ON A MODIFICATION OF THE GENE PRODUCT *P*23 ACCORDING TO ITS USE AS SUBUNIT OF EITHER NORMAL CAPSIDS OF PHAGE T4 OR OF POLYHEADS

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Received 28 March 1970

Previous results have shown that the major protein subunits of the capsids of normal T4-heads and of the capsoids of head related variants are identical with, or derived from, gene product *P*23. In SDS gel electrophoresis, two distinct mobilities are found, suggesting a molecular weight difference of about 20%.

The product of gene 23 (*P*23) is used in the assembly of several morphologically defined head related variants [1–4]. These variants - enumerated in the order of an increased number of other genes obligatorily cooperating in the assembly - are as follows: multilayered polyheads, single-layered polyheads, isometric τ-particles, prolate τ-particles, isometric heads, prolate heads [3]. By different methods, evidence was provided that the major component of all these particles is identical with, or derived from, the same protein produced by gene 23:

1) Amber mutants of gene 23 alone or in combination with other appropriately chosen morphopoietic genes are unable to build any of these variants [3, 6].

2) Dissociation of all the particles into subunits by different reagents (6 M guanidine hydrochloride, 67% acetic acid, pH 13–8 M urea) and subsequent urea gel electrophoresis always shows the