HORMONE-RECEPTOR INTERACTIONS: A STUDY OF THE BINDING OF HORMONE-SUBSTITUTED
TOBACCO MOSAIC VIRUS TO MEMBRANE VESICLES BY DYNAMIC LIGHT SCATTERING AND BY
TRANSIENT ELECTRIC BIREFRINGENCE

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Abstract – Tobacco mosaic virus has been covalently conjugated with 500 –
600 molecules of different hormones. The particles react with antisera
against the hormones and with hormone receptors in target organs, on
target cells, and on membrane vesicles. The compounds were used for
cooperative affinity labelling and for the isolation of receptor-bearing
vesicles. The binding of a tobacco mosaic virus/angiotensin II conjugate
to adrenal cortex membrane vesicles was studied by dynamic light scattering
and transient electric birefringence. Despite the complexity of the poly-
disperse systems and the fact that the membrane vesicles exhibit a Kerr
effect and depolarize the light, methods were developed that allowed the
observation and theoretical treatment of the effects due to specific
binding.

INTRODUCTION

Tobacco mosaic virus (TMV) is an easily accessible, crystalline nucleoprotein with a
molecular weight of about 40,000,000 Daltons. The virus particle is a hollow cylinder
(diameter 180 $\overset{\circ}{A}$, length 3,000 $\overset{\circ}{A}$) consisting of a ribonucleic acid genome and a capsid of
2,130 identical polypeptide capsomers. The ε-amino groups of the lysine residues No. 68 of
each capsomer are the only amino groups available for substitution in the intact virus (for
a recent review of TMV structure and chemistry, see Ref. 1).

In order to study hormone-receptor interactions, to localize hormone receptors, and to
facilitate receptor isolation, we have prepared covalent conjugates between TMV and hormones.
About 500 – 600 amino groups per virus particle were substituted with mercaptopropionic acid
and the introduced mercapto groups reacted with biologically active derivatives of bromo-
acetyl α-melanotropin and ε-maleiimidohexanoyl angiotensin II to produce 'TMV-S-hormone'
(Ref. 2 & 3).

The compounds were biologically active, were aggregated by hormone antisera, and strongly
bound by receptor-bearing membrane vesicles from target organs. The latter reaction allowed
the separation of TMV-S-hormone/vesicle aggregates from unreacted virus particles and
vesicles by density gradient centrifugation (Ref. 3). All of the observations suggested an
especially strong, specific association between TMV-S-hormone and receptors on the plasma
membrane.

We have interpreted this phenomenon as possibly arising from cooperative affinity inter-
actions (Ref. 2 & 3). A rough calculation shows that the TMV-hormone particle is large
enough to cover a cell surface area that might contain between about 1 and 5 receptors. The
hormone molecules attached to a single virus would then be able to react simultaneously with
more than one receptor, resulting in a potentiation of the over-all association constant.
(The situation is that of a polyvalent ligand reacting with a polyvalent acceptor.)

The physical studies reported here in a preliminary manner support this view. The binding of
a (3-mercapto-1-succinyl)-TMV/ε-maleimidohexanoyl-[1-asparagine,5-valine]angiotensin II
conjugate (TMV-S-ANG') to bovine adrenal cortex cell membrane vesicles containing angiotensin

binding sites (VESICLES) (Ref. 3) was studied by <u>dynamic light scattering</u> (Ref. 4) and
<u>signal-averaging transient electric birefringence</u> (Ref. 5; for more recent literature, see
Ref. 6). The results shall be published in detail elsewhere.

