In either case, linear relations can be expected only at low levels of sodium impregnation where the term $[Ca]_{solid}$ remains essentially constant. When the data are plotted according to equations 3 and 4 as in Fig. 5, a mole for mole exchange is indicated. Such a non-equivalent exchange can be expected either to reduce the positive charge on the crystals and/or result in a loss of anions associated with the crystals. It has, indeed, already been shown[15] that sodium impregnation sharply reduces the number of surface phosphate groups as measured by exchange techniques.

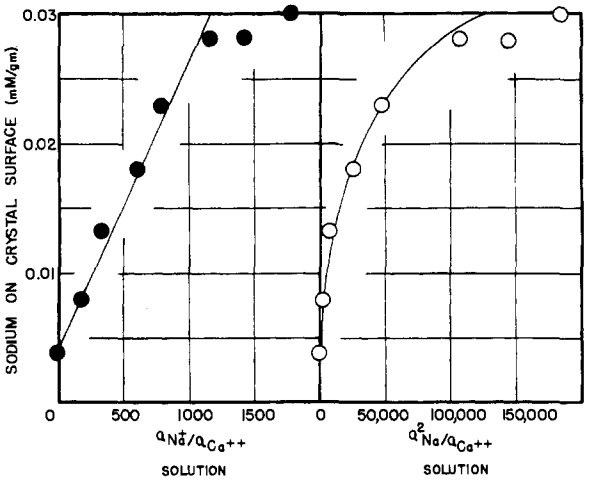


Fig. 5.—Indication of a mole for mole ionic competition between sodium and calcium with hydrated hydroxyapatite crystals; $\mu = 0.162$.

The data obtained in these studies *in vitro* cannot be extrapolated readily to the physiological state of bone *in vivo*. It is worth noting, however, that the sodium content of bone can be reasonably explained in terms of the hydration shell:crystal surface exchange concept. The crystals of bone are smaller than the crystals of apatite used in these studies (68 m.²/g.) and therefore present a greater surface area,[9,20,21] probably approaching 200 m.²/g. On the other hand the crystals in bone are not fully hydrated averaging only about 0.4 g. $H_2O$/g. apatite.[11] The sodium content of bone can thus be calculated from the results obtained here.

Crystal surface exchange = 0.04 mmole/g. $\times \frac{200 \text{ m.}^2/\text{g.}}{68 \text{ m.}^2/\text{g.}} = 0.12$ mmole/g. (apatite or ash)

Hydration shell = 0.16 mmole/g. $\times \frac{0.4 \text{ g. } H_2O/\text{g.}}{0.8 \text{ g. } H_2O/\text{g.}} = 0.08$ mmole/g.

Total predicted Na content = 0.2 m$M$/g. (apatite or ash).

Total predicted Na content = 0.2 m$M$/g. (apatite or ash). A number of analyses by flame photometry of various specimens of bone gave values for total sodium content varying between 0.2 and 0.25 mmole/g. of bone ash, in excellent agreement with the value predicted from the data obtained *in vitro*.

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[CONTRIBUTION FROM THE VIRUS LABORATORY, UNIVERSITY OF CALIFORNIA, BERKELEY]

# Composition of an Abnormal Protein Present in Tobacco Plants Infected with Tobacco Mosaic Virus[1,2]

BY PHILIP NEWMARK[3] AND DEAN FRASER[4]

RECEIVED MARCH 18, 1955

An abnormal nucleic acid-free protein is present in tobacco plants infected with tobacco mosaic virus. Though lacking infectivity, this abnormal protein resembles the virus closely in appearance and in other physical properties. In the present study the abnormal protein and virus have been shown to contain the same amino acids. No quantitative differences in amino acid composition have been detected by column chromatography on hydrochloric acid hydrolysates of the two proteins. These results emphasize the close chemical relationship of the abnormal protein to the virus.

