glycine (GG) hydrolysis, and glycyl transfer from GG to phenylalanine (P) were of most interest. It had proved difficult by other methods to separate these hydrolytic and transferring activities, and the questions had consequently arisen whether they were two inseparable functions of a single enzyme, or whether an enzyme (or enzymes) might exist able to catalyse one of the reactions but not the other. The electrophoretic analysis illustrated in the figure provides a partial answer to both these questions. Thus the results show that one enzyme is present (with its maximum activity at slice #41) that will catalyse *only* hydrolysis of GG and is unable to catalyse the transfer reaction in the presence of P under the conditions used. A different enzyme is present (maximum at slice #50) that will catalyse *both* the hydrolysis of GG and the transfer of a glycyl residue to P, although this faster migrating enzyme still shows GG hydrolytic activity in the presence of P. Nevertheless this faster migrating enzyme shows a greater ratio of transferring to hydrolytic activity than did any preparation purified by other means, and the results suggest (although they do not prove) that it is a single enzyme able to catalyse both reactions.

The present observations do not exclude the possibility that the solely hydrolytic enzyme is derived from an enzyme with catalytic power for both types of reaction, which has lost one function of its activity during preparation (with a simultaneous change in its electrophoretic properties). However the electrophoretic analysis demonstrates that hydrolytic activity in an enzyme is not always accompanied by transferring activity, and serves to illustrate the value of such an analysis in investigations of enzyme function. The symmetry of the zones and their discreteness indicate the freedom from troublesome adsorption effects which makes starch gel such a satisfactory medium for electrophoretic analyses of protein mixtures.

We should like to point out that this type of investigation is not restricted to enzyme or protein studies but may be applied to the analysis of any property or substance in complex mixtures for which a suitable micro-method of assay is available.

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The reaggregation of the A-protein of tobacco mosaic virus

SCHRAMM and his co-workers[1,2,3] have made detailed studies of the break-down of tobacco mosaic virus (TMV) and of the reaggregation of the protein obtained in this way. They have observed a series of discrete fractions in the disaggregation and reaggregation experiments, one of which, common to both processes, consists of disc-shaped particles in which a central hole is clearly visible in the electron micrographs. The particles have a rather uniform molecular weight of about one million, and from the length of shadows on the electron micrographs, the thickness of the discs has been estimated to be about 70 A. Discs with central holes similar to these have also been observed by Berkeley workers[4,5] in reaggregation experiments.

The disc thickness therefore appears to be similar to the axial repeat period of 69 A determined by X-ray diffraction[6,7] and SCHRAMM has already drawn attention to this fact. The 69 A axial period, however, relates to the helical arrangement of protein sub-units in the TMV particle[7], and there is no obvious reason why this length of particle should form a stable fragment. 69 A is simply the shortest length of the virus rod which contains both a whole number of turns of the protein helix and a whole number (or very nearly a whole number[8]) of protein sub-units. In the present paper we put forward a hypothesis that would account for the relative stability of a disc of this length.

Reaggregation to discs. The molecular weight of the protein sub-units of TMV is approximately 17,000[9]. However, under the conditions used by both the Tübingen and Berkeley workers to disaggregate TMV, the end-product is a protein of mol.wt. about 100,000, which SCHRAMM has called A-protein. It is this protein that has been used as the starting material in the reaggregation experiments[3,5]. There thus appears to be a stable protein molecule of this size. It is relevant

here to point out that the protein-X[10] isolated from infected plants also appears to occur with a molecular weight of about 100,000 and that SCHRAMM finds it to be identical in all its properties[3] with A-protein, in particular, in aggregating to form first discs, then rods in acid solutions[3,4].

We suggest, therefore, that the A-protein molecule consists, for the most part, of a group of six protein sub-units and, moreover, that these are arranged in 3 layers of 2 as shown schematically in Fig. 1. The basis for this suggested arrangement of sub-units within a molecule is that we can show that the polymerisation or aggregation of such molecules may be expected to lead to the formation of discs of thickness 70 A as observed.

Consider the aggregation of two such molecules in the first step of the polymerisation process. The energetically most favourable way for them to unite is likely to be side-by-side as shown in Fig. 2a (with a rather less stable variant as shown in Fig. 2b), rather than end-to-end as in Fig. 2c. Further molecules can then join on in a similar way until, when 8 molecules have come together, a closed-ring structure will be formed, in which three turns of the protein helix are complete but for one protein sub-unit on the third, as shown schematically in Fig. 3. (The latest X-ray diffraction studies[11] indicate that in TMV there are 49 protein sub-units on 3 turns of the helix.) The last molecule of the eight might not go in very easily, but there are alternative paths leading to the same final closed-ring structure. Alternatively an incomplete ring containing 7 molecules might be expected to show enough flexibility to facilitate the entry of the eighth.