DYNAMIC LIGHT SCATTERING

Our original expectations of the system TMV-S-ANG'/VESICLES were: (i) Vesicles as roughly
spherical particles will not depolarize the incident light. (ii) The rotational diffusion
constants of 'free' and 'bound' virus particles should be markedly different ($D_R^F > D_R^B$).
Hence, the titration of TMV-S-ANG' with VESICLES should cause the appearance of a 'slow'
component in the intensity autocorrelation function. However, it was found that vesicles do
depolarize light and that our TMV, especially TMV-S-ANG' preparations, already contain
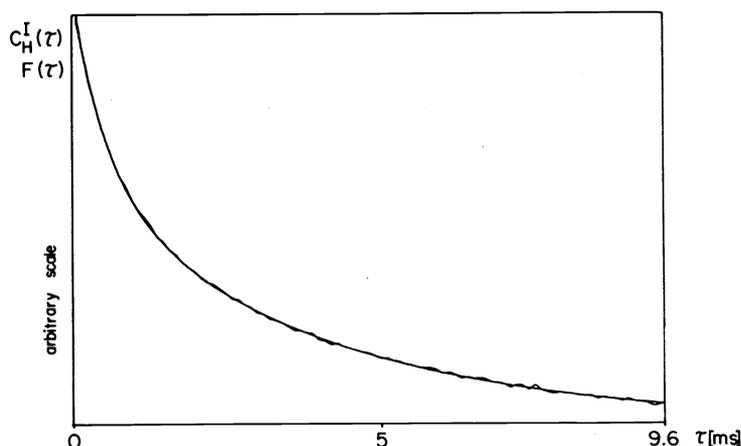about 25 % of a 'slow' component (Fig. 1).

Fig. 1. Depolarized intensity autocorrelation function,
$c_H^I(\tau)$, obtained with the heterodyne method for a TMV pre-
paration containing 0.5 g/l of TMV in 1 mM HEPES buffer
('noisy' curve). Smooth curve, $F(\tau)$, fitted to $c_H^I(\tau)$ with
2 exponentials, equation (1).

$$F(\tau) = B_1 + B_2 \left[B_3 \exp(-\tau/B_4) + (1-B_3)\exp(-\tau/B_5) \right] \tag{1}$$

$$D_R^1 = 1/6B_4 \tag{2}$$

$$D_R^2 = 1/6B_5 \tag{3}$$

From the fitted curve parameters of (1), the rotatory diffusion constants were calculated
according to equations (2) and (3). They correspond to unaggregated TMV ($D_R^1 = 287$ s^{-1},
$1 \simeq 3000$ Å) and an end-to-end aggregated species ($D_R^2 = 43.4$ s^{-1}, $1 \simeq 6000$ Å) that can also
be seen by electron microscopy. The results agree roughly with those reported in the
literature (<u>e.g.</u> Ref. 7 & 8).

Similar measurements with VESICLES gave $D_R = 20.4$ s^{-1}, which would correspond ideally to an
assembly of spheres with diameters of about 450 nm.

Because VESICLES also depolarize the incident light and because their rotational diffusion
constants are so close to those of the slow components of TMV and TMV-S-ANG', it was

impossible to devise an experiment that would 'see' only the virus particles. It was there-
fore decided to use the intensity decrease of the 'fast' virus component as a measure for
binding. In the actual experiments, increasing amounts of VESICLES were admixed to TMV and
TMV-S-ANG', and B_3/B_3^0 (ratio of the relative amplitude of the 'fast' component <u>with</u>
VESICLES to the relative amplitude of the 'fast' component without <u>VESICLES</u>) plotted against
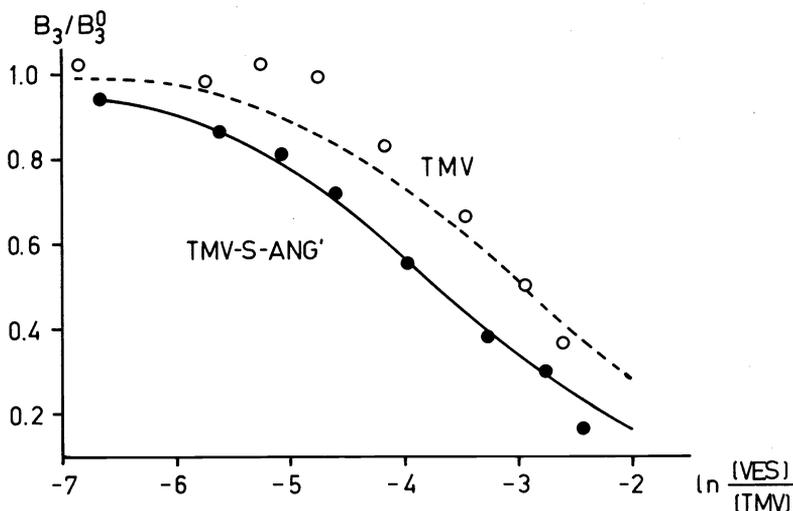the ratios of VESICLE/TMV or VESICLE/TMV-S-ANG', as in Fig. 2.

