A SPIN LABEL EPR STUDY OF TOBACCO MOSAIC VIRUS PROTEIN

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1. Introduction

Tobacco Mosaic Virus (TMV) is a rodlike particle composed of coat protein and RNA. The coat protein consists of 2130 identical subunits with mol. wt 17 500. These subunits itselves are able to form well-defined oligomers, such as trimers, double disks and helices [1], dependent on protein concentration, pH, ionic strength and temperature.

Recently a 0.5 nm resolution three-dimensional model of the double disk is published, based on an X-ray diffraction study [2]. From this model it is seen that the single SH-group, which is present in the protein subunit [3], is located at the side of the subunit that has interaction with adjacent subunits in the oligomers.

In a previous paper spin labeled TMV protein with maleimide spin label I attached to this SH-group is the subject of a saturation transfer EPR study [4]. In this communication conventional EPR spin label results are presented with TMV-protein that is labeled with maleimide spin labels with different chain length (I and II), given in fig.1. It is demonstrated that these spin labels provide information about the dissociation-association equilibrium of the spin labeled protein subunits as a function of pH and monitor a conformational change in the protein subunits above pH 9.5.

2. Materials and methods

TMV-protein was labeled with spin labels I and II as described previously [4]. The labeling was carried out in the presence of 4 M urea and was about 100% for spin labels I and II. The spin labels were obtained from Syva (Calif. USA).

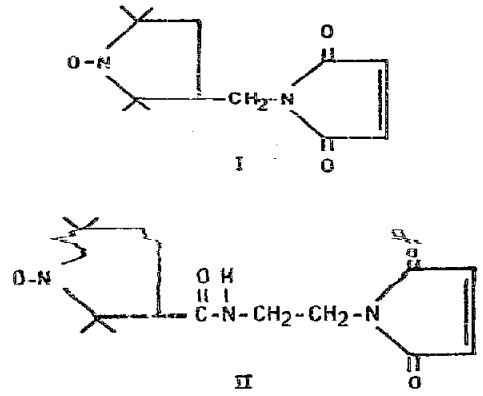

Fig.1. The structure of the maleimide spin labels I and II.

EPR measurements were carried out on a Varian E-6 spectrometer equipped with variable temperature controller. Protein samples were contained in quartz capillaries [4]. The temperature was measured with a copper-constantan thermocouple and is accurate within ± 1°C.

For the EPR measurements the spin labeled protein samples were dialysed at 4°C against water (pH 5.5) or appropriate buffers: 12 mM Tris–HCl (pH 8.8), 12 mM $NaHCO_3$–NaOH (pH 9.5) and 10 mM NaOH (pH 11.7).

3. Results

Typical EPR spectra of TMV-protein labeled with spin labels I and II are shown in fig.2. The spectra contain two components: A sharp three-line spectrum arising from mobile spin labels on the protein, super-

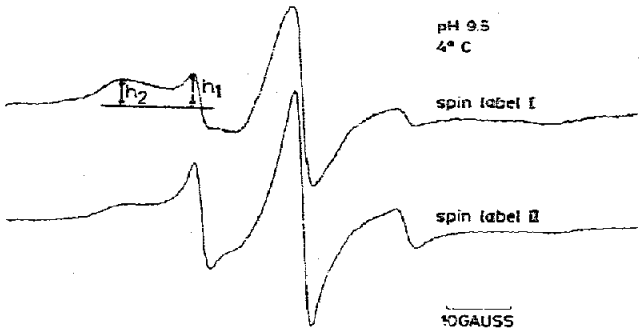

Fig.2. EPR spectra of TMV protein labeled with spin labels I and II. Protein concentration approx. 6 g/liter, in 12 mM $NaHCO_3$–NaOH buffer, pH 9.5. h_1 and h_2 are the heights of the low-field lines of the sharp and broad spectral components, respectively. The samples were contained in small capillaries. EPR conditions: frequency 9.12 GHz, microwave power 50 mW, modulation amplitude 1.6 G, temp. 4°C.

imposed on a broad spectrum arising from immobilized spin labels. The sharp spectral component of the longest spin label II has a larger amplitude with respect to the broad component than this component of spin label I.

