THERMAL SOLUBILITY OF GENE 8 COAT PROTEIN IN 2 M UREA FROM f_d PHAGE

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SUMMARY

A detailed procedure is outlined here for the thermal solubilization of the gene 8 coat protein from f_d phage. The pronounced effect of metal ions such as Na^+, Mg^{++}, Zn^{++}, Mn^{++}, Ca^{++} on the thermal solubility of this coat protein is described. Although we are able to solubilize gene 8 coat protein, this material exists in neutral buffer as aggregates of numerous coat protein subunits which prove resistant to further dissociation to the monomeric form. Circular dichroism studies indicate that the thermally solubilized coat protein exhibits a characteristic β structure at 217 mμ.

INTRODUCTION

The filamentous, male specific bacterial phage f_d is a flexible, helical rod 8500 to 9500 Å in length with a diameter of 60 to 70 Å (1,4,5,6) which adsorbs at the F-pili appendages of the male strain of E. coli. f_d phage (like M13, f1 and ZJ/2) has a circular, single-stranded DNA genome (1,2,3) and a DNA replication process which somewhat resembles that of ϕX174 (7,8).

The f_d phage contains two coat proteins, the major one being gene 8 coat protein and the minor gene 3 protein (A-protein) (9,11). The gene 3 protein, with a molecular weight of 70,000 daltons, is present in one copy per virion and is responsible for phage attachment to the host cell (5,9). Gene 8 coat protein, the smallest known protein to have a biological function, has a molecular weight of 5169 daltons, contains 49 amino acid residues and consists of 2900 identical subunits per virion (10).

The most common procedure currently used for separation of coat protein and DNA is phenol extraction. The portion of gene 8 coat protein recovered from the phenol layer is insoluble, except after treatment with 0.1 M NaOH or a high concentration of urea, guanidine HCl or 1% SDS solution.

In this short communication, we report on a new procedure for the simple thermal solubilization of f_d gene 8 coat protein and on the pronounced effect which metal ions have on its solubility.

MATERIALS AND METHODS

Isolation of f_d phage. f_d phage was prepared according to the procedure of Yamamoto et al. (12). The phage was further purified by CsCl density gradient centrifugation and banded at a CsCl density of 1.30.

The concentration of purified f_d phage was determined by an extinction coefficient of 36.7 (13). In purified f_d phage, the optical density of 260/280 mμ is 1.0. One optical density at 280 mμ is equal to 1.3×10^{13} PFU/ml. PFU (plaque forming unit) values were determined by plaque assay.

Isolation of gene 8 coat protein from f_d phage and thermal solubilization. Gene 8 coat protein was obtained from purified f_d phage by 6X phenol extraction similar to the method of Knippers and Hoffmann-Berling (2). Twenty ml of Na_2-EDTA-borate buffer, pH 9.2, was added to 10 ml of purified f_d phage (approximately 100 mg of phage), then 30 ml of freshly distilled phenol which had been previously saturated with the same buffer were added and the mixture was shaken gently at room temperature for 10 to 15 minutes.

The solution was centrifuged at 6000 RPM using a JA-20 rotor for 10 minutes at 4°C. The phenol and aqueous layers were extracted three times by the procedure described above. The phenol layer was reextracted with 20 ml of EDTA-borate buffer saturated with phenol, and the process repeated three times at room temperature. This layer was dialyzed against four different systems at 4°C, following the addition of two volumes of methanol. These systems in the order in which they were used are:

1. 1:1 methanol: EDTA-borate buffer, overnight with several changes.
2. 1:3 methanol: EDTA-borate buffer, several days.
3. EDTA-borate buffer, overnight with several changes.
4. Water for one week, with several changes each day.

