Protein Components of a Mycobacteriophage

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SUMMARY

The protein components of a mycobacteriophage were dissociated with various concentrations of KOH. The tails disintegrated at concentrations leaving the heads intact. The process of disintegration of the heads was gradual and increased when the normality of the potassium hydroxide was augmented. Following dialysis of this alkaline degraded material against distilled water at +4° and adjustment of pH to 5 a reaggregation of protein fragments very similar to tails was observed. This also occurred before disintegration of the heads. The alkaline-degraded material was studied by immunodiffusion before and after dialysis; the ability to precipitate with antibodies was lost after treatment with strong KOH but was recovered after dialysis.

INTRODUCTION

The purpose of this research was to analyse the protein components of a mycobacteriophage, phage Phlei, isolated by Penso & Ortali (1949) and highly specific for strains of Mycobacterium phlei. The head of this phage presents a hexagonal profile, each side of the hexagon measuring 380 Å, while the distance between two parallel sides is 660 Å. The tail is helical; the proximal extremity ends in a knob and the distal extremity in an end plate. The length of the tail is 1650 to 1700 Å, the diameter 100 Å and the pitch of the helix 45 Å (Pl. 1 a). The tail has never been observed in a contracted state even when the phage is attached to the surface of the host cell.

The coat of the phage particle is composed of aggregates of one or more protein subunits linked together by secondary bonds; in order to dissociate these subunits it is necessary to use reagents which weaken the secondary bonds. The substances generally capable of interfering with such bonds are urea, guanidine, detergents, phenols, organic solvents, heat and changes of pH. A high pH weakens the hydrogen bonds which stabilize secondary and tertiary protein structures. We found the coat of phage Phlei very resistant to various chemicals in spite of repeated attempts to disrupt it.

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METHODS

Purification. Phage Pflei was produced on a strain of Mycobacterium phlei, strain TIMOTHY, which had been grown in Sauton medium. The phage was purified by 4 cycles of fractional centrifugation (10,000g for 1 hr and 53,000g for 4 hr) using a Spinco centrifuge, model L2-65B. The final titre of the purified phage was $3 \times 10^{11}$ p.f.u./ml. The degree of purity was estimated by immunodiffusion: the phage was allowed to react against an antiserum to phage Pflei and against antiserum to a cell extract of the host organism. It was thus possible to demonstrate the presence of any antigenic components of the host cell in the phage suspensions. Purification was continued until these components were no longer visible.

Reagents used. The following reagents were used for dissociating the coat: 20% (w/v) sodium dodecylsulphate, 4% (w/v) formaldehyde, 8 M-urea and various concentrations of KOH.

Immunization. Each batch of antisera against phage Pflei was obtained by inoculating three rabbits with purified phage suspension. Before inoculation the sera of the individual rabbits were tested for the presence of antibody against any components of the host cell or against DNA. The first inoculation of 0.5 ml. was given intravenously, and was followed by four subcutaneous injections, administered every other day, increasing from 1 to 2 ml. After an interval of 10 days an intravenous inoculation of 1 ml. was given, followed by four subcutaneous injections beginning with 1.5 ml. and increasing to 2 ml. This second series of inoculations was given in the pad of the foot; 8 days after the final injection the rabbits were bled.

Immunodiffusion. Double immunodiffusion was carried out using small plastic moulds with a depth of 0.3 cm. The moulds contain holes 0.2 cm. in diameter and equidistant 0.5 cm. The moulds were placed on glass plates and 0.8 Difco Noble agar containing 0.15 M-NaCl buffered with veronal buffer at pH 8.2 was pipetted between the mould and the glass plate. When the gel had set, the phage suspensions and the antisera were inoculated into the holes. The plates so prepared were then stored in a humidified chamber at 37° and examined every 24 hr for at least 5 consecutive days until no more precipitation lines appeared.

Electron microscopy. The samples were placed on the grids, dried and then negatively stained with 1.5% (w/v) phosphotungstic acid, pH 7.

RESULTS

Treatment for 12 hr with 20% (w/v) sodium dodecylsulphate, 4% (w/v) formaldehyde or 8 M-urea detached the heads from the tails: many empty heads and tails with

EXPLANATION OF PLATE

PLATE 1

a. Electron micrograph of two phages, one of which has an empty head. Note the hexagonal profile and the helical shape of the tail.
b. Electron micrograph showing the action of 0.07 N-KOH on phage: whole phages, empty heads and detached tails are visible.
c. Electron micrograph showing the action of 0.5 N-KOH on phage: empty heads and a few detached tails are observable.
d. Electronmicrograph showing the action of N-KOH on phage: note the early alteration of the capsid, the disappearance of the tails and the appearance of some amorphous aggregates.
normal appearance could be observed. The addition of KOH at various concentrations for a period of 12 hr at 4°C produced the following results: With 0.07 N-KOH most of the heads were empty and the tails were detached, but both structures appeared normal in outline (Pl. 1b). With 0.5 N-KOH all the heads were empty and the number of tails visible was greatly reduced; only 1% of tails was observable (Pl. 1c). With N-KOH the empty heads began to show a somewhat altered coat. The tails were no longer visible, while it was possible to observe the appearance of amorphous aggregates arranged in a characteristic pattern (Pl. 1d). With 1.5 N-KOH the heads showed an increased alteration in morphology and tails were not visible. With 2 N-KOH all structures were completely disintegrated while the number of the amorphous aggregates was increased (Pl. 2a).