## Introduction

The presence of an abnormal protein, other than the virus in tobacco plants infected with tobacco mosaic virus, was reported by Commoner, Newmark and Rodenberg,[5] Takahashi and Ishii,[6] and Jeener and Lemoine.[7] Takahashi and Ishii[8] showed that this protein contained no nucleic acid, but yet could be polymerized into rod-like particles which under the electron microscope appeared to be identical in width and in general very similar to the virus. As part of an over-all study on the relationship of the abnormal protein to the virus, the amino acid composition of both were compared. The results are reported here.

## Experimental

Tobacco mosaic virus is readily obtained in high purity by means of well established procedures.[9] Isolation and purification of the abnormal protein is more difficult. By means of a procedure which we have outlined elsewhere,[10] the abnormal protein was obtained from a finely ground sus-

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(2) Aided by a grant from the National Foundation for Infantile Paralysis.

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(4) Department of Chemistry, Indiana University, Bloomington, Ind.

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pension of infected tobacco leaves as a centrifugal fraction, of which 90% migrated as a single symmetrical electrophoretic component with an average mobility of $-3.6 \times 10^{-5}$ cm./sec./v./cm. in 0.1 $\mu$ cacodylate/sodium chloride (0.2/0.8) buffer at *p*H 7.0. The major electrophoretic component, after separation in a Tiselius cell, was shown to be virtually free of nucleic acid by its ultraviolet absorption spectrum ($\epsilon$ 280 m$\mu$/260 m$\mu$ = 1.69, $\epsilon$ 280 m$\mu$/250 m$\mu$ = 2.35, and $\epsilon$ 280 m$\mu$/300 m$\mu$ = 7.39), as well as by a negative orcinol test. Virtual freedom from intact virus was shown by the absence of characteristic rod-like particles in electron micrographs of the protein preparation (courtesy of Dr. R. C. Williams of this Laboratory) and by absence of infectivity when tested on *Nicotiana glutinosa*.

In order to compare the amino acid composition of the abnormal protein (major electrophoretic component) with that of the virus protein, samples of both were hydrolyzed with 6 *N* hydrochloric acid at 113° for 17 hr. The hydrolysates were chromatographed on Dowex 50 columns, with separate columns used for the basic and for the neutral plus acidic amino acids; and the individual amino acids in the effluents were estimated by the ninhydrin color reaction according to the procedures of Moore and Stein.[11]

Assays for the neutral and acidic amino acids were carried out on four separate samples of the same strain of virus. One of the virus samples was isolated from the infected tobacco leaves which were the source of one of the assayed samples of abnormal protein. The other three virus samples, obtained from Dr. C. A. Knight of this Laboratory, represented three different preparations of the same strain of tobacco mosaic virus. The average recovery of neutral and acidic amino acids comprised 84% of the initial virus weight, equivalent to 89% of the weight of the protein moiety when corrected for the 6% nucleic acid content of the virus.

Analyses were obtained on only two separate samples of the abnormal protein which were isolated at different times from virus-infected tobacco leaves. The difficulty in obtaining the abnormal protein in the desired degree of purity precluded additional analyses. Eighty per cent. of the initial weight of abnormal protein was recovered from the columns as neutral and acidic amino acids.

Different samples of the virus and abnormal protein from those used in the analyses above were used for assaying the basic amino acids on separate columns. Only single determinations were made. The total quantities of the two amino acids (arginine and lysine) recovered in the effluents from these columns represented 10% of the initial weight of the virus and 8% of the initial weight of abnormal protein.

## Results

The results presented in Fig. 1 show a remarkable parallelism of composition between the virus and abnormal protein. As was noted earlier in two-dimensional paper chromatograms of acid hydrolysates of the proteins,[12] both the virus and abnormal protein contain the same 14 amino acids. Neither protein contains detectable amounts of histidine or methionine. Furthermore, no significant quantitative differences in amino acid composition are noted.

## Discussion

The amino acid compositions of various strains of tobacco mosaic virus have been determined by Black and Knight,[13] primarily by means of microbiological assays. The procedures used by these workers to hydrolyse the virus were similar but not identical with ours. Nevertheless, when the data of Black and Knight for the strain of virus which we used (TMV) are recalculated so as to be comparable with our data, the agreement between the two sets of values is very good.

