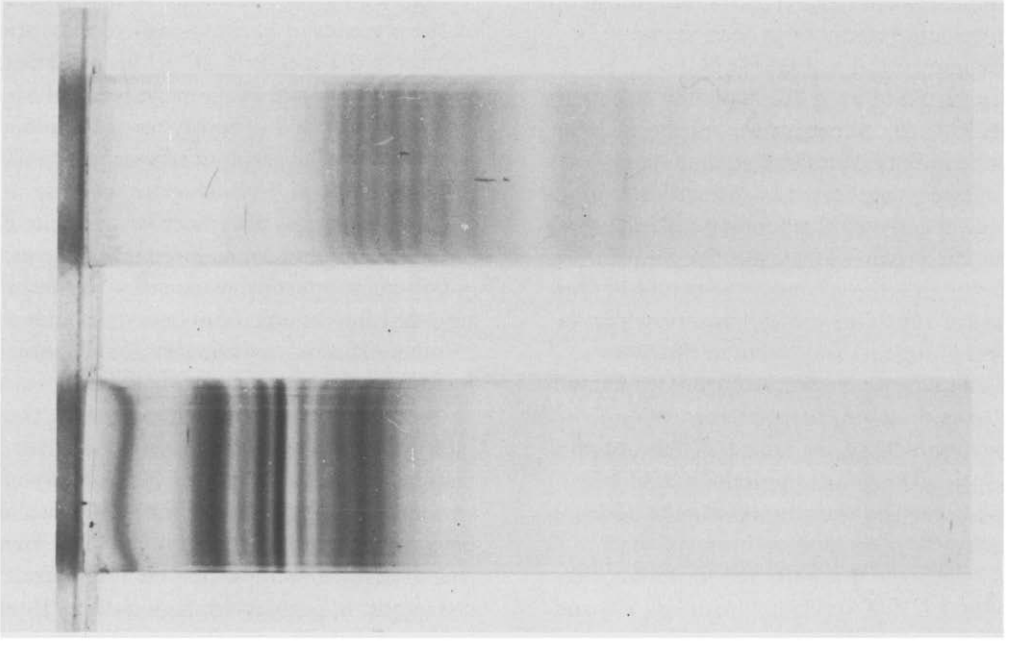

Fig. 2. Electrofocusing pattern for IgD 'A' (top tube) and IgD 'B' (bottom tube). pH range 4–9, anode on left.

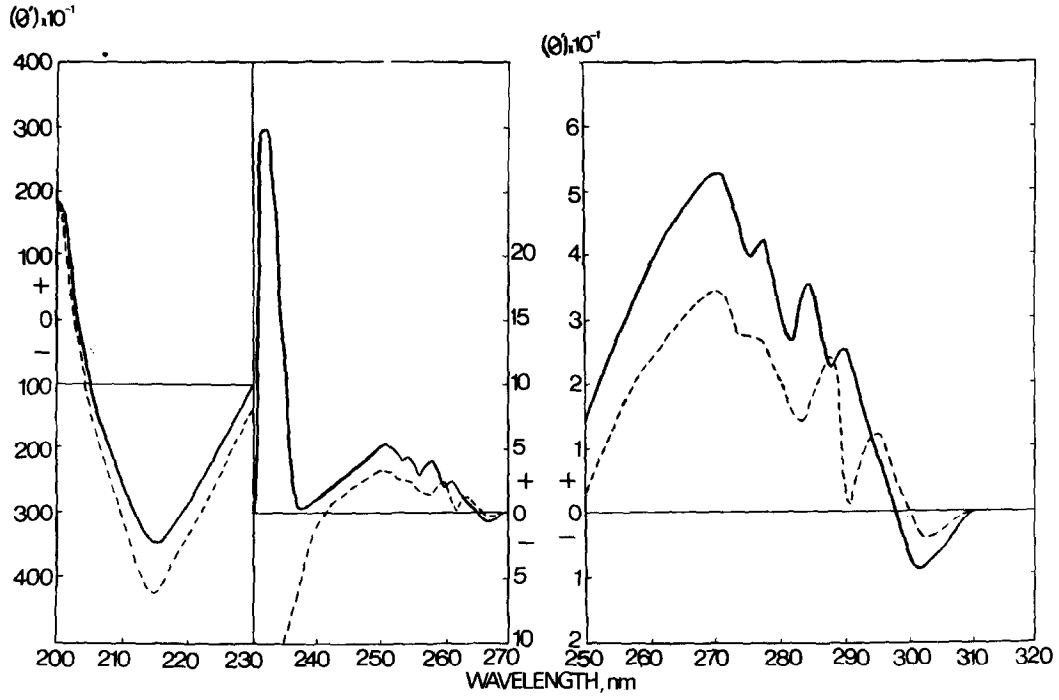

Fig. 3. Circular dichroism spectra of IgD 'A', (– – – –); and IgD 'B' (————) The ellipticity scale is expanded in the right hand figure to show greater spectral detail between 250 nm and 320 nm.

to the high sialic acid content [12]. Indeed, the nature of the digosaccharide content has been shown to be analogous for human IgA and IgD [13].

