SLOW CONFORMATIONAL CHANGE IN ANTI-DANSYL ANTIBODY AS A CONSEQUENCE OF HAPTEN BINDING

Demonstration by ESR spectra

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1. Introduction

Attempts to detect conformational changes in antibody molecules following antigen binding led to various results depending on the particular antibody–antigen model system (reviewed in [1,2]). One of the most sensitive tools for the study of changes of protein conformation is the spin-label method. Changes of ESR spectra of spin-labelled antibodies induced by binding of protein antigens were determined in [3].

Here we demonstrate that occupation of combining sites of pig anti-Dns antibody by ε-Dns-lysine results in a change of ESR spectrum of spin-labelled antibody. Whereas the binding of the hapten is instantaneous, as evidenced by the measurement of fluorescence enhancement, the changes of the ESR spectrum proceed for several minutes after addition of the hapten. Presumably, the change of the ESR spectrum reflects a relatively slow reversible conformational change in the antibody molecule following the interaction with the hapten.

2. Materials and methods

Anti-Dns antibodies were isolated from sera of pigs immunized by Dns-bovine immunoglobulin using Sepharose-bound Dns-porcine serum albumin. Purified anti-Dns antibodies (immunoglobulin G class) were spin-labelled by SL I (2,2,6,6-tetramethyl-*N*1-oxylpiperidine-4-amino-(*N*-dichlorotriazine)) in 0.05 M phosphate buffer (pH 7.3) for 3 days at 4°C, or by SL II (2,2,6,6-tetramethyl-*N*1-oxylpiperidine-4-iodoacetamide) in 0.1 M phosphate buffer (pH 8.0) for 7 days at 4°C. The unreacted SL I or SL II were removed by exhaustive dialysis and subsequent gel filtration on a Sephadex G-25 column [4]. The amount of bound spin-label was determined as in [4] and was found to be 2 mol label/mol antibody.

The rotational correlation times were calculated from the shifts of outer wide extrema of ESR spectra from the central line as in [4]. The ESR spectra were run at 20°C on a E-104A Varian spectrometer. Fluorescence spectra were recorded on a Hitachi MPF-2A spectrofluorimeter. ε-Dns-L-lysine was purchased from Calbiochem.

Abbreviations: Dns- or dansyl-, 1-dimethylamino-naphtalene-5-sulfonyl; Dnp, 2,4-dinitrophenyl

3. Results and discussion

Figure 1 shows the ESR spectra SL I-labelled anti-Dns antibody before and after interaction with ε-Dns-lysine. These ESR spectra are in general similar to the ESR spectra of other SL I-labelled immunoglobulins [4], with the exception of additional outer wide extrema I and IV. These extrema are not resolved well and are obviously due to more immobilized spin labels. The addition of ε-Dns-lysine results in disappearance of the extrema I and IV with a simultaneous increase of intensity of all other extrema. This

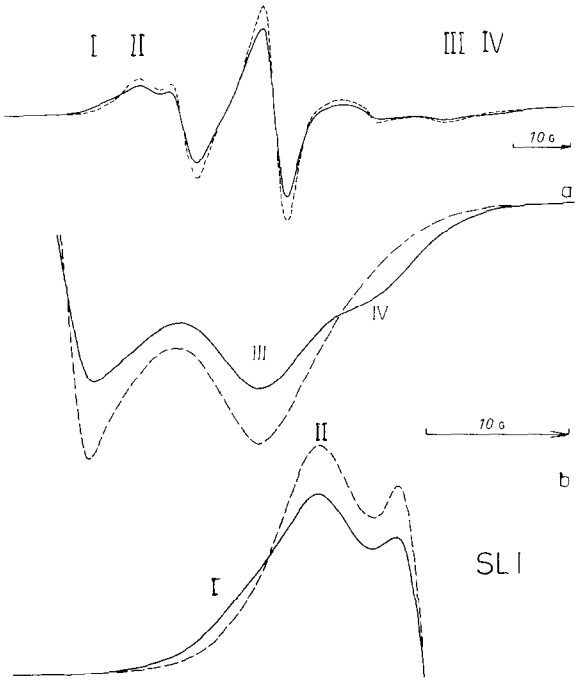

Fig 1 ESR spectra of SL I-labelled anti-Dns antibodies in presence (- - -) and absence (—) of ϵ-Dns-lysine ϵ-Dns-lysine was added in 20-fold molar excess Enlarged high-field (a) and low-field (b) parts of the ESR spectra

