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A hydrogen bond study in tobacco mosaic virus using Moessbauer spectroscopy

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Abstract. The Moessbauer method was applied to obtain information on a suggested hydrogen bond in tobacco mosaic virus (TMV), between the hydroxyl group of Tyr 139 and a carboxyl oxygen of Glu 22 in a neighbouring subunit. Spectra of ^{129}I were taken of 3,5-di-iodo-L-tyrosine as a free amino acid and in situ in TMV. The increase of the pK value of 3,5-di-iodo-L-tyrosine by 0.8 units at position 139 in TMV compared to the free value is a strong argument in favour of the existence of a hydrogen bond via the relevant hydroxyl group.

The reported study demonstrates the surprising sensitivity of the observable Moessbauer parameters to details of the electronic configuration in the neighbourhood of the probe nucleus.

Key words: Tobacco mosaic virus, hydrogen bonds, ^{129}iodine, Moessbauer spectroscopy

1. Introduction

In order to understand the mechanism of protein assemblies it is useful to study the electronic state of those atoms that participate in particular and essential bonds. The three dimensional structure of proteins is (in addition to peptide bonds) determined and stabilized by non-covalent interactions. The most specific of the latter are hydrogen bonds. The making and breaking of these hydrogen bonds is often important for the physiological functions of proteins (see, e.g. Perutz and TenEyck 1971).

Proton nuclear magnetic resonance (NMR) spectroscopy has gone some way to dealing with studies of such bonds but unfortunately a large class of interesting biological macromolecules have such high molecular weights that the NMR resonance lines are very broad. This broadening is essentially due to relaxation phenomena allowed by the hindered rotational freedom of the amino acid residues in proteins and protein assemblies.

Similar line broadening effects occur in electron paramagnetic resonance (EPR) measurements on free radicals or paramagnetic ions when molecular motion is very slow, as it is in proteins of high molecular weights or at low temperatures.

Moreover, for proton NMR it is, in general, difficult to identify the signal due to the bond of interest. Therefore other specific spectroscopic methods applicable to macromolecular structures are of considerable interest.

During the last two decades Moessbauer spectroscopy has proved itself as a method of a high specificity with no restriction on molecular weight. In most Moessbauer bond studies on proteins the binding conditions of the reporter atoms itself were of interest. The most prominent Moessbauer nucleus in these investigations was the isotope ^{57}Fe. It served for studying the function and binding of iron e.g. in heme proteins, iron transport- and storage-proteins, and iron-sulphur proteins. For a survey, see e.g. (Dickson and Johnson 1980).

In the following we report studies on tobacco mosaic virus (TMV) using the isotope ^{129}I introduced into a specific tyrosine residue. This nucleus had been used earlier to investigate iodine bonding in iodine-containing hormones (Groves et al. 1973). With the naturally occurring isotope, ^{127}I, measurements on some metabolically important amino acid residues have been reported (Oberley and Ehrhardt 1975).

In this paper we explore the use of ^{129}I as a reporter atom that is not primarily involved in the bond under study but senses a neighbouring bond change. The results demonstrate the surprising sensitivity of the observable Moessbauer parameters to the details of the electronic configuration near the probe nucleus.

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2. The problem

The molecular weight of the TMV is about 4×10^7. The single strain RNA is surrounded by a protein coat of 2,200 identical subunits of molecular weight 17,800 each, arranged in a flat helix.

The subunits consist of 158 amino acids including four tyrosines and one cysteine. Only tyrosine 139 can be iodinated in the intact virus (Fraenkel-Conrat and Sherwood 1967). It is the OH group of this molecule which is the subject of the studies reported here. The protonated and the deprotonated state were expected to have characteristic Moessbauer spectra for the reporter atom, ^{129}I.