Below pH 9.5 the widths of the low and high-field lines of the sharp spectral component are approximately equal for spin labels I en II and do not change with pH. This also applies to the broad spectral component. This means that the mobility of the mobile and immobile spin labels remains constant and that the amplitudes of the sharp and broad spectral components represent approximately the concentration of mobile and immobile spin labels, respectively. Thus the ratio h_1/h_2, where h_1 and h_2 are the heights of the low-field lines of the sharp and broad components, respectively (see fig.2), is a suitable parameter to express the relative number of mobile spin labels.

When the pH of a spin labeled TMV-protein sample is raised from 5.5–9.5 at equal settings of the EPR spectrometer and equal protein concentrations, the amplitude of the sharp component increases at the cost of the broad component. This indicates that at increasing pH spin labels go over from the immobile state to the mobile state.

A plot of h_1/h_2 against pH is given in fig.3 for TMV protein labeled with spin labels I and II. Note that h_1/h_2 increases when the pH is raised and that the longer the spin label, the higher the value of h_1/h_2. At

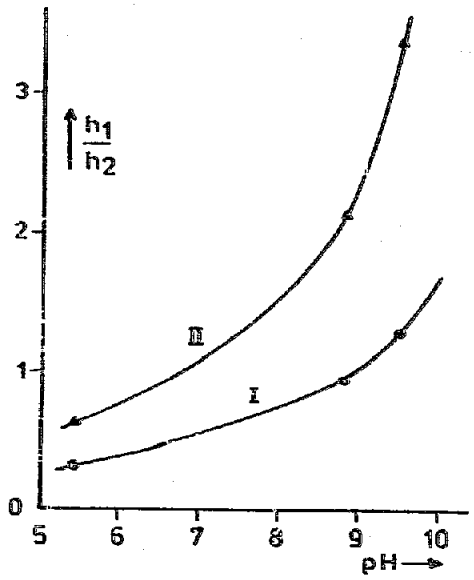

Fig.3. Variation of h_1/h_2 with pH for TMV protein labeled with spin labels I and II. The error limits in h_1/h_2 are about ± 0.1.

pH 11.7 a sharp three-line spectrum is present only and h_1/h_2 goes to infinity. At pH 5.5 the sharp spectral component is almost absent for spin label I. The curves in fig.3 are reversible.

At pH 11.7 the line width of the low and high-field lines of the three-line spectrum, which is similar for spin labels I and II, are about 15% smaller than for the corresponding lines at pH 5.5–9.5. This indicates an increase of motion of the mobile spin labels when the pH is raised from 9.5–11.7.

4. Discussion

The EPR spectra in fig.2 indicate the presence of two states of the spin labels I and II on the surface of spin labeled TMV protein. In one state the spin label is very mobile with a rotational correlation time $\tau_c \approx 10^{-9}$ s, whereas in the other state the spin label is immobilized with $\tau_c \gtrsim 5 \times 10^{-8}$ s [5].

The results of fig.2 and 3 have led to the model given in fig.4, to explain the effect of pH and different chain lengths of spin labels I and II. In a single subunit of spin labeled TMV protein the spin label extends in solution and is mobile. When a subunit