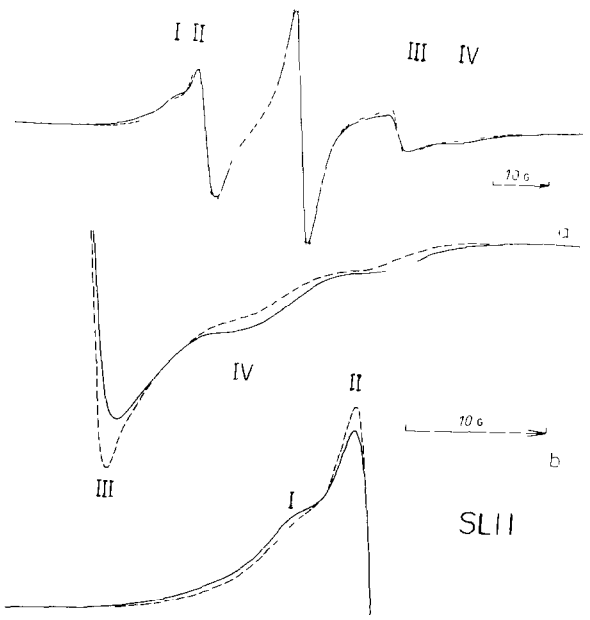

Fig 2 ESR spectra of SL II-labelled anti-Dns antibodies in presence (- - -) and absence (——) of ϵ-Dns-lysine ϵ-Dns-lysine was added in 20-fold molar excess Enlarged high-field (a) and low-field (b) parts of the ESR spectra

effect may be explained by the increase of mobility of the spin labels responsible for the extrema I and IV

Figure 2 shows the ESR-spectra of SL II-labelled antibody The shape of the spectra points to a more mobile state of the attached SL II label as compared to the SL I label bound to the same antibody Upon interaction with the hapten the spectral extrema become more narrow and their intensity increases presumably due to an increase of the mobility of the label

In contrast to the situation with pig anti-Dnp antibodies [5] it appears that no label is non-covalently attached to the antibody combining site The interaction of SL I-labelled antibodies with ϵ-Dns-lysine does not result in changes of the ESR spectrum that would indicate a presence of a displaced free label

In other experiments we investigated the time dependence of the changes of the ESR spectrum following the addition of a 20-fold molar excess of ϵ-Dns-lysine The intensity of the extrema III (SL I-labelled antibodies) or II (SL II-labelled antibodies) were used as quantitative parameters Figure 3 shows that the changes of the intensity of the extrema reach their maxima after 4–5 min following the addition of the hapten

The intensity of the ϵ-Dns-lysine fluorescence is enhanced upon binding to the antibody combining site [6] The magnitude of fluorescence enhancement was used to investigate the velocity of the interaction of both spin-labelled and intact antibodies with the hapten added in a 20-fold molar excess With both antibody samples the enhancement of fluorescence reached its maximum instantaneously As expected, the velocity of the hapten binding to the antibody combining site is very high and cannot explain the slow changes of the ESR spectrum

We suggest that the observed changes of the ESR spectrum are due to conformational changes of the antibody as a consequence of hapten binding If this is the case, hapten binding should produce a similar quantitative effect on the fluorescence enhancement

