CCXXII. THE DISINTEGRATION OF TOBACCO MOSAIC VIRUS PREPARATIONS WITH SODIUM DODECYL SULPHATE

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IT is well known that many bacteria are lysed and that toxins and antigens are modified by treatment with surface-active materials such as soaps and bile salts. Recently the commercially available wetting and spreading agents have been used in this way. Dean has summarized the chemical nature of these substances [1937]. The inactivation of diphtheria toxin by lauryl sulphate and the lysis of Gram-negative organisms have been studied by Bayliss [1936, 1937], and other workers, continuing the work of Larson *et al.* [1925], have prepared bacterial antigens by incubating suspensions of bacteria with these substances. This technique probably has no general application, for we have found that many large molecules are destroyed under these conditions. The destruction of preparations of potato virus "*X*" and of bushy stunt virus by incubation with the sodium salt of the sulphuric half ester of dodecyl alcohol has already been described [Bawden & Pirie, 1937; 1938, 1], and in this paper some observations on the disintegration of tobacco mosaic virus preparations will be reported.

Of this group of substances sodium dodecyl sulphate (Imperial Chemical Industries) and the apparently similar product called "Sulphonated" Lorol (Ronsheim and Moore) are the most efficient virus-inactivating agents that have been tested but the corresponding esters of cetyl alcohol and of the alcohols derived from palm oil, and the related amide, Igepon T, have some action; only sodium dodecyl sulphate (called SDS in future) has been studied in any detail.

The rate of action depends on temperature, concentration of SDS and *p*H. When tobacco mosaic virus preparations are disintegrated by SDS they become more acid, as they do when boiled [Bawden & Pirie, 1937]; it is therefore necessary to add a buffer to the system. Buffer solutions containing phthalates, phosphates or borates cannot be used, for even when dilute these form insoluble crystalline complexes with SDS. These complexes have not been investigated further. Veronal does not apparently combine with SDS and this buffer system [Michaelis, 1930] has been used. The action proceeds very slowly if at all in fluids more acid than *p*H 7·0; at *p*H 8·0 disintegration takes place and this *p*H value has been used in most experiments for in more alkaline solutions, although the action proceeds faster, there is greater spontaneous breakdown of the virus preparation.

The course of the action may be followed in five different ways, for on disintegration tobacco mosaic preparations lose their infectivity, serological activity, anisotropy of flow and sedimentability in a high-speed centrifuge and phosphorus and carbohydrate are no longer found in the precipitate which separates when the solution is one-third saturated with ammonium sulphate. An experiment in which the loss of infectivity and serological activity of a potato virus "*X*" preparation were followed has already been published [Bawden & Pirie, 1938, 1] and substantially similar results have been obtained with tobacco mosaic virus.

In this work a good preparation of virus, made by the method already described [Bawden & Pirie, 1937], was used. This preparation was colourless and a 2% neutral solution was nearly clear and wholly in the liquid crystalline state; it gave specific precipitation with antisera at a dilution of 1 : 8,000,000 and contained 0·5% phosphorus and 2·5% carbohydrate. The protein shows strong anisotropy of flow when a 1% solution is made in *M*/20 veronal buffer at *p*H 8·0; this solution gives an immediate precipitate when mixed with an equal volume of a 1% solution of SDS in buffer. The precipitate has the sheen and "crystalline", or more correctly fibrous [Bernal & Fankuchen, 1937], appearance under the microscope that characterizes the precipitates given by tobacco mosaic virus with acids, strong salt solutions or clupein. After about ½ hr. at room temperature or after a shorter time at 37° the precipitate dissolves and the anisotropy of flow can now be followed by shaking the mixture in a tube about 1 cm. wide between crossed Nicols. The intensity of the anisotropy of flow falls off rapidly and after about 2 hr. at 37° it is barely perceptible, i.e. it corresponds to that given by a 0·04% solution of the original preparation. The anisotropy of flow disappears completely after about 10 hr. incubation. Nothing can be sedimented from a solution treated in this way by centrifuging for 3 hr. at 14,000 r.p.m. (17,000 times gravity). If the incubation is stopped before the anisotropy of flow has disappeared completely a small birefringent pellet with the properties of tobacco mosaic virus is sedimented and material can be isolated, which contains carbohydrate and phosphorus and which precipitates specifically with tobacco mosaic antisera.

If samples of a buffered mixture containing 0·5% of both virus preparation and SDS are withdrawn after various periods of incubation and added to 2 vol. of ½ saturated ammonium sulphate solution containing enough ammonia to make the solution alkaline to phenol red, it is found that the amounts of carbohydrate and phosphorus in the supernatant fluid after centrifuging increase as the anisotropy of flow disappears and that the ammonium sulphate precipitate is free from nucleic acid when it separates from a solution giving no anisotropy of flow. This shows that in addition to inactivating the virus and breaking down the protein of high molecular weight the SDS separates the nucleic acid from the protein to which it was attached.

The action, as measured by any of these methods, is very greatly affected by changes in the concentration of SDS and in the ratio of its concentration to that of the virus preparation. If the concentration of SDS is doubled the disruption of the virus is complete in 1 hr. at 37°, whereas if the concentration is halved the anisotropy of flow does not disappear completely even after many days if the initial virus concentration is 0·5%, but if the virus concentration is reduced to 0·2% the anisotropy of flow disappears in the usual way.