TMV protein exists in two major polymorphic forms: helix and double-disk. Structural studies of the crystalline disk form of the TMV coat protein may be used to make accurate predictions about the atomic structure of the helical virus, which is only known to limited resolution (Stubbs et al. 1977; Holmes 1979). Recent studies of the relationship between the disk and helix (Mondragon 1984) suggested a hydrogen bond in the virus between the hydroxyl group of Tyr 139 and a carboxyl oxygen of Glu 22 in a neighbouring subunit. A direct determination of the pK of Tyr 139 would therefore be of interest.

3. Parametrization of Moessbauer spectra

The parameters used for the evaluation of Moessbauer spectra have been introduced to quantify details of the magnetic and electric hyperfine interaction between the electron shell and nuclear states of the probe atom.

In biophysical and biochemical investigations the chemical shift and the electric quadrupole splitting are the dominating parameters. The chemical binding of the Moessbauer atom determines the exact γ-transition energy. Different chemical binding in source and absorber results, because of characteristic s-electron densities at the position of the nuclei, in an energy difference δ, the chemical or isomeric shift

$$\delta = \frac{4}{5} \pi Z \cdot e^2 \{ |\Psi_s(0)_A|^2 - |\Psi_s(0)_S|^2 \} \cdot R^2 \frac{\Delta R}{R}$$

with

Z = charge number of nucleus
$|\Psi_s(0)_{S,A}|^2$ = s-electron density at the position of the nucleus in source and absorber, respectively
R = nuclear radius
$\frac{\Delta R}{R}$ = relative difference of nuclear radii of ground and excited state.

In the case of an axially symmetric electric field gradient, V_{zz}, acting on the nuclear electric quadrupole moment Q, the nuclear states split into energy substates

$$E(I,m) = e\,Q\,V_{zz} \frac{|3m^2 - I(I+1)|}{4I(2I-1)}$$

with

I = nuclear spin, and
m = z-component of I.

It is the essential feature of Moessbauer spectroscopy that, besides its selectivity, the parameters δ, and $e\,Q\,V_{zz}$ are extremely sensitive to details of the characteristic electron arrangement in the neighbourhood of the probe atom. In particular, small changes of the electron configuration due to varying physical or chemical probe conditions may be observed.

4. The probe nucleus ^{129}I

Most studies in biological molecules have, until now, been based on the properties of the probe atom ^{57}Fe. Data have been obtained on the chemical state and the environment for iron containing compounds such as heme proteins or iron-sulphur proteins (Johnson 1975). Normally, coordination compounds are formed between metal atoms and proteins and, therefore, the iron atom is not well suited as a reporter atom – unless its own electronic state is of interest.

By contrast, iodine offers the advantage of easily forming covalent bonds with proteins or can be introduced into substrate analogues for enzymes. For Moessbauer experiments two iodine isotopes are suitable, namely ^{127}I and ^{129}I. As compared to ^{127}I (which is 100% abundant in nature) ^{129}I offers a line width which is smaller by a factor 4 and allows one to observe completely resolved lines in the spectra. The Moessbauer level in ^{129}I with an excitation energy of 27.8 keV is populated by an isomeric transition in ^{129m}Te with a half life $T_{1/2} = 33.6$ d and a subsequent β-decay. The groundstate of ^{129}I is not stable but decays with a half life of 1.6×10^7 a, which is sufficiently long to be considered a stable absorber nucleus. A simplified decay scheme of ^{129m}Te and the electric quadrupole splitting of the excited state and the groundstate of ^{129}I are shown in Figs. 1 and 2, respectively.

5. Sample preparation

Two types of absorbers were prepared: 3,5-di-iodo-L-tyrosine *a)* as a free amino acid and *b)* in situ in