During the first two hours of step (1), a precipitate forms within the dia-

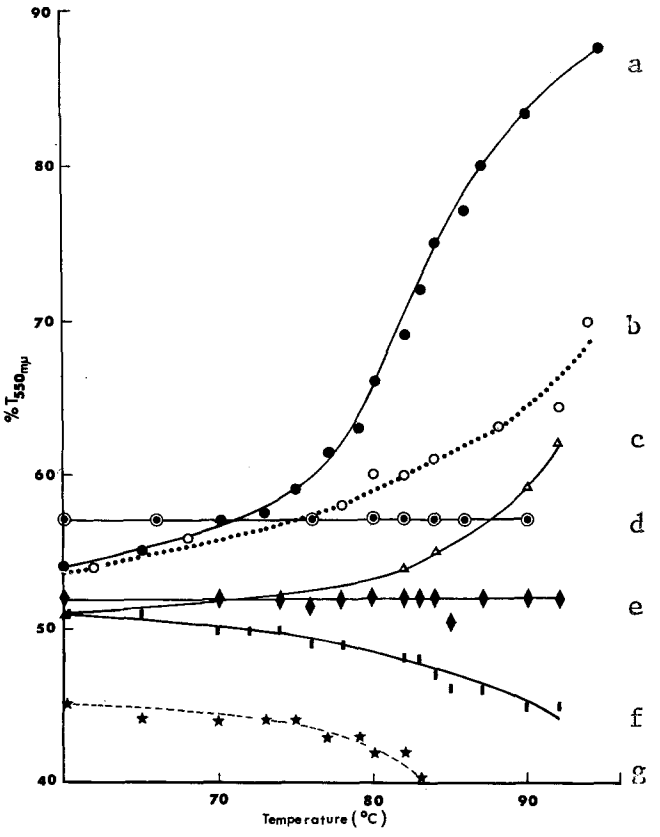

Fig.1

The effect of metal ions on thermal solubility of gene 8 coat protein in 2 M urea. Disappearance % of turbidity at 550 mu as a function of temperature.

a. 0.15 mg/ml of coat protein in 0.1 M $MgCl_2$
b. 0.14 mg/ml of coat protein in water
c. 0.14 mg/ml of coat protein in 0.033 M NaCl
d. 0.12 mg/ml of coat protein in 0.033 $MgCl_2$
e. 0.12 mg/ml of coat protein in 0.033 M $CaCl_2$
f. 0.12 mg/ml of coat protein in 0.033 M $MnCl_2$
g. 0.1 mg/ml of coat protein in 0.033 M $ZnCl_2$

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lysis bag; thus, the dialysis bag must be constantly rotated. After removal of phenol, the solution was reduced to 15 ml (4.4 mg/ml of coat protein), using PM2 filter paper on the Amicon filtering apparatus. The precipitated

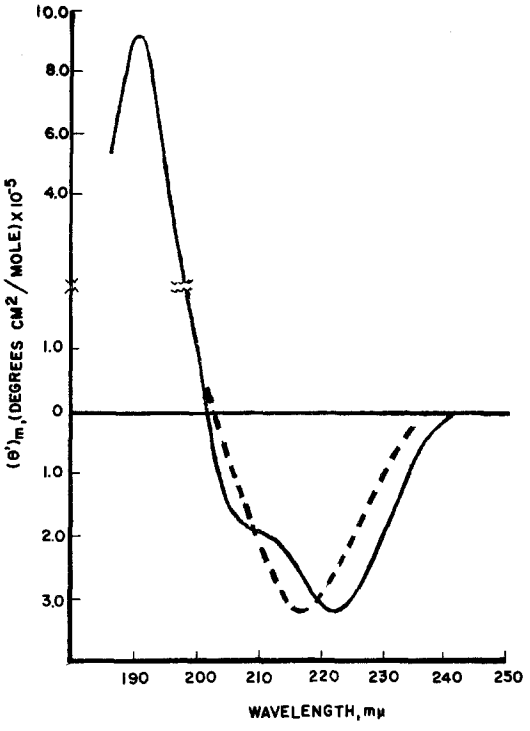

Fig.2

Circular dichroism of f_d phage and thermally solubilized gene 8 coat protein. Solid line representes intact particles. Broken line represents thermally solubilized coat protein (Optical density of both samples at 280 mu was 1.0, dialyzed against 0.1 M NaCl).

suspension of gene 8 coat protein was stored at 4°C. The concentration of protein was determined by dissolving the precipitate in 0.1 M NaOH, giving an optical density at 280 mμ of 0.625, which is equivalent to 1 mg/ml (2). The suspended gene 8 coat protein was brought to approximately 5.0-8.0 mg/ml using PM2 filter paper.

