COMPARISON OF THE CIRCULAR DICHROISM SPECTRA OF THE SUBCLASSES OF HUMAN IMMUNOGLOBULIN A

Walter F. RIESEN, Hans HUSER
Basel Institute for Immunology, Grenzacherstrasse 487, Postfach, 4005 Basel 5, Switzerland

and

F. SKVARIL
Institute for Clinical and Experimental Cancer Research, University of Berne, Tiefenau-Hospital, 3004 Berne, Switzerland

Received 1 December 1975

1. Introduction

Human immunoglobulin A is known to occur in at least two different subclasses, called IgA_1 and IgA_2 [1,2]. These two subclasses are present in normal human serum in a ratio of about 9 IgA_1 to 1 IgA_2. The IgA_2 subclasses has been reported to exist in two different genetic variants A_2m (+) and A_2m (–) [3,4]; IgA_2 myeloma proteins carrying the A_2m (+) genetic marker posses a unique structure with the heavy chains not covalently linked to the light chains while in the A_2m (–) type heavy and light chains are covalently linked by disulfide bridges in the usual manner [5]. Recently, a second genetic marker on IgA_2 proteins of A_2m (–) type has been discovered and proposed to be called A_2m (1–, 2+) [6]. The complete amino acid sequence of an IgA myeloma protein belonging to the IgA_1 subclass has recently been published [7]. However, with the exception of the partial amino acid sequence of the IgA_2 hinge region [8] and of the mentioned genetic variants in IgA_2 the structural differences of the IgA subclasses are still unknown. Since circular dichroism has proved a valuable technique in assessing the conformation of biological macromolecules [9] it might be expected that the different forms of human immunoglobulin A could be discerned according to their circular dichroism spectra.

2. Materials and methods

IgA M-components were isolated from the sera of patients with multiple myeloma by Pevikon block electrophoresis, followed by Sephadex G-200 gel filtration. Purity of the isolated M-components was assessed by immunoelectrophoresis at a concentration of 20 mg/ml. The purified IgA proteins were typed with IgA subclass specific antisera raised in rabbits [10] and with rabbit anti-kappa and anti-lambda antisera. Am markers on IgA_2 proteins were kindly determined by Dr Erna van Loghem, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service (Amsterdam). Circular dichroism measurements were performed on a Jasco J-20 recording spectropolarimeter at room temperature (22°C). All experiments were done in triplicate. The mean residue ellipticity $[\theta]$, in units of degree cm^2 per decimole, was calculated as described elsewhere [11]. Protein concentrations of 0.7 to 0.8 mg per ml were used in cells of 1.0 cm path length. The mean residue weight was taken as 110. The solvent used was 0.1 M Tris–NaCl, pH 7.5. Protein concentrations were determined spectrophotometrically using a value of:

$$E^{1\%}_{1\ cm} = 13.4 \text{ at } 280 \text{ nm for IgA [12].}$$

3. Results

The CD spectra of eight different IgA M-components are illustrated in fig.1A (two IgA_1 K and two IgA_2 K proteins) and in fig.1B (two IgA_1 L and two IgA_2 L proteins). Three of the IgA_2 proteins were of the genetic variant A_2m (1+, 2−), one (protein San) belonged to the A_2m (1−, 2+) type. All IgA proteins studied show essentially similar spectra in the wavelength region between 190 and 300 nm characterized by a major negative transition around 217 nm, and by the absence of any positive transitions. The wavelength of the negative trough and the corresponding mean residue weight ellipticity for the eight different IgA proteins and the corresponding values for human IgG, IgM, IgD and IgE taken from the literature [13–16] are given in table 1. Apparently protein San, which belongs to the A_2m (1−, 2+) genetic variant displays a much smaller mean residue weight ellipticity value than any other human immunoglobulin of either class or subclass. Moreover, the wavelength of this maximum negative transition is shifted from 217 nm to 220 nm. Variations in minor bands among the spectra of the various IgA proteins are also found between 250 nm and 300 nm which, however, could not be correlated with the IgA subclasses or with the genetic variants of the IgA_2 subclass.