Fig. 2. Titration of TMV (o) and TMV-S-ANG' (●) with VESICLES.
Calculated curves for no binding (-----) and for the binding
(———) of about 30 TMV-S-ANG' particles per VESICLE (see text).

The model that was developed to interpret these findings had to take into account that the
intensity of the light scattered by a particle is proportional to the square of the particle
volume. Therefore the intensity of a system consisting of one vesicle and several virus
particles increases as soon as the virus particles become bound to the vesicle. Equations
(4) and (5) were assumed to describe adequately the situations of 'no binding' and 'binding',
respectively:

$$\frac{B_3}{B_3^0} = \frac{1}{1+\varepsilon \dfrac{<N_v>}{<N_t>}} \tag{4}$$

$$\frac{B_3}{B_3^0} = \frac{1}{1+(\varepsilon+\gamma r) \dfrac{<N_v>}{<N_t> - r <N_v>}} \tag{5}$$

Therein ε is the ratio of the intensity produced by one 'free' VESICLE to that of one 'free'
TMV or TMV-S-ANG' particle, γ is the ratio of intensities of bound to free virus particles,
r the number of virus particles bound per vesicle, and $<N_v>$ and $<N_t>$ the number of
vesicles and viruses, respectively, within the scattering volume.

With the help of the ratio $<N_v>$ / $<N_t>$ at the point $B_3/B_3^0 = 0.5$ (determined from the
concentrations at that point of the TMV titration curve of Fig. 2), ε was approximated
(4180 in this, and 6640 in another example). Insertion of this value into (5) and similar
treatment yielded an approximation for r. An estimate of γ was obtained from ε and r. The
average number of TMV-S-ANG' bound per VESICLE, r, was found to be in the order of 30 (31
and 28 for the two experiments, respectively).

SIGNAL-AVERAGING TRANSIENT ELECTRIC BIREFRINGENCE

Pulses of 48 V, 1 ms, were used to produce a transient <u>Kerr</u> effect. Studies with our TMV preparations gave $D_R^1 = (336 \pm 6.6)$ s^{-1} and $D_R^2 = (52.8 \pm 3.0)$ s^{-1} corresponding to calculated cylinder lengths of about 300 and 600 nm (Ref. 9). The values for TMV-S-ANG' varied with the age of the sample. Just after preparation, they were very similar to those for TMV: $D_R^1 = (342.5 \pm 17.6)$ s^{-1} and $D_R^2 = (54.2 \pm 3.2)$ s^{-1}; after standing for one month, some degradation had apparently occurred: $D_R^1 = (599 \pm 24.0)$ s^{-1}, $D_R^2 = (67.6 \pm 5.4)$ s^{-1}. It was found that <u>vesicles also exhibited electric birefringence</u>. The observed decay curves were characteristic of a polydisperse system in agreement with the electron-microscopic findings (Ref. 10). They were approximated with two exponentials. The apparent rotational diffusion constants, "D_R" (that are not necessarily caused by Brownian rotation alone) were: "D_R^1" = (474.0 ± 28.5), (543.5 ± 27.0) s^{-1} and "D_R^2" = (26.8 ± 3.2), (30.1 ± 2.4) s^{-1} for two different preparations. Measurement of the rotational diffusion constants of various mixtures of TMV or TMV-S-ANG' with VESICLES gave the results exemplified by the experiment of Fig. 3.
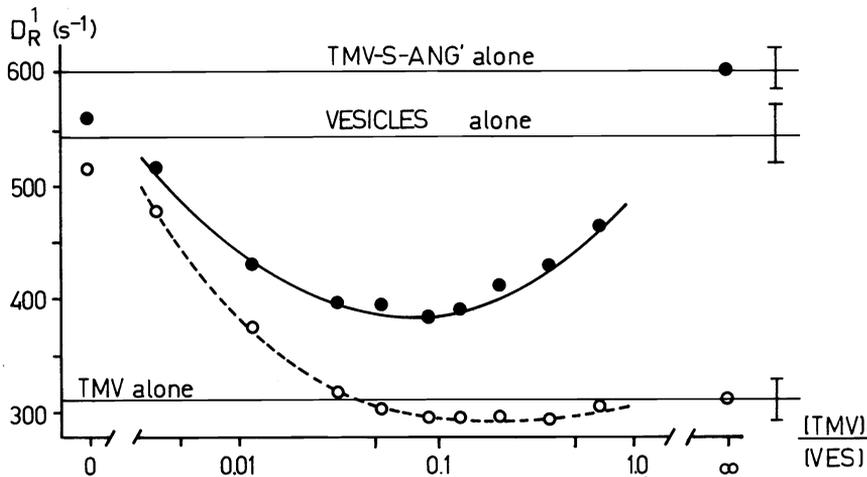