To prepare a quantity of the nucleic acid-free protein 50 ml. of a 1% solution of virus preparation are mixed with 15 ml. of 5% SDS. It is unnecessary to buffer the mixture but *N*/10 NaOH is added from time to time to keep the *p*H between 8·0 and 8·5. After incubation for 24 hr. at 37° the mixture is filtered on a grade 4·5 Bechhold membrane (Schleicher & Schull). Most, but by no means all, of the SDS is found in the filtrate. The filter residue is dissolved in 5–10 ml. of water and dialysed thoroughly in a cellophane tube; this is necessary for the SDS is easily precipitated by ammonium sulphate. After a few days the dialysis sac contents no longer froth strongly. The *p*H of the fluid is now adjusted to 8–9 and saturated ammonium sulphate solution is added; with about 1/10 of a volume there is copious precipitation and on centrifuging the precipitate separates as a clear, gelatinous, coherent mass; this is redissolved and repreci-

pitated in the same way. If the disruption of the virus preparation has been complete there will be no further precipitation on the addition of more ammonium sulphate but if any protein remains undisrupted it will be precipitated. Nucleic acid precipitates from the supernatant fluid on acidification; if however the ammonium sulphate precipitation is carried out under slightly acid conditions part of this nucleic acid will precipitate with the protein.

The nucleic acid-free protein may be precipitated many times with ammonium sulphate but it is found that this treatment converts it in part into an insoluble material and in part into protein that is very easily precipitated by ammonium sulphate. After four or five reprecipitations it precipitates with 1–2 % solutions of ammonium sulphate and of certain other salts. This apparent denaturation also takes place if the neutral protein is kept at room temperature for some days and even more rapidly if it is made slightly acid. The insoluble protein, like the denatured protein made from tobacco mosaic virus preparations in other ways, is readily hydrolysed by trypsin preparations, whereas the protein prepared from disrupted virus by one or two precipitations with ammonium sulphate resembles "native" proteins in its resistance to proteolysis. It would appear that the products of disruption contain undenatured protein in an unstable state, they therefore resemble in many ways the "metaprotein" prepared by Theorell [1937] as a result of the removal of the lactoflavinphosphoric acid from Warburg's "yellow enzyme". In each case the protein which is made by splitting the starting material into a protein and a non-protein part is less stable than the intact molecule, but the unstable protein from tobacco mosaic virus can be kept at 0° for some weeks without apparent change. If however solutions which contain both protein and nucleic acid, but from which the SDS has been removed by dialysis, are kept, a gelatinous or slimy precipitate separates containing both protein and nucleic acid and only soluble if the *p*H of the fluid is raised above 8·5. The nucleic acid-free protein gives all the usual protein colour reactions and precipitates with the usual protein precipitants; its absorption spectrum resembles closely that of other proteins, i.e. there is an absorption maximum at 2750–2800 Å. and general absorption below 2500 Å. This supports the view that the intense absorption maximum at 2600 Å. which is found in tobacco mosaic and bushy stunt virus preparations [Bawden & Pirie, 1938, 2] is due to nucleic acid, for this absorption maximum is absent from the nucleic acid-free protein preparations.

Many attempts at fractionation have been made using acid, ammonium sulphate and similar agents but so far it has not been found to be heterogeneous if the incubation with SDS has been continued for a time sufficient to inactivate the virus completely. This observation sheds some light on the constitution of the protein of high molecular weight, which is the only recognizable component of a fully active virus preparation, for it seems to disintegrate into a nucleic acid and a protein that is too large to pass through a cellophane membrane but too small to sediment in a few hours in a centrifugal field of 17,000 times gravity. Bernal & Fankuchen [1937] have stated that tobacco mosaic virus preparations have not been made to give true crystals *in vitro* but that the submicroscopic rods which are responsible for the physical properties of virus preparations have an internal regularity which might be described as crystalline. It has been suggested [Bawden & Pirie, 1937] that these rods are artefacts made by the irreversible linear aggregation of the virus particles as they occur in the sap of young plants. Study of the disruption of tobacco mosaic virus preparations suggests that these units (which have as yet only been studied by filtration methods), from which the rod-shaped aggregates are built up, are themselves built from a number of

similar or identical proteins of normal molecular weight. The methods that have been used so far for studying these products are however too crude to give a definite result. Some attempts have been made to prepare antisera using the disrupted virus as an antigen but these have been unsuccessful.

SUMMARY

The kinetics of the disintegration of a tobacco mosaic virus preparation by sodium dodecyl sulphate are described and also the preparation and properties of the resulting unstable protein.

REFERENCES

Bawden & Pirie (1937). *Proc. roy. Soc.* B, **123**, 274.
—— —— (1938, 1). *Brit. J. exp. Path.* **19**, 66.
—— —— (1938, 2). *Brit. J. exp. Path.* **19**, 251.
Bayliss (1936). *J. infect. Dis.* **59**, 131.
—— (1937). *J. Lab. clin. Med.* **22**, 700.
Bernal & Fankuchen (1937). *Nature, Lond.*, **139**, 923.
Dean (1937). In *Wetting and Detergency.* London.
Larson, Holvarson, Evans & Green (1925). *Colloid Symp. Monogr.* **3**, 152.
Michaelis (1930). *J. biol. Chem.* **87**, 33.
Theorell (1937). *Biochem. Z.* **290**, 293.