4. Discussion

Circular dichroism has successfully been used by Johnson et al. [13] to show conformational differences among the subclasses of IgG, notably between IgG_3 and the other three IgG subclasses. In the present report the CD spectra of purified human IgA M-components belonging to the two known subclasses IgA_1 and IgA_2 were compared in an attempt to assess conformational differences between these two subclasses and to test the usefulness of this method for subclasstyping of isolated IgA proteins.

The CD spectra of all studied monoclonal IgA immunoglobulins showed a large negative transition in the region of 217 nm which is a common feature of human immunoglobulins belonging to other classes and which seems to be characteristic of the β-pleated sheets in polypeptides [17]. The smaller transitions at higher wavelength are characteristic of the asym-

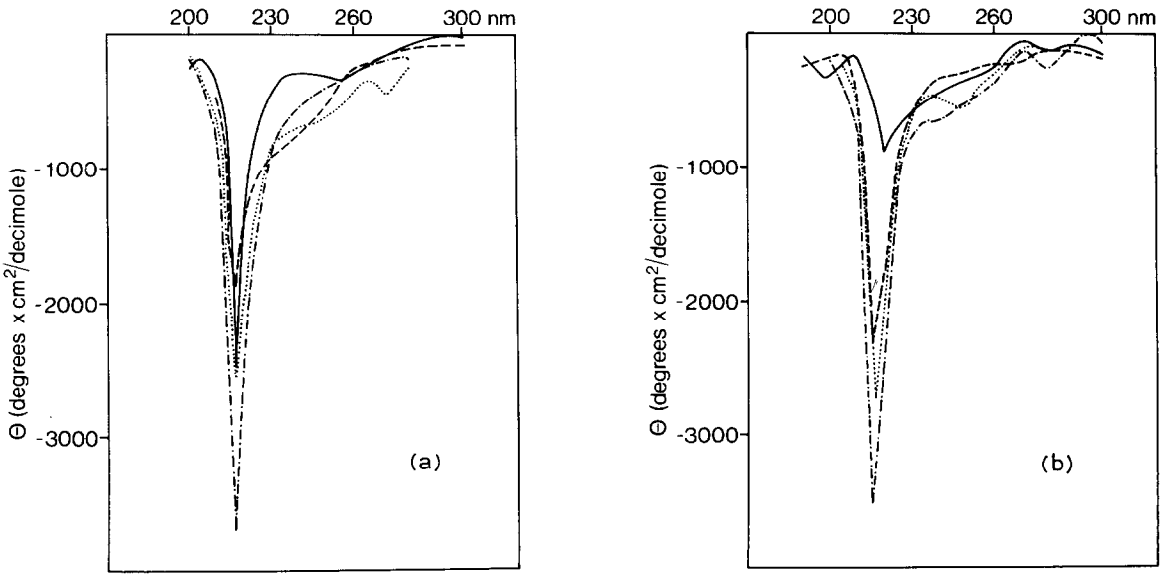

Fig.1. (a) CD spectra of two IgA_1 K (protein Lem —— and protein Stu —— · ——) and two IgA_2 K myeloma proteins (protein 13 ----- and protein Sat · · · ·). (b) CD spectra of two IgA_1 L (protein Tro ----- and protein Per) and two IgA_2 L myeloma proteins (protein Zbi —— · —— and protein San ——).

Table 1
Wavelength of the maximum negative transition (nm) and corresponding mean residue weight ellipticities (θ) of human immunologulins