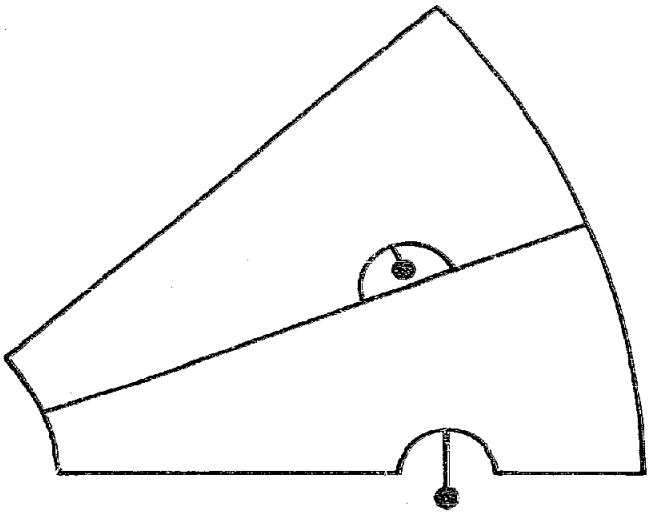

Fig.4. Schematic model to explain the presence of the sharp and broad spectral components in the EPR spectra in fig.2. The shape of the TMV protein subunit and the position of the spin label (indicated by a black dot), which is attached to the SH-group, are derived from [1,2]. The spin label at the outside of the two subunits is mobile, whereas the spin label that is squeezed between the subunits is immobile. This spin label has a rotational correlation time corresponding to the overall rotational motion of the two subunits.

interacts with another one, the mobility of one spin label will be reduced by the presence of the adjacent protein surface (see fig.4). An increase of pH will dissociate oligomers of spin labeled TMV protein and increase the mobile component at the cost of the immobile component. This gives an increase of h_1/h_2 in accordance with fig.3. An increase of the length of the spin label will reduce the interaction between the subunits and enhance dissociation, also leading to an increase of h_1/h_2.

At pH values above 9.5 an increase in motion of the spin labels on the protein surface is detected. This can not arise from dissociation of the oligomers, but results from a change in the environment of the spin labels. This demonstrates that in the pH region 9.5–11.7 a conformational change takes place in the spin labeled protein subunits.

From computer simulations of superpositions of mobile and immobile EPR spectra by [6], it is possible to express h_1/h_2 in terms of f, the fraction of mobile spin labels. Because the line widths in the computer simulated [6] and experimental spectra are different, they must be taken into account by the relation $h_1\Delta_1^2 = \text{constant}$ [7], where Δ_1 is the width of the low-field line of the sharp component in the spectra. This gives $h_1/h_2 \approx 16.3\, f/1-f$, for $h_1/h_2 \leqslant 5$. It is calculated from fig.3 that at pH 9.5 the values for f are approx. 7 and 17% for TMV protein labeled with spin labels I and II, respectively. When it is assumed that the protein oligomers consist of linear chains of n subunits, it is easily seen that $n = 1/f$, by extending the model in fig.4. This yields values for n of about 14 and 6, respectively. In the case that the subunits polymerize in two or more layers [1], the approximate values of n are larger, so that the calculated numbers are minimum values of n. The molecular weight of these oligomers at pH 9.5 is sufficiently high to explain the presence of the broad spectral component in fig.2 arising from spin labels squeezed between the surfaces of the subunits.

It is interesting to make a comparison between the behaviour of native TMV protein as a function of pH [1] and the spin labeled protein. At low ionic strength and a temperature of 5°C native TMV protein forms helical oligomers at pH ≈ 5, which dissociate into mainly trimers at pH ≈ 9 [1]. The spin label results also indicate a dissociation of oligomers of spin labeled TMV protein at increasing pH, in agreement with the behaviour of native TMV protein. However, since the smallest number of subunits of spin labeled TMV protein in oligomers at pH 9.5 is about 6, the spin labeled protein has a tendency to associate to larger oligomers than native protein. This is in agreement with previous experiments, using saturation transfer EPR, where the same effect is found at pH 7 [4].

It is obvious from the present experiments that association of TMV protein is very sensitive to changes at the surface of the protein. Chemical modification of the TMV protein, by attaching a maleimide spin label at the SH-group, changes its association properties, but also increasing the length of the spin label at the SH-group has large effects on the protein–protein interaction.

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