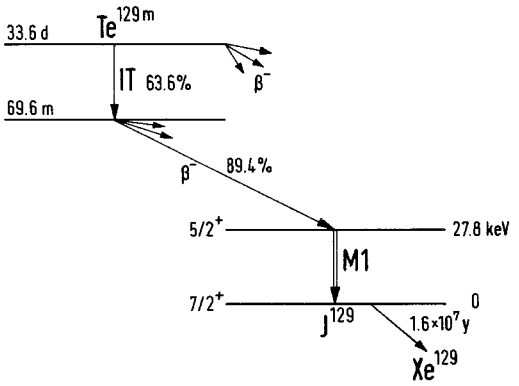

Fig. 1. Simplified level scheme of the ^{129m}Te decay

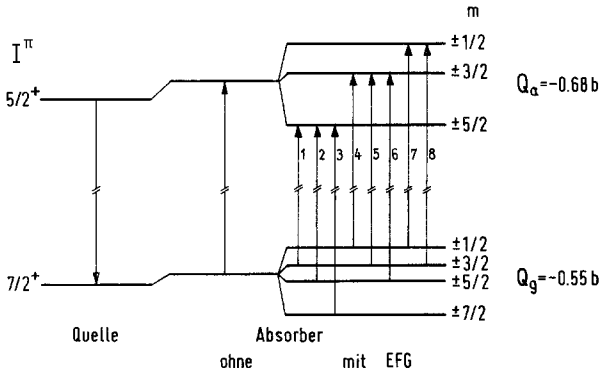

Fig. 2. Electric quadrupole splitting of the groundstate and first excited state in ^{129}I. The transition scheme is correct for an axially symmetric field gradient

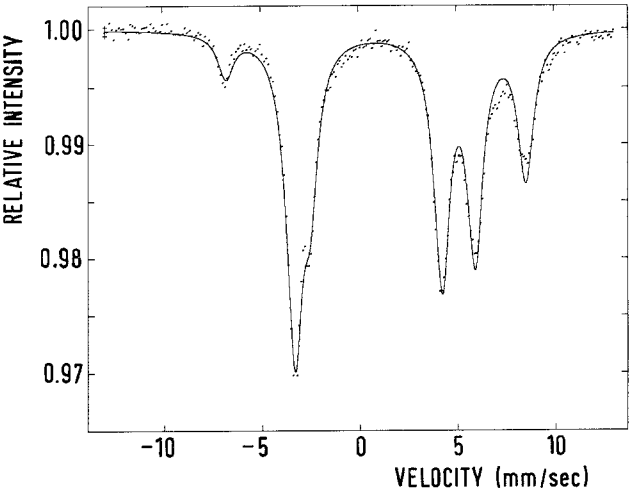

Fig. 3. Moessbauer spectrum of TMV taken at pH = 8 with tyrosine 139 iodinated at positions 3 and 5 of the benzene ring and the hydrogen in the sulph-hydryl group of cysteine 27 blocked by methyl mercury nitrate

TMV. In tyrosine it is possible to substitute iodine for hydrogen at positions 3 and 5 of the benzene ring. These positions are particularly favourable, since after substitution the Moessbauer atoms are next to the OH group, which is the hydrogen bond donor.

Tyrosine 139 in TMV can be iodinated selectively since, at 20 °C *in the intact virus*, it reacts with iodine much more readily than all the other tyrosine side chains. Since the reaction time of the sulphydryl group of cysteine 27 with iodine is comparable to that of tyrosine 139, the former was blocked by methyl mercury nitrate (MMN) (Fraenckel-Conrat and Sherwood 1967). Following the procedure outlined in detail in (Haffner 1976) 15 absorbers were prepared from TMV covering the pH range from 5.5 through 9.5 and 2 absorbers from L-tyrosine at pH 2 and pH 10.

TMV offers favourable conditions for this type of investigation, since

- TMV is a stable system which is easy to prepare in comparatively large amounts,
- the virus consists of approximately 2,200 identical protein subunits, whose sequence of amino acids in the polypeptide chain is well known (Anderer and Handschuh 1962), the structure has been thoroughly examined by X-ray crystallographic methods (Stubbs et al. 1977; Holmes 1979), and
- the iodination technique for the amino acid tyrosine 139 is straightforward.

6. The experimental procedure and results

Experimental details of Moessbauer spectroscopy devices are extensively described in the literature (see e.g. Gruverman 1965). Special experimental techniques for biochemical studies are dealt with by Lang (1976).

