THE INTERACTION BETWEEN PROTEIN A AND IMMUNOGLOBULIN G AS STUDIED WITH THE Fc-FRAGMENT OF A MYELOMA PROTEIN BY CIRCULAR DICHROISM

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1. Introduction

Protein A is a cell-wall constituent from *Staphylococcus aureus* [1,2] with the ability to react with non-immune immunoglobulins from several mammalia [3,4]. The active binding site for protein A is situated in the Fc-region of human IgG1, IgG2 and IgG4 [5,6]. As a consequence of the reaction, the complement system, for instance, is activated, as well as certain immunological reactions are initiated in vivo [7–9].

Tyrosine-modifying reagents inhibit the reactivity of protein A, as shown by nitration, acetylation and iodination [10–12]. The circular dichroism (CD) spectrum of protein A is very characteristic with strong, well separated bands at 261 and 268 nm originating from the phenylalanine residues [13]. The conditions to detect changes of the intrinsic Cotton effects resulting from the protein A–IgG interaction can therefore be considered as relatively good, especially as an isolated Fc-fragment can be used instead of the complete γ-globulin. Thereby, the ellipticity background from the non-reacting Fab-fragments can be avoided, which means that any specific conformational changes can be detected more easily. The present paper describes the CD-changes seen, when protein A interacts with the Fc-fragment of a human myeloma protein.

2. Materials and methods

Protein A was obtained after lysostaphin digestion of the bacteria *S. aureus*, strain Cowan I, as described earlier [10,14].

A myeloma IgG (GN) was kindly provided by Professor J. Sjöquist, Uppsala. It was digested with papain according to Porter [15] and the monomer of the Fc-fragment isolated by chromatography on DEAE-Sephadex A-50 and gelfiltration on Sephadex.

CD measurements were performed on an automatic spectropolarimeter, JASCO J-20, Japan Spectroscopic Co., Tokyo, in a thermostated cellholder at 25°C. The proteins were solved in 0.1 M KCl at pH 7.3–7.4. The concentration was checked in a recording double-beam spectrophotometer, Cary 15, after filtration. The scanning was started at least 20 min after protein A and the Fc-fragment were mixed. The ellipticity is expressed as molar ellipticity, θ, degrees $\times$ cm^2 $\times$ dmol^{-1} for the separate protein samples, with the mol. wt 42 000 and 55 000 of protein A and the Fc-fragment, respectively. For the mixtures, the ellipticity, θ or $\Delta\theta$ is expressed in degrees and the concentrations of the components given as mg per ml.

3. Results

Fig.1 shows the CD spectra of the protein A and the Fc-fragment preparations used in the present studies. The spectrum of protein A corresponds closely to those earlier published [11–13], and that of the Fc-fragment has the same general characteristics as the CD-spectrum of Fc_γ shown by Ghose [16].

Protein A and the Fc-fragment of the myeloma IgG-GN form soluble complexes. Fig.2 shows the spectrum of a mixture of the Fc-fragment and protein A in a molar ratio of 1:2.45 (or 0.408:1), which means that protein A is added in a concentration far over the

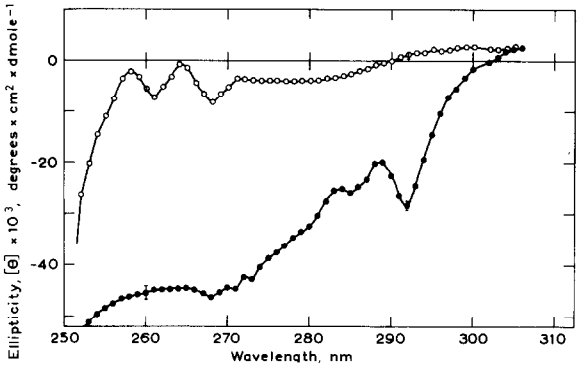

Fig.1. Circular dichroism spectra of protein A (o–o–o) and the Fc-fragment of a myeloma IgG (GN) (•–•–•) at pH 7.3 and 25°C in 0.1 M KCl. The vertical bars show the noise level.