The CD spectrum of both IgD myeloma proteins is shown in fig. 3. The large negative transition at 217 nm is a common feature of other human immunoglobulin classes and has been implicated as characteristic of the β-structure of the polypeptide bonding [14]. The size of this transition and that of the positive transition at 200 nm indicate that the secondary structure of both these human IgD myeloma proteins is very similar in nature and magnitude to that found in the other human immunoglobulin classes. At higher wavelengths, the smaller transitions are characteristic of the asymetric environment of the aromatic and disulphide groupings. Both IgD myeloma proteins exhibited a similar series of positive transitions between 250 and 310 nm. Such a CD spectrum in this wavelength region is also similar to that reported for rabbit IgG [15] and human IgE [16]; both human IgG [9] and human IgM (unpublished observations) show predominantly negative transitions in this wavelength region.

The two IgD proteins differed in their CD spectra by the presence of a strong positive absorption at 232 nm in the spectrum of IgD 'B'. A transition at this wavelength and of the same order of magnitude has been observed previously for IgG 3 subclass proteins [9]. The origin of this transition has been discussed [9] and, by analogy to IgG myeloma proteins, a subclass difference between these two IgD myeloma proteins can be inferred. However, we could not obtain supporting evidence by the detection of any 'spurring' of precipitin lines on double radial immunodiffusion analysis against antiserum monospecific for δ heavy chains.

We conclude from the CD analysis of two human IgD myeloma proteins that there are no exceptional features characteristic of either secondary or tertiary structure that may account for the unusual biophysical properties of this immunoglobulin class. Hence, the lack of many effector functions could result from differences in primary amino-sequence. However Kehoe et al. [17] have recently suggested that the complement-binding site is dependent on the con-

formation of at least part of the C_H2 domain. Our IEF results suggest that the binding of carbohydrate moieties to IgD may be crucial to its physiological role since they appear to bestow IgD myeloma proteins with a low pI value and high degree of microheterogeniety.

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References

[1] Rowe, D. S. and Fahey, J. L. (1965) J. Exp. Med., 121, 185.
[2] Henney, C. S., Welscher, H. D., Terry, W. D. and Rowe, D. S. (1969) Immunochemistry, 6, 445.
[3] Rowe, D. S., Hug, K., Forni, L. and Pernis, B. (1973) J. Exp. Med., 138, 965.
[4] Perry, M. B. and Milstein, C. (1970) Nature, 228, 934.
[5] Saha, A., Chowdrey, P., Sambury, S., Behelak, Y., Heiner, D. C. and Rose, B. (1970) J. Immunol., 105, 238.
[6] Spiegelberg, H. L., Prahl, J. W. and Grey, H. M. (1970) Biochemistry, 9, 2115.
[7] Howard, A. and Virella, G. (1970), in: Protides of the Biological Fluids (H. Peeters, ed), 17th Colloquium, pp. 449–453, Pergamon, Oxford.
[8] Wrigley, C. W. (1968) Science Tools, 15, 17.
[9] Johnson, P. M., Scopes, P. M., Tracey, B. M. and Watkins, J. (1974) Immunology, 27, 27.
[10] Williamson, A. R., Salaman, M. R. and Kreth, H. W. (1973) Ann. N.Y. Acad. Sci., 209, 210.
[11] Cwynarski, M., Watkins, J. and Johnson, P. M. (1975), in: Isoelectric Focusing of Proteins and Related Substances (J. P. Arbuthnott and J. A. Beeley, eds.), Ch.27, Butterworths, London. In press.
[12] Leaback, D. H., Rutter, A. C. and Walker, P. G. (1970), in: Protides of the Biological Fluids (H. Peeters, ed), 17th Colloquium, pp. 423–426, Pergamon, Oxford..
[13] Clamp, J. R. and Johnson, I. (1972), in: Glycoproteins (A. Gotteschalk, ed.) Vol. 1, 2nd Edn., pp. 612–652, Elsevier, Amsterdam.
[14] Ross, D. L. and Jirgensons, B. (1968) J. Biol. Chem., 243, 2829.
[15] Cathou, R. E., Kulczycki, A. and Haber, E. (1968) Biochemistry, 7, 3960.
[16] Dorrington, K. J. and Bennich, H. H. (1973) J. Biol. Chem., 248, 8378.
[17] Kehoe, J. M., Bourgois, A., Capra, J. D. and Fougereau, M. (1974) Biochemistry, 13, 2499.