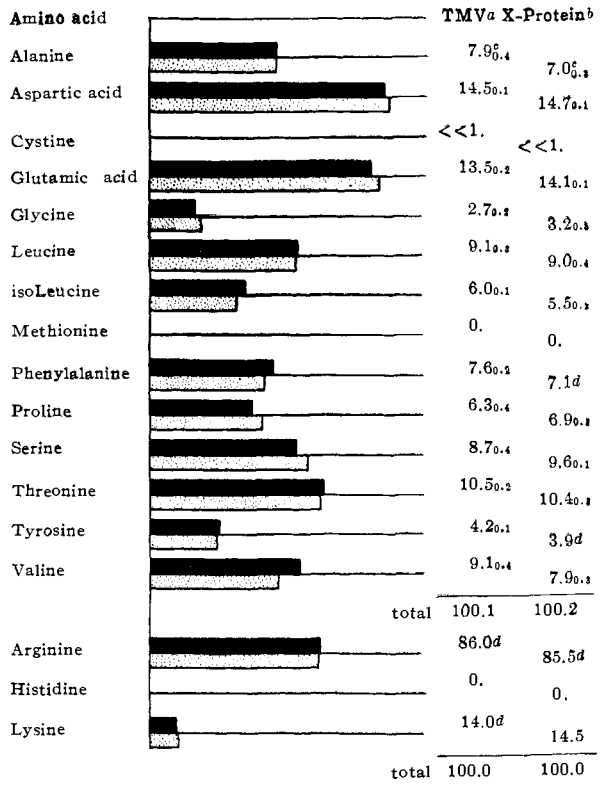


Fig. 1.—Amino acid composition of tobacco mosaic virus (TMV) and of a nucleic acid-free abnormal protein isolated from infected tobacco plants: (a) mean of analyses of four samples of the same strain of TMV as used to infect Turkish tobacco plants from which the abnormal protein was isolated. Small numbers indicate average deviation from mean; (b) average of analyses of two samples (except where noted). Small numbers indicate spread; (c) values expressed as per cent. of the total neutral and acidic amino acids recovered from one column and of the basic amino acids recovered from another column; (d) single determinations.

Our findings on the composition of the abnormal protein are not in accord with Commoner's contention[14] that this protein contains two more amino acids than does the virus. Commoner's results are reasonable in light of the reported ultraviolet absorption spectrum of his abnormal protein (component $B_3$), giving absorbancy ratios of $\epsilon$ 280 m$\mu$/250 m$\mu$ = 1.26, $\epsilon$ 280 m$\mu$/260 m$\mu$ = 1.22, $\epsilon$ 280 m$\mu$/300 m$\mu$ = 2.41, which are much too low for a pure protein preparation (*e.g*, compare these values with the absorbancy ratios quoted in the Experimental section of this paper).

Marked similarities in the X-ray crystallographic patterns of the polymerized abnormal protein as compared with the virus have been observed and have been reported elsewhere.[15] Even more striking similarities are evident when the patterns obtained from the abnormal protein are compared with those obtained recently by Franklin on a polymerized preparation of nucleic acid-free virus protein prepared by alkaline degradation of the virus.[16]

All of these results emphasize the close physical

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and chemical relationship of the abnormal protein to the virus. However, it must not yet be assumed that the abnormal protein and the virus protein are necessarily identical. Unlike intact tobacco mosaic virus, the abnormal protein cannot be heated to 60° without being denatured.[12] Even at 0–5° the abnormal protein does not appear to be as stable as either intact virus[12] or nucleic acid-free virus protein.[17] Preliminary experiments by Dr. H. Fraenkel-Conrat and Mrs. B. Singer of this Laboratory[12] showed that the abnormal protein, like the intact virus or nucleic acid-free virus protein, contained no detectable N-terminal amino acids. However, unlike the intact virus or virus protein, treatment of the abnormal protein with carboxypeptidase yielded glutamic acid and traces of several other amino acids in addition to the C-terminal threonine. Finally, the abnormal protein contained only $^2/_3$ as much of the masked sulfhydryl group as did the nucleic acid-free virus protein or intact virus.[18]

Comparisons of particle sizes of the unpolymerized abnormal protein with the unpolymerized nucleic acid-free virus protein are contained in another report.[19] The possible biological relationships of the abnormal protein to the virus, based on isotope studies, have been considered elsewhere.[10,20]

**Acknowledgments.**—We are indebted to Mrs. Terry Andrews, Miss Mary K. Brown and Mr. Don de Fremery for valuable technical assistance.