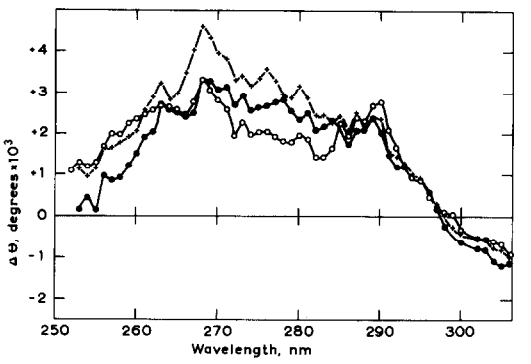

Fig.3. Difference circular dichroism spectra of protein A–Fc complexes at pH 7.3 and 25°C in 0.1 M KCl. The concentration of Fc-fragment is 0.657 mg/ml and that of protein A 0.19 mg/ml (o–o–o), 0.35 mg/ml (•–•–•) and 0.56 mg/ml (+–+–+). The spectra shown are obtained by subtracting the sum of the ellipticity contribution of the different components from the experimental spectra obtained of the mixtures. The cell pathlength was 20 mm.

equivalent amount. The spectrum is compared with the theoretical spectrum obtained by a summation of the ellipticities from each of the separate components. As is evident from the figure, there are significant differences between the two spectra. The most striking effect is the partial loss of the characteristic negative peaks at 268 and 261 nm, which will be shown clearly also in the difference spectra (fig.3). Below about 292 nm, the whole spectrum has been less negative. The relative decrease is about 40% at 289 nm and almost 20% at 264 nm. The same qualitative changes have been seen with two different combinations of different Fc- and protein A-preparations.

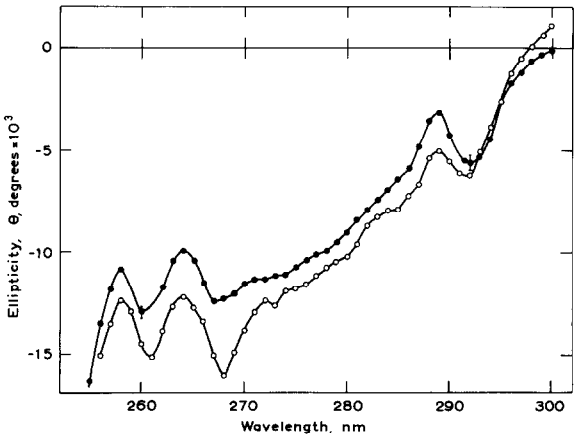

Fig.2. Circular dichroism spectra of a mixture of protein A (1.28 mg/ml) and Fc-fragment (0.66 mg/ml) at pH 7.3 and 25°C in 0.1 M KCl (•–•–•) compared with a calculated spectrum constructed from the ellipticity of the respective components (o–o–o). The cell pathlength was 20 mm. The vertical bars show the noise level.

Addition of increasing amounts of protein A to the Fc-fragment will gradually change the CD spectrum. In fig.3, the difference spectra have been calculated as the difference between the experimental CD spectra and the theoretical ones for different mixtures of the Fc-fragment and protein A. In these experiments, the concentration of the Fc-fragment was kept constant (0.66 mg/ml), while that of protein A was increased from 0.19 to 0.56 mg/ml. The molar ratio between Fc-fragment and protein A thus changes from 1:0.395 to 1:0.709 to 1:1.11 (or 2.53:1, 1.41:1 and 0.90:1, respectively) in the mixtures. The spectra will show the changes of the intrinsic Cotton effects resulted from the conformational changes brought about during the complex formation. In the wavelength region under study the ellipticity is given by the aromatic amino-acid side-chains (from phenylalanine, tyrosine and tryptophan), as well as by disulfide bonds.

The noise level of the primary spectra was low as shown in figs.1 and 2, and the difference spectra shown in fig.3 can thus be considered to be significant. They show that the conformation of the proteins is

considerably affected by the complex formation. The spectra show maxima at 262–263, 268, 275, 280, 289 nm and possibly a negative one at 305 nm. This means that all the aromatic amino acids are involved in the conformational changes. Several studies with model compounds or proteins have detected separated transitions of these amino acids in this region. The two lower peaks (262–263 and 268 nm) most probably originate from phenyl-alanine residues [13,17,18] and tyrosine residues show several transitions from 273 nm up to 288.5 nm [19,20]. Finally, tryptophan also will show several transitions between 288–305 nm [21].