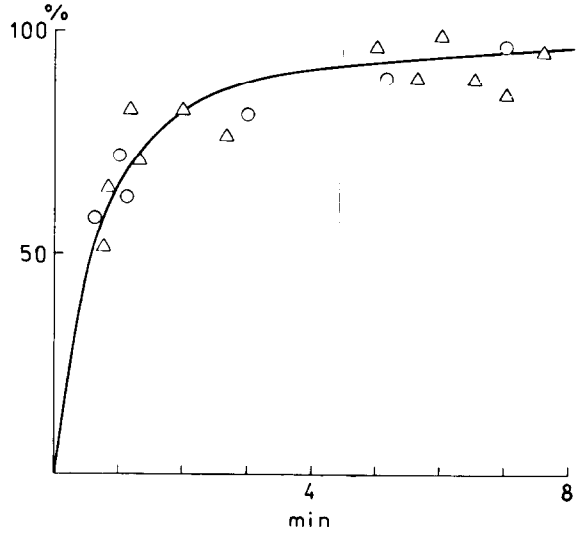

Fig.3. Time dependence of the intensity of extrema III from fig.1 (○) and extrema II from fig.2 (△) after addition of ε-Dns-lysine in 20-fold molar excess.

and on the increase of the ESR spectrum intensity. Indeed, both effects depend in a similar way on the amount of added hapten (fig.4).

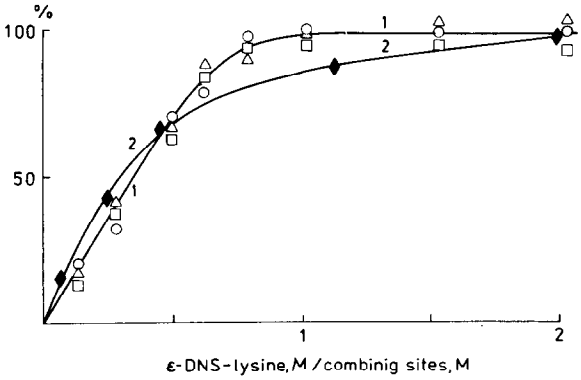

Fig.4. Fluorescence and ESR spectra of complexes of anti-Dns antibody and ε-Dns-lysine prepared at various degrees of saturation. Curve 1: Fluorescence enhancement after addition of different amounts of ε-Dns-lysine to intact (○). SL I-labelled (△) and SL II-labelled (□) antibodies. Excitation 340 nm, emission 480 nm. Protein conc. 0.8 mg/ml. The intensity of fluorescence was corrected for volume changes during the titration. Curve 2: Changes of the intensity of extrema III (♦) (see fig.1). Protein conc. 13 mg/ml. The intensity of the extrema was corrected for changes of volume during the titration.

The observed changes of the ESR spectrum of spin-labelled antibodies are reversible. A prolonged dialysis of the hapten–binding complex resulted in appearance of the extrema I and IV; they disappear again after a second addition of the hapten.

The conformational changes presumably occurring in anti-Dns antibodies upon hapten binding must involve a relatively large part of the antibody molecule since they can be found with antibodies labelled by two different labels (SL I and SL II), which are likely to be attached to different amino acid residues. Iminoxyl radicals quench the fluorescence, if the fluorescent substance is located at a distance <0.5 nm [7]. No quenching was observed upon addition of ε-Dns-lysine to SL I-labelled antibodies (as compared to intact antibodies) and therefore we conclude that the changes of the ESR spectrum are not due to the close contact between hapten and the spin label. The binding of hapten does not change the rotational correlation time which is equal to 26 ns for SL I-labelled antibodies, as it was calculated from the shift of the extrema III.

4. Conclusions

Our findings favour conformational changes occurring upon binding of ε-Dns-lysine to pig anti-Dns antibodies. We are not able, at present, to determine which domains of the antibody molecule are involved in the conformational change since the position of the spin label attachment has not been established. There are certain indications, however, that the change does not occur in the close vicinity of the binding site. The conformational change proceeds much slower than the binding of hapten to the combining site: the maximum effect is reached ~5 min after the hapten has been added.

Acknowledgement

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