Immunoglobulin	Wavelength	(θ) (deg cm^2 decimole^{-1})	Ref.
IgA_1 K (Lem)	217	−2500	
IgA_1 K (Stu)	217	−3700	
IgA_1 L (Tro)	216	−2300	
IgA_1 L (Per)	216,5	−2800	
IgA_2 K (13)	217	−1900	
IgA_2 K (Sat)	216	−2600	
IgA_2 L (Zbi)	216	−3500	
IgA_2 L (San)	220	− 890	
IgG (normal human)	217	−2900	[13]
IgM	217	− 300	[14]
IgD (A)	217	−3500	[15]
IgD (B)	217	−4200	[15]
IgE (PS)	217	−2800	[16]
IgE (ND)	217	−3400	[16]

metric environment of the aromatic and disulphide groupings. Whereas human IgD [15] and IgE [16] exhibit a series of positive transition between 250 and 300 nm, similar to rabbit IgG [18], human IgG [13], and IgA ([19] and this report) show predominantly negative transitions in this wavelength region. No transitions which could be assigned to subclass specific conformational differences were observed among the IgA_1 and IgA_2 proteins between 240 and 300 nm. The differences in the CD spectra within this wavelength region rather have to be attributed to individual specific conformational features, which might be associated with the variable domains of these proteins.

A remarkable difference, however, may be noted for protein San, which belongs to the rare A_2m (1−, 2+) variant of IgA_2. The CD spectrum of protein San displays a much smaller negative transition at 217 nm than any other immunoglobulin studied so far. This peculiarity which might prove to be suitable for genetic typing of immunoglobulins belonging to the IgA_2 subclass, however, will have to be confirmed with other proteins belonging to this rare genetic variant.

Acknowledgements

The authors thank Dr P. Moser (Ciba-Geigy Ltd.) for giving the opportunity to use his spectropolarimeter.

This work was supported in part by the Schweizerischer Nationalfonds zur Förderung der Wissenschaftlichen Forschung.

References

[1] Kunkel, H. G. and Prendergast, R. A. (1966) Proc. Soc. Exp. Biol. Med. 122, 910–913.

[2] Vaerman, J. P. and Heremans, J. F. (1966) Science 153, 647–649.

[3] Vyas, G. N. and Fudenberg, H. H. (1969) Proc. Nat. Acad. Sci. USA 64, 1211–1216.

[4] Kunkel, H. G., Smith, W. R., Joslin, F. G., Natvig, J. B. and Litman, S. D. (1969) Nature 223, 1247–1248.

[5] Jerry, L. M., Kunkel, H. G. and Grey, H. M. (1970) Proc. Nat. Acad. Sci. USA 65, 557–563.

[6] van Loghem, E., Wang, A. C. and Shuster, J. (1973) Vox Sang. 24, 481–488.

[7] Frangione, B. and Wolfenstein-Todel, C. (1972) Proc. Nat. Acad. Sci. USA 69, 3673–3676.

[8] Schol, R. and Hilschmann, N. (1975) Hoppe-Seyler's Z. Physiol. Chem. 356, 1333–1335.

[9] Beychok, S. (1966) Science 154, 1288–1299.

[10] Skvaril, F. and Morell, A. (1974) in: The Immunoglobulin A System, (J. Mestecky and A. R. Lawton eds.) p. 433, Plenum Press New York and London.

[11] Dorrington, K. J. and Smith, B. R. (1972) Biochim. Biophys. Acta 263, 70–81.

[12] Schultze, H. E. and Heremans, J. F. (1966) Molecular Biology of Human Proteins with special Reference to Plasma Proteins. Vol. I. pp. 175–181, Elsevier, Amsterdam.

[13] Johnson, P. M., Scopes, P. M., Tracey, B. M. and Watkins, J. (1974) Immunology 27, 27–31.

[14] Lintwin, G. W., Frommel, D., Rosenberg, A. and Good, R. A. (1971) Biochim. Biophys. Acta 236, 647–654.

[15] Johnson, P. M., Howard, A. and Scopes, P. M. (1975) FEBS Lett. 49, 310–313.

[16] Dorrington, K. J. and Bennich, H. H. (1973) J. Biol. Chem. 248, 8378–8384.

[17] Ross, D. L. and Jirgensons, B. (1968) J. Biol. Chem. 243, 2829.

[18] Cathou, R. E., Kulczycki, A. and Haber, E. (1968) Biochemistry 7, 3958–3964.

[19] Underdown, B. J. and Dorrington, K. J. (1974) J. Immunol. 112, 949–959.