SATURATION TRANSFER ELECTRON PARAMAGNETIC RESONANCE SPECTROSCOPY OF SPIN LABELED TOBACCO MOSAIC VIRUS PROTEIN*

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Received October 14, 1977

SUMMARY

The coat protein of Tobacco Mosaic Virus is covalently labeled with a maleimide spin label at the single SH-group of the protein. Saturation transfer electron paramagnetic resonance spectroscopy, a technique that is sensitive to very slow molecular motion with rotational correlation times τ_c in the range 10^{-7} to 10^{-3} sec, shows the dissociation of large oligomers of spin labeled protein with $\tau_c \sim 10^{-4}$ sec at pH 5.5 to smaller oligomers at higher pH.

INTRODUCTION

Nitroxide spin labels have been widely used to study molecular motion in biological systems (1). In most cases the application of the spin label technique has been restricted to rotational correlation times τ_c shorter than about 10^{-7} sec. At larger values of τ_c the EPR spectrum is almost insensitive to the effect of motion. During the past few years an increasing interest has grown in the saturation transfer electron paramagnetic resonance (ST-EPR) technique. With this method very slow motion can be studied with τ_c in the range 10^{-7} to 10^{-3} sec (2). In particular ST-EPR employing out-of-phase second harmonic absorption has been used to study the dynamic behaviour of nitroxide spin

*Abbreviations used: TMV, Tobacco Mosaic Virus; ST-EPR, saturation transfer electron paramagnetic resonance.

ISSN 0006-291X

labels in a variety of biological systems, such as proteins, DNA and membranes (2).

In this communication we report ST-EPR spectra of spin labeled Tobacco Mosaic Virus (TMV) protein. The subunits of native TMV-protein have a molecular weight of 17 500. At low pH TMV-protein forms large oligomers with molecular weight up to 20 x 10^6, which dissociate to smaller oligomers on increasing the pH (3). With the ST-EPR technique a similar behaviour is found for spin labeled TMV-protein that has a maleimide spin label covalently attached to the single SH-group (4) of the protein.

MATERIALS AND METHODS

TMV strain Vulgare was purified according to Leberman (5). TMV-protein was prepared as described elsewhere (6). Protein stock solutions were stored in water at 5°C and pH ∿ 5. Spin labeled TMV-protein was prepared as follows: The protein was dialysed for 8 h against 12 mM Tris-HCl buffer, pH 8.8, followed by dialysis for 8 h against 12 mM Tris-HCl buffer, pH 7.0, containing 4 M urea. Then the dialysis bags were opened and a 25% excess of maleimide spin label (3-(maleimidomethyl) - 2,2,5,5-tetramethyl-1-pyrrolidinyloxyl) was added as a 30 mM solution in ethanol. To remove non-reacted spin label the reaction mixture was dialysed against several changes of 12 mM Tris-HCl buffer, pH 8.8 and finally against several changes of water. All buffers were saturated with nitrogen, to prevent oxydation of the SH-group of the protein. The maleimide spin label was purchased from Syva (California, USA). The amount of labeling of the SH-group was about 100% as determined with Ellman reagent (7). Protein concentrations were determined spectrofotometrically with $E^{0,1\%}_{280} = 1.27$.

ST-EPR measurements were carried out on a Varian E-6 spectrometer equipped with reference arm and variable temperature accessory. Second harmonic ST-EPR was performed with a Brookdeal lock-in amplifier set connected with the spectrometer, consisting of a 452 ac amplifier, 422 reference unit and 411 phase sensitive detector. The lock-in amplifier uses the 100 kHz field modulation of the spectrometer and detects saturation transfer spectra at 200 kHz. Details of this second harmonic extension of the spectrometer will be described elsewhere (De Jager, P.A., and Hemminga, M.A., manuscript in preparation). To record out-of-phase second harmonic saturation transfer spectra, the spectrometer and lock-in amplifier were adjusted according to the self-null method (8).

The protein samples were contained in quartz capillaries with an inner diameter of 1.0 mm. The temperature was measured with a copper-constantan thermocouple and is accurate within ± 1 °C.

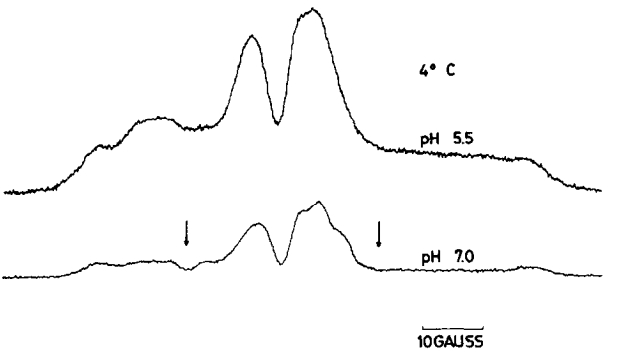

Fig. 1. Out-of-phase second harmonic absorption saturation transfer spectra detected at 200 kHz of Tobacco Mosaic Virus protein labeled with maleimide spin label, at pH 5.5 and 7.0. The temperature is 4 °C. The approximate protein concentration is 70 g/l at pH 5.5 and 40 g/l at pH 7.0 (concentrations in nitroxide are 4.0 and 2.3 mM, respectively). About 20 µl protein sample is contained in a quartz capillary. Varian E-6 spectrometer settings: microwave power 63 mW, peak-to-peak modulation amplitude 5.0 Gauss, modulation frequency 100 kHz, scan time 8 min. The setting of the time constant on the Brookdeal 411 phase sensitive detector is 0.3 and 1.0 sec at pH 5.5 and 7.0, respectively. The arrows indicate resonances arising from very small amounts of freely rotating spin labels.

RESULTS

The saturation transfer spectra of spin labeled TMV-protein at pH 5.5 and 7.0 are shown in fig. 1. The protein concentration is 70 and 40 g/l, respectively. At these concentrations and pH values the samples are in a gel state. This behaviour of spin labeled TMV-protein is different from the behaviour of native protein, due to the effect of the maleimide spin label attached to the protein.

At both pH values the line shape of the saturation transfer spectrum is different, which can be interpreted as an increase of molecular motion with an increase of pH. From a comparison of the experimental spectra in fig. 1 and saturation transfer spectra in the literature (8-10), the rotational correlation times τ_c are roughly

estimated to be about 10^{-4} and 10^{-5} sec at pH 5.5 and 7.0, respectively. By using the Stokes-Einstein relation (11) and assuming a spherical shape for the oligomers, it can be calculated that these τ_c values correspond to oligomers consisting of approximately 20 000 and 2 000 protein subunits, respectively.

DISCUSSION

The behaviour of spin labeled TMV-protein with pH as found with ST-EPR is in qualitative agreement with the phase diagram of native protein (3). The size of the oligomers of spin labeled TMV-protein does not fit with these data. Native TMV-protein with a concentration of 5 g/l forms a range of oligomers varying from trimers to disk-like oligomers consisting of 34 subunits in the pH range 5.5 to 7.0 at 5 °C (3). The presence of oligomers of spin labeled TMV-protein with about 2 000 subunits at pH 7.0 can partly be explained by considering the high concentration of the spin labeled protein samples. However, the main reason is that the maleimide spin label influences the interactions between the subunits giving rise to a change in the phase diagram of spin labeled protein. This disturbing effect of the spin label is also found with spin labeled TMV-protein in the pH range 7 to 10, using normal EPR (Hemminga, M.A., Van den Boomgaard, T., and De Wit, J.L., manuscript in preparation).

The results in this paper show that ST-EPR is a suitable technique to study the assembly of TMV-protein to large oligomers. It can be expected that spin label ST-EPR spectroscopy applied to TMV and other virusses may give interesting information about the mechanism of virus assembly.

ACKNOWLEDGEMENTS

The excellent technical assistence of Miss M. Boerjan with the preparation of TMV-protein is gratefully acknowledged. The authors wish to thank Mr. T. van den Boomgaard for his contribution to the preparation of spin labeled TMV-protein in the early stage of the work. We are indebted to the Department of Virology for providing laboratory facilities.

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