4. Discussion

When protein A is added to IgG-Fc at pH 7.3 the CD spectra show significant deviations from the theoretical ones in all the wavelength region studied (250–306 nm). The deviations are easily detected and are expressed as a decreased negative ellipticity amounting to almost 20% at 264 nm and about 40% at 289 nm. Moreover, both phenylalanine, tyrosine and tryptophan residues seem to be involved as judged by the composition of the difference spectra (fig.3). All the results, considering the magnitude of the changes and the amino acids involved, indicate that the interaction is accompanied by extensive conformational changes.

The protein A–IgG(GN)–Fc complex does not form precipitates (and nor does the complex with complete IgG (GN)), but precipitation studies with non-immune, normal IgG showed that the molar ratio of IgG to protein A was 2.1:1 in the equivalence zone [14]. This means that almost all IgG molecules are engaged in the reaction already at the lowest protein A concentration used at a molar ratio of 2.53:1 (or 1:0.395) of Fc to protein A (fig.3). With increasing concentrations of protein A, the CD spectra at 285–306 were not further changed. Thus, most probably this part of the spectrum illustrates the events taking place at Fc-excess. The wavelength localization of this spectral change indicates that tryptophan residues are involved. As protein A does not contain tryptophan, the spectral change detected has arisen from changes in the primary active binding site of the Fc-fragment or from parts of the Fc-fragment, the conformation of which is influenced by the binding site. As changes of the ellipticity are detected also in the rest of the wavelength region, tyrosines and phenylalanines should be involved, too.

When the concentration of protein A is increased over the equivalence concentration, the ellipticity deviation successively increases from the theoretical ellipticity below 285 nm. This means that a secondary reaction takes place at protein A-excess or that protein A is bound to the complex at one more site on the Fc-surface. In this reaction tyrosine and phenylalanine residues are involved.

It is certainly not possible to definitely conclude which component in the system the amino acids participating in the interaction belong to. However, all the four tyrosines in protein A have such a position on the surface that they indeed can take part in the reaction [13] and they have also been shown to be essential for the reaction [10–12]. Consequently, the ellipticity changes seen at least partly should originate from them. Protein A, moreover, exhibits very typical CD bands ascribed to the phenylalanine residues [13]. In the complexes these bands are largely lost and if the ellipticity is not exactly matched by an induced ellipticity in the Fc-fragment, also at least some phenylalanine residues from protein A are involved in the conformational change. If that is the case, the changes seem to be radical, as the phenylalanine residues are deeply buried, as they are not easily affected by neither temperature nor high salt concentration, as judged by CD studies [13].

The results thus indicate that extensive conformational changes take place in both the protein components, when protein A interacts with the Fc-part of IgG molecules. When protein A reacts with intact human IgG1, IgG2 and IgG4, the interaction is followed by the same immunological reactions as a common antigen-antibody reaction both in vitro and in vivo [7–9]. As the structure of the Fc-fragments are constant, the CD changes seen thus should reflect conformational changes similar to those taking place in a normal antigen-antibody reaction. In the system studied, phenylalanine, tyrosine and tryptophan residues have been shown to be involved.

Also in studies on hapten–antibody systems, tyrosine and tryptophan residues have been shown to be involved. Thus, e.g. Holowka et al. [22] showed by CD studies that three antibody populations isolated by isoelectric focusing bound a capsular hexasaccharide

in such a way, that the conformation around tryptophan and tyrosine residues was changed. Litman et al. [23] concluded from difference spectroscopy studies on turtle and duck antibodies towards dinitrophenol groupings, that tyrosine residues were situated at the binding sites. The amino acid residues mentioned are thus parts of the IgG binding sites. If tyrosines also are present on the Fc-sites for protein A in addition to those in protein A taking part in the reaction cannot be concluded from the present results. It should, however, be mentioned in this context, that active and apparently monovalent fragments of protein A containing one tyrosine residue have been isolated after trypsin digestions (Hjelm, H., Sjödahl, J. and Sjöquist, J. (1974) personal communication). Further CD studies on the Fc-complex with these fragments or chemically modified protein A fragments should provide better possibilities for a differentiation of the CD effects seen.

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