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[CONTRIBUTION FROM DEPARTMENT OF AGRICULTURAL BIOCHEMISTRY, UNIVERSITY OF MINNESOTA]

# A Study of the Interaction of *n*-Octylbenzene-*p*-sulfonate with β-Lactoglobulin[1,2]

BY ROBERT M. HILL AND D. R. BRIGGS

RECEIVED AUGUST 19, 1955

The binding of *n*-octylbenzene-*p*-sulfonate by β-lactoglobulin in 0.1 ionic strength phosphate buffer, *p*H 6.8, occurs in three distinct stages as the equilibrium concentration of the detergent is increased. At low detergent concentrations the protein molecule interacts according to the mass action law to bind 2 or 3 molecules of detergent, probably in a tail first manner, the intrinsic dissociation constant being $1.59 \times 10^{-5}$. At this point the protein molecule undergoes a change in which its ability to bind detergent is greatly increased. This increased binding is believed to take place in a head first manner and also proceeds according to the mass action law, the intrinsic dissociation constant being $2.32 \times 10^{-4}$ and the number of available sites being approximately 22. Simultaneously, however, a third type of binding occurs which is micellar in nature and involves interaction between the detergent molecules already bound to the protein and the detergent in solution. A limit to this binding occurs when the free detergent concentration reaches its critical micelle concentration. This micellar binding does not produce any physical change in the protein structure and the equilibrium is the same when approached from either direction. Binding at the lower levels, dependent upon an "opening-up" of the protein molecule, shows a hysteresis effect upon reversal indicating that the new protein species formed is stabilized by different conditions than are required in its formation. The fact that the "opening-up" of all of the protein molecules does not occur at a single free detergent concentration is taken to indicate that the native molecules vary in their ability to withstand this action of the detergent. An equation is proposed which adequately describes the experimentally determined interaction isotherm.

During recent years a number of investigators have reported on the interaction of proteins with detergent ions. Such studies have been directed toward obtaining information about the nature of the reactive sites of protein molecules in solution. It has been recognized that protein–ion interaction is dependent upon both the charge and the chemical nature of the ion involved. Anionic detergents have been of particular interest because of their bactericidal activity and because of the changes they produce on the physical properties of various proteins. The organic sulfates and sulfonates, because of their favorable solubilities, have received considerable attention. Since the measurement of light absorption in the ultraviolet region has become a routine operation, the use of detergents containing aromatic groups has become preferred because of the ease and accuracy with which their concentrations can be determined by this method.

(1) Paper No. 3385, Scientific Journal Series, Minnesota Agricultural Experiment Station.

(2) A part of a thesis submitted by Robert M. Hill to the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the Ph.D. degree. This study was aided by a research grant from the National Institute of Health, Public Health Services.

At low equilibrium concentrations of detergent, it has been found[4,5] that an interaction occurs, describable in terms of a simple mass action relationship,[3] based on the concept of a homogeneity of interaction sites. However, upon increasing the equilibrium concentration of detergent, deviations from this relationship are readily observed. Karush and Sonenberg[4] explained these as being due to a heterogeneity of binding sites. The combined use of equilibrium dialysis and electrophoretic methods has provided a means for a more complete study of such interactions and, subsequently, a more complete description of the interaction process has evolved.

Pallansch and Briggs,[5] studying the interaction of highly purified sodium dodecyl sulfate with bovine serum albumin at free detergent concentrations well below that required for micelle formation, observed that when the moles of anion bound per mole of protein exceeded 10, a faster moving protein component appeared in the electrophoretic patterns.

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