The disintegration of phage structures was therefore a gradual process: the tails began to disintegrate at a concentration of KOH which apparently left the heads still intact, and then disappeared completely while heads were still present. Disintegration of tails occurred in the first 10 min. of contact between these structures and KOH.

The characteristic amorphous aggregates which appeared after treatment with 1.5 N-KOH and which were more evident after treatment with 2 N-KOH could, theoretically, be protein aggregates originating from either the head or the tail of the phage. It was decided to study these aggregates further in an attempt to identify their origin. Phage suspensions previously treated with different concentrations of KOH (0.5 N, N, 1.5 N, 2 N) were dialysed against distilled water at 4°C for 48 hr. The final pH of each solution was pH 5.

Electron-microscopic examination of these solutions showed many protein fragments with a helical structure closely resembling normal tails. These protein fragments were always smaller than normal tails, their maximum length never exceeding 1100 to 1300 Å while the average length of a normal tail is 1650 to 1700 Å. The majority of the fragments measured 1000 Å, but some were only 500 Å in length. The diameter was 1000 Å, the same as the normal tail, while the pitch of the helix was 45 Å, identical with that of the normal tail. None of these fragments were ever observed to have either a proximal knob or a distal end plate. The protein fragments were seen singly, in pairs joined together at one extremity or grouped in bundles with the long axis parallel (Pl. 2b, c). Amorphous aggregates arranged in a characteristic pattern were also present. The number of protein fragments observed was greater in phage suspensions treated with N-KOH.

Increasing the time of dialysis resulted in the appearance of a greater number of protein fragments. The fragments began to appear after 12 hr of dialysis and gradually increased in number with time. Because these protein fragments appeared in phage suspensions previously treated with 0.5 N-KOH, N-KOH, 1.5 N-KOH, and then
dialysed, i.e. in suspensions where the heads were not yet disintegrated while the tails were no longer visible, we believe that these reaggregates, so similar to intact tails, may be tail proteins and not head proteins. On the other hand the characteristic amorphous aggregates which appeared when the heads had begun to disintegrate after treatment of the phage suspension with N-KOH, and which increased in number when the heads were completely disintegrated after treatment of the phage suspension with 2 N-KOH, could very well be head proteins.

The reaggregation of the alkali-degraded material into fragments with a structure similar to tails showed the great tendency of the virus proteins to reaggregate after alkali degradation and after dialysis to form oligomers, polymers and, finally, defined structures.

We studied this protein material in greater detail by observing the alkali-degraded material by immunodiffusion and ascertaining the point at which the ability to react with antibodies was lost. Analyses were made on this material both before and after dialysis. Treatment with either 0.07 N-KOH or 0.5 N-KOH left intact the ability to precipitate with antibodies while treatment with N-, 1.5 N- and 2 N-KOH abolished this ability. To obviate the possibility that precipitation was inhibited by the high pH values of the samples, they were first dialysed against distilled water for a short time until neutral pH was attained. At this point electron microscopy showed no evidence of reaggregation in any of the samples. This material tested by immunodiffusion did not show any precipitation lines. Absence of precipitation could not therefore be attributed to the pH of the sample. However, the alkali-degraded material which had been subjected to longer dialysis and which had reached pH 5 produced visible precipitation with antibodies. It is interesting to note that the material which had undergone treatment with strong concentrations of KOH was no longer capable of producing a visible reaction with antibodies but that this capability returned after dialysis.

DISCUSSION

Our results seem to agree with those of Poglazov, Borhsenius & Belavtseva (1965), who, after having completely disintegrated the sheaths of T2 by alkaline treatment, were able to reconstitute them by dialysing the degraded material against distilled water in the cold and readjusting to an acid pH value.

The protein fragments which we obtained after dialysis are very similar to normal tails although they are shorter, not always of constant length and never show a proximal knob or a distal end plate. Nevertheless the reconstitution of structures very similar to normal tails, although incomplete, seems to confirm that the process of reaggregation is possible for some bacteriophage proteins. The results of Poglazov et al. (1965) with the sheaths of T2 and those of Fraenkel-Conrat & Williams (1955) and Fraenkel-Conrat & Singer (1957, 1959) with TMV show that disaggregation is a reversible and general process for many viruses. We have now shown that this is possible also for the bacteriophage.
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REFERENCES


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