Addition of a further molecule of A-protein to this ring structure will be a less energetically favourable process than aggregation of this molecule with other A-protein molecules as described for the earlier stages of the polymerisation. For it can make contact with the existing ring fragment

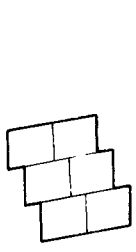

Fig. 1.

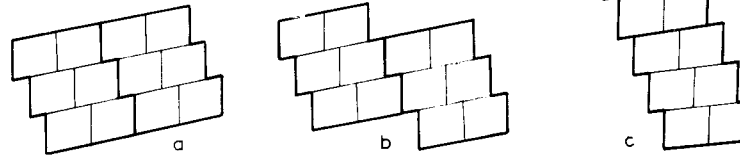

Fig. 2.

Fig. 1. Schematic representation of an A-protein molecule composed of 6 protein sub-units.

Fig. 2. Modes of aggregation of two A-protein molecules: (a) side-by-side combination; (b) a less favourable variant of (a); (c) end-to-end combination.

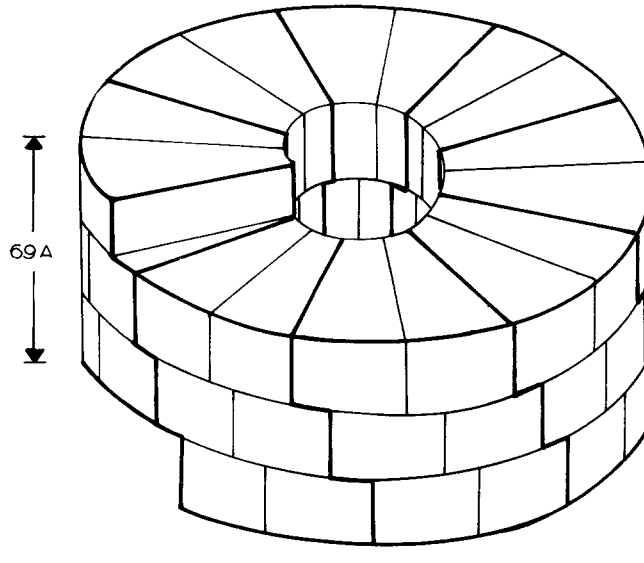

Fig. 3. Schematic model of disc with central hole, regarded as made up of 8 molecules of A-protein. Each protein sub-unit is drawn as a truncated wedge.

only on one step of the protein helix compared with three for another A-protein molecule. Even when the new "horizontal" surface contact in the first possibility is taken into account, the extent of contact between sub-units is smaller than in the second possibility.

We should therefore expect that, if polymerisation proceeds from A-protein molecules in the way we suggest, the process would have some tendency to stop after the formation of disc-like particles 69 A thick. In geometrical terms, the break in the polymerisation process arises from the fact that the pitch of the protein helix is only one-third the height of the effective building unit. This leads to the formation of a closed ring containing a screw dislocation.

Polymerisation to rods. In order for the polymerisation to proceed any further we should expect that different experimental conditions would be required. It is indeed found that to obtain a degree of polymerisation higher than that represented by the disc-like aggregates a lower pH is required[3]. We should, moreover, predict that the next stage of polymerisation would proceed in two steps. Firstly the disc-shaped particles would tend to stack end-to-end in piles; and next the individual discs would gradually come into the correct rotational position for their protein helices to fit together as in the complete virus rod.

One isolated observation of ours lends some support to this view. An X-ray diagram of a freshly-prepared gel of orientated rods of reaggregated A-protein showed meridional scattering at a spacing of about 70 A. No such spot is given by TMV itself nor by reaggregated A-protein that has been "aged". This observation therefore suggests that, at least in this one instance, parallel stacking of the protein discs had occurred, but that the discs were not yet correctly orientated with respect to one another to form a continuous helix of protein sub-units.

Discussion. The mode of aggregation suggested here is intended to explain the formation of a rather homogeneous fraction of disc-shaped particles of thickness about 70 A from A-protein. It does not in itself explain the occurrence of such particles when TMV is broken down by mild alkali[2]. Recent work by HARRINGTON AND SCHACHMAN[12], however, indicates that when such particles are found in disaggregation experiments, they are, in fact produced by *reaggregation* from A-protein.

Finally, we should like to comment on the implications of the suggestion that the A-protein molecule is a relatively stable unit consisting of 6 sub-units. While there is always the possibility that a group of 6 protein sub-units might simply be a favoured equilibrium state (*cf.* other proteins such as haemoglobin and insulin), the existence of such a group could also be due to the protein sub-units not being all identical. The arrangement of substances indicates that there might be 2, 3 or 6 slightly different kinds of sub-units. There is as yet no chemical evidence for lack of uniformity in the protein sub-units. The possibility of such differences should however be borne in mind in subsequent work.

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