CHEMICAL TYPING OF THE IMMUNOGLOBULINS IgM, IgA1 and IgA2*

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A simple chemical method has been employed to classify different classes and subclasses of human immunoglobulins. It is based on the fact that the interchain S–S bonds are variable and characteristic for each of them.

During the characterization of the interchain disulfide bonds of γG myeloma proteins belonging to the 4 subclasses, it became apparent that the number of bonds as well as the sequences around them differed [1]. Since these differences could be easily detected by high voltage electrophoresis of peptic–tryptic digests of the partially reduced proteins, a method was developed to chemically type the 4 subclasses of IgG and the 2 types of light chains [2]. With the development of a similar approach to the study of IgA myeloma proteins belonging to the α 1 and α 2 subclasses and IgM macroglobulins, it is now possible to recognize at least 7 types of immunoglobulins by a simple chemical method.

IgA myeloma proteins were isolated from the sera of patients with multiple myeloma by starch zone electrophoresis followed by gel filtration on Sephadex G-200 in 0.3 M saline. Macroglobulins were isolated from the sera of patients with macroglobulinemia of Waldenstrom by precipitation of the euglobulin followed by filtration of the redissolved precipitate on Sephadex G-200 in 0.3 M NaCl. Proteins were tested for purity by immunoelectrophoretic analysis using rabbit antisera to whole human serum and antisera specific for γ, α 1, α 2 and μ chain determinants. The method for chemically typing the isolated immunoglobulins was described previously although in the present study better resolution was obtained when the proteins were reduced with DTT at concentrations between 1 and 5 mM instead of 0.65 mM [2].

Seven proteins were used in this study: 2 IgA1 proteins (Pat and Oso), 2 IgA2 proteins (Avi and Rya) carrying the genetic marker Am2(+) and 3 Waldenstrom macroglobulins. All of them had κ light chains. IgA2 myeloma proteins belonging to the rare Am2(–) subclass [3–6] were not available.

Fig. 1 is a composite diagram showing the position of radioactive bands containing the interchain S–S bonds of the 2 types of IgA proteins, IgM proteins, and also the 4 subclasses of IgG which were studied previously [2]. The most striking difference between the IgA2, Am2(+) proteins, and the IgA1 proteins is the absence of the band corresponding to the peptide containing the half-cysteine joining the heavy to the light chain. The only band common to all of them is a band running with glutamic acid which represents the carboxyl end of the κ light chain. This band is absent in molecules with λ light chains. The H–H, and in some cases H–L bands are specific for each class or subclass of heavy chain and permit easy classification. Table 1 shows the sequences and function of relevant carboxymethylcysteine peptides characteristic of IgA1, IgA2 and IgM.

This method is reproducible and relatively easy to interpret. Because of the difficulties often encountered in preparing subclass specific antisera it presents an alternative and clearcut approach for classifying Ig's.

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Table 1
Sequence and function of the main peptic–tryptic carboxymethylated proteins characteristic of IgA1, IgA2 and IgM proteins.

Protein	Sequence	Function
	CHO	
Pat*, IgA_1	Val–Thr–Val–Pro–Cys–Pro–Val–Pro–Ser–Thr–Pro–Pro–Thr–Pro–Ser–Pro–Ser–Thr	
	(Ser_2, Pro_{3-4}, Cms, His) Arg	H–H [ref. 7]
	Ser–Leu–Cys–Ser–Thr–Glx–Pro–Asx–Gly–Asx	H–L [ref. 7]
Avi, IgA_2, Am_2(+)	Val–Thr–Val–Pro–Cys–Pro–Val–Pro–Pro–Pro–Pro–Pro(Pro_{2-3}, Cms, His) Arg	H–H [ref. 8]
Hel, IgM	Ala–Ser–Ile–Cys–Glu–Gln	I–S [ref. 9]
	Ser–Met–Cys–Val–Pro–Asx–Glx–Asx–Thr–Ala–Ile	H–H [ref. 9]
	Ile–Cys–Glu	I–S [ref. 9]
	Ser–Asx–Thr–Ala–Gly–Thr–Cys–Tyr	COO^- [ref. 9]
	Ser–Cys(Glu, Asp_2, Ser_3, Thr, Pro)	H–L [ref. 9]

*The sequence of the other peptides present in IgA are shown elsewhere [7].
Cms: s–carboxymethylcysteine sulphone.
For key to abbreviations on right see fig. 1 legend.

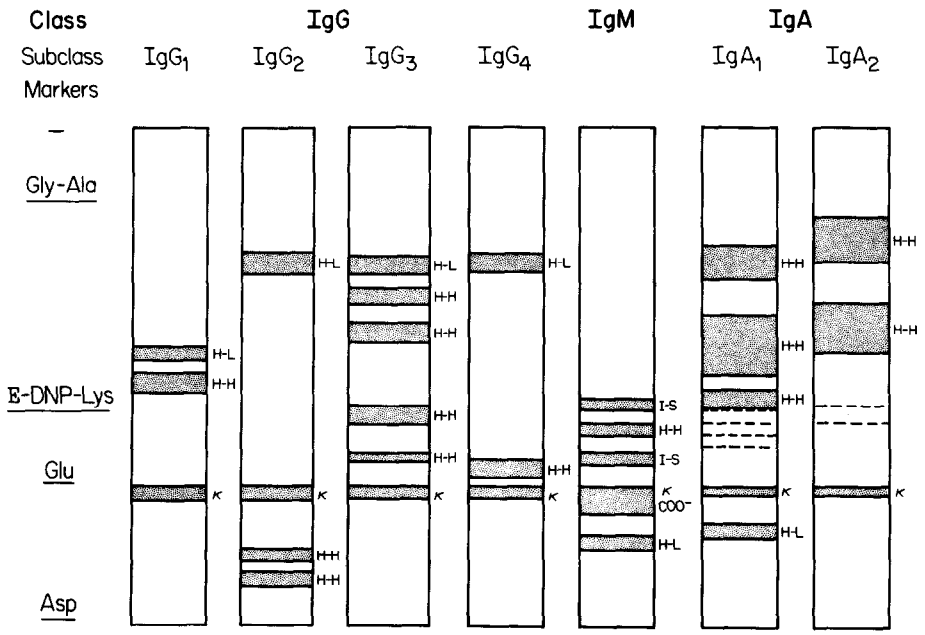

Fig. 1. Composite diagram of radioautograph showing the position of radioactive bands containing the interchain S–S bonds of IgG, M and A and their subclasses. The proteins were partially reduced and alkylated, and after peptic–tryptic digestion run on electrophoresis at pH 3.5. Because of heterogeneity, mainly due to peptic cleavage, the H–H peptide divides into a major and 2 minor related peptides in IgA1, and into 2 bands in IgA2. These differ slightly in mobility from those present in IgA1. The function of the dotted bands is unknown. Abbreviations: H–H: interheavy; H–L: interheavy–light; I–S: intersubunit (intrachain S–S bond); COO: carboxyl end; K: κ light chain type.

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