Fig. 3. Rotational diffusion constants of various mixtures
of TMV (o) or TMV-S-ANG' (●) with VESICLES measured by signal-
-averaging transient electric birefringence. [TMV]/[VES] in-
dicates the mixing ratio of solutions containing 0.12 g/l of
TMV or TMV-S-ANG' and 0.1 g/l VESICLES, respectively, in 1 mM
HEPES buffer, pH 6.8. This corresponds to roughly $1.5 \cdot 10^{12}$
virus particles and $2 \cdot 10^{10}$ vesicles per ml

The results were explained in the following manner: The values for "D_R^1" (VESICLES) are changed to those for D_R^1(TMV) or D_R^1(TMV-S-ANG') when these components are in excess. With native TMV the values decline rather rapidly because the signal produced by the virus is much more intense than that of the vesicle. The curves were not changed, if aged VESICLES were used that had lost most of their binding capacity (Ref. 10). This indicates that TMV is not bound appreciably.

The fact that aged TMV-S-ANG' has a somewhat larger D_R^1 than VESICLES proved to be very fortunate. With VESICLES that had lost most of their binding capacity, the D_R^1 values re-mained always between 550 and 600 s^{-1} if TMV-S-ANG' was admixed. However, with viable, angiotensin II-binding VESICLES the results shown in Fig. 3 were obtained. The distinct minimum indicates the appearance of more slowly relaxing particles, such as a VESICLE/TMV--S-ANG' complex. After saturation of the binding sites, D_R^1 begins to rise again as a result of the excess TMV-S-ANG'. The ratio of particles at the minimum gives an idea of the mean number, r, of TMV-S-ANG' bound per VESICLE if it is assumed that binding is saturable and virtually irreversible. In this case, r = 8.

DISCUSSION

The strong, selective binding of a TMV/angiotensin II conjugate to bovine adrenal cortex membrane vesicles containing angiotensin II binding sites that had been observed earlier by density gradient centrifugation and electron microscopy (Ref. 3) has been confirmed by dynamic light scattering and signal-averaging transient electric birefringence. The polydispersity of the systems and the fact that vesicles depolarize the light and actually show a Kerr effect caused difficulties. These were overcome by developing a model for depolarized light scattering that takes into account the intensity increase caused by the binding of a virus particle to a vesicle. With transient electric birefringence, the appearance of components with longer relaxation times were observed directly. The results indicate that on the average 10 - 30 TMV/angiotensin II conjugate particles are strongly bound per vesicle; the binding is much less with unsubstituted TMV or with aged vesicles that have lost their binding capacity for angiotensin II. These results suggest that the binding is caused by membrane components that are specific for angiotensin (receptors? other binding sites?) and do not contradict the idea of the interaction being due to cooperative affinity. The results are still semiquantitative and could be made more accurate by decreasing the polydispersity of the systems.

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