Sulfuric acid tests (14) were performed to detect any DNA contaminant present in the preparation and the Lowry test (15) was used for determination of protein. The still turbid solution suspended in 2 M urea was placed in

a water bath and the temperature gradually brought to 90°C over a period of one hour with constant stirring. The % $T_{550\ m\mu}$ as an indication of turbidity was measured using a Bausch and Lomb Spectrophotometer 20. Similar experiments were performed in 4M, 6M and 8M urea. In all cases after dialysis to remove urea, the coat protein is in solution, with a percent transmittance of 95 to 100. The solubilized coat protein may be kept at 4°C indefinitely, and it remains soluble when dialyzed against any buffer system above neutral pH.

If the insoluble coat protein precipitate is suspended before heating in 2 M urea in the presence of metal ions (such as NaCl, $MgCl_2$, $MgSO_4$, $CaCl_2$, $ZnCl_2$, or $MnCl_2$), the solubility is decreased by 80-90% or more, as shown in Figure 1, indicating that the thermal solubility of coat protein is highly dependent on the type of metal ions present.

In the absence of metal ions, when 0.1 mg/ml of insoluble coat protein was suspended in 4 M urea, the percent transmittance increased to 75-80%, while in 6 to 8 M urea, percent transmittance rose to 100%. Increasing the temperature to 95°C, the percent transmittance was nearly 100% at all concentrations of urea. The value did not change upon cooling and dialysis against water.

When the thermally solubilized coat protein in 1% SDS solution was examined by SDS gel electrophoresis, only a single band with some streaking was observed. The mobility of this band corresponds to a molecular weight of about 14,000, a great deal higher than the reported molecular weight of 5,200 for the monomer (10).

Results of circular dichroism measurements shown in Figure 2 indicate that the native phage particles exhibit 80-85% helicity. However, thermally solubilized gene 8 coat protein shows a trough at 217 mμ which is characteristic of a β structure. None of our results indicate that the structure of the coat protein is helical in nature.

The molecular weight of thermally solubilized coat protein in 0.055 M PO_4 buffer, pH 7.0 is found to be 43,000 ± 1,500, as compared to 42,500 ±

1,350 for insoluble coat protein in 8 M urea, both at infinite dilution, as obtained by sedimentation equilibrium measurements.

Insoluble gene 8 coat protein exhibits apparent sedimentation coefficients of 2.6 S_{app} in 1% SDS, 4.6 S_{app} in 0.01 M NaOH and 3.5 S_{app} in 8 M urea. The thermally solubilized coat protein in 0.1 M NaCl, pH 7.0, however, shows a higher degree of aggregation, with an apparent sedimentation coefficient of 13.5 S_{app}. These results indicate that the state of aggregation of gene 8 coat protein varies greatly with the solvent conditions. Our observations are in agreement with the results reported by Knippers and Hoffmann-Berling (2).

In working with f_d phage, we found we are dealing with one of the least soluble of phage proteins. Its coat protein remains highly aggregated even after the most drastic treatment.

The thermal solubility procedure which we have described alleviates this problem of protein solubility, and promises wide applicability in phage research. As long as no metal ions are present, this procedure would yield a soluble protein product which will remain in solution even after dialysis against water or prolonged storage.

Although we were able to solubilize the gene 8 coat protein, all our results indicate that even in solution this material remains highly aggregated. We were unable to dissociate the gene 8 coat protein into monomeric units of 5200 in any of the solvent systems tested. This thermal solubility procedure remains, however, the only way to solubilize f_d coat protein. Still to be determinedare the nature and degree of structural change which may occur in the protein as a result of this procedure.

Gene 8 coat protein has been sequenced by Asbeck et al. (10). Based on sequence analysis, this protein has been divided into three regions: the acidic (N-terminal, amino acid residues 1 to 20) or region I; the central hydrophobic (θ^h region; 21-38 amino acid residues) or region II; and the basic (C-terminal, 39-49 amino acid residues), region III.

The central hydrophobic region is of particular interest because of the

arrangement of two tyrosine and one tryptophan residues in close proximity (tyr^{21}-ile^{22}-gly^{23}-tyr^{24}-ala^{25}-$tryp^{26}$). These three aromatic amino acids of the coat protein may interact with the base of f_d-DNA to form a structure stabilized by a π-cloud of electrons. A second possibility is that the phosphate groups of f_d-DNA may form a salt linkage with the C-terminus carboxyl group of region II (16).

In either case, once this interaction is broken by alkali treatment or phenol extraction, we speculate that the hydrophobic region may assume an irreversible, fully-exposed conformation. This would explain the high degree of aggregation of thermally solubilized coat protein.

ACKNOWLEDGMENT

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