The spectra presented in this paper were taken in standard transmission geometry. The source was mounted on an electromechanical drive system operated in constant acceleration mode. Thus during one cycle the whole range of Doppler-velocities necessary for the observation of the essential part of the spectrum was covered in linear mode. The registered intensity as a function of the relative velocity between source and absorber was stored in a multichannel analyser.

A Zn ^{129m}Te single-line source was used. Both source and absorber were maintained at a temperature of 4.2 K during the measurements. The β^--decay of the absorber ($T_{1/2} = 1.6 \times 10^7 a$) is succeeded by a 39.6 keV γ-transition in ^{129}Xe, which is strongly converted. The resulting 30.4 keV X-ray background was reduced by a Sn critical absorber of 70 mg/cm^2.

A typical spectrum is shown in Fig. 3. The quadrupole splitting is well resolved. Only six lines

out of the eight occurring transitions lie within the velocity range covered. The smooth curve drawn in the figure was obtained by a least squares fit of the theoretical quadrupole spectrum to the data. The background, intensity, quadrupole coupling constant eQV_{zz}, asymmetry parameter η, chemical shift δ (relative to the ZnTe source), line widths Γ, and recoilless absorption anisotropy χ were the parameters varied to obtain the minimum chi-square. The asymmetry parameter η was incorporated into the fit program using the approximation method reported by Shenoy and Dunlap (1969).

Studies were performed on TMV samples where tyrosine 139 was modified to 3,5-di-iodo tyrosine as outlined in Sect. 5. Spectra were taken within the range pH = 5.5 through 9.5. Line widths, asymmetry parameter, and absorption anisotropy (e.g. at pH = 8: $\Gamma = 0.98 \pm 0.02$ mm s^{-1}, $\eta = 0.06 \pm 0.01$, and $\chi = 0.94 \pm 0.04$) are within the error margins constant throughout the whole pH range. The important parameters from an electron configurational point of view, are the quadrupole splitting, eQV_{zz}, and the chemical shift, δ. They vary with the pH value of the absorber. While the values of eQV_{zz} change markedly within the studied pH range, the chemical shift δ alters to a much lesser extent. The dependence on eQV_{zz} is plotted in Fig. 4.

Using free 3,5-di-iodo-L-tyrosine as an absorber, spectra were taken for two extreme pH values only: pH = 2 and pH = 10. The parameters eQV_{zz} and δ extracted from the data are given in Table 1.

Table 1. Parameters eQV_{zz} and δ evaluated for free 3,5-di-iodo-L-tyrosine

	eQV_{zz} [MHz]	δ [mm · s^{-1}]
pH = 2	1,350 ± 10	0.30 ± 0.03
pH = 10	1,268 ± 10	0.16 ± 0.03

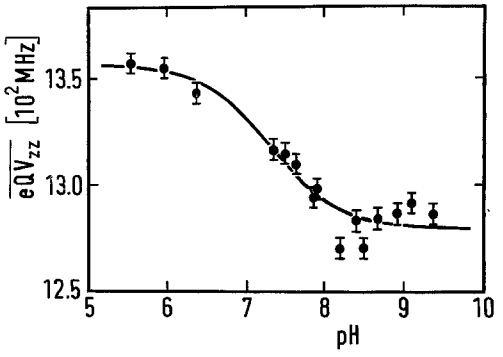

Fig. 4. Average quadrupole splittings measured on iodinated tyrosine 139 in TMV as a function of the pH value. The solid line represents a titration curve parameterised on the basis of model assumptions and fitted to the measured values

There is a clear relation between the different electronic configurations of an iodinated tyrosine molecule and the degree of protonation: At pH = 2 the protonated state predominates in the solution by a factor 10^5, for pH = 10 the non-protonated state by a factor of 10^3. Thus the Moessbauer parameters for quadrupole splitting and isomeric shift obtained for pH = 2 and pH = 10 are characteristic for the two extreme states of the molecule.

Close to the pK value of the 3,5-di-iodo-L-tyrosine 139 in TMV the protonated and the non-protonated states of the OH group are present in equal amounts in the solution. The corresponding two quadrupole spectra cannot be separated by the fit program. We assume that the measured spectrum is a weight average of the two configurations:

$$\overline{eQV_{zz}} = n \cdot eQV_{zz,1} + (1-n) \cdot eQV_{zz,2},$$

where n and $(1-n)$ are the concentrations of the protonated and non-protonated state of the OH group, respectively, and $eQV_{zz,1}$ and $eQV_{zz,2}$ the relevant quadrupole splittings. The program evaluated the average quadrupole splitting from the unresolved spectra.

This average value is plotted in Fig. 4. The solid line drawn is a titration plot

$$\mathrm{pH} = \log \frac{(1-n)}{n} + \mathrm{pK}$$

having the form

$$n = \frac{1}{(10^{(\mathrm{pH}-\mathrm{pK})}+1)},$$

which was fitted to the data. This allows one to derive the pK value of the hydroxyl group of 3,5-di-iodo-tyrosine 139 in TMV as 7.33 ± 0.15. The pH values assigned to the frozen samples were adjusted in solution at temperatures close to 0 °C. By rapid quenching to liquid nitrogen temperatures, it is assumed that the relevant ion concentration was maintained and stabilized. The cited pH values are therefore to be attributed to 0 °C.

The pK value of free 3,5-di-iodo-L-tyrosine has been determined from chemical studies to be 6.53 at 0 °C (Winnek and Schmidt 1935). Thus the pK of the iodinated tyrosine residue 139 appears to be significantly shifted in an alkaline direction (0.8) in the virus as compared with the free amino acid. The result is consistent with the existence of a hydrogen bond between iodinated tyrosine 139 and the carboxylate group of glutamic acid residue 22 in a neighbouring subunit of the TMV.

For non iodinated free tyrosine the pK value lies around 10 (Winnek and Schmidt 1935). An interpretation of the results taken from iodinated TMV in analogy to normal TMV seems to be justified

since the carboxylate group of the glutamic acid residue is not subject to a change within the relevant pH range. The pK value of this side group lies significantly lower (Simms 1928).

7. Discussion

The results of the studies reported allow the following statements to be made:

- the increase of the pK value of 3,5-di-iodotyrosine when it forms a bond at position 139 in TMV compared to the free value is a strong argument in favour of the existence of a hydrogen bond via the hydroxyl group.
- the variation of quadrupole splitting as a function of the pH value, as shown in Fig. 4, represents a normal titration plot. Any cooperativity between the hydrogen bonds of the hydroxyl group of iodinated tyrosine 139 in TMV would necessarily result in a titration within a narrower pH range, which is not observed.

It is interesting that the changes in the electronic configuration in the neighbourhood of the hydroxyl group connected with the making and breaking of the hydrogen bond manifest themselves considerably more clearly in the quadrupole interaction, eQV_{zz}, than in the chemical shift, δ. This is readily understood because the bond change is essentially connected with alterations in the $2p$ electron shell configuration of the aromatic ring which, in turn, affects predominantly the $5p$ electron configuration in ^{129}I. The hydrogen bond introduces a preferred electronic asymmetry in the neighbourhood of the reporter atom leading to a more pronounced quadrupole splitting. (The field gradient turns out to be nearly axially symmetric: $\eta = 0.05 \pm 0.01$). The s-electron density in ^{129}I which determines the value of the chemical shift parameter is not immediately affected and thus is altered to a much smaller extent. In the absence of magnetic effects the two parameters eQV_{zz} and δ reflect, in general, the characteristic electronic configuration in the neighbourhood of the reporter atom. Which of the two parameters is more sensitive with respect to specific alterations has to be individually examined and will be dependent on the special type of the electronic configurational change.

The study reported here is one of the very few that have been carried out so far where the bond state of the Moessbauer atom is not primarily of interest but it senses the general electronic environment. The reporter atom has in such cases to be introduced into a uniquely defined site of a molecule. As has been demonstrated by this TMV study, the application of the Moessbauer method can thus be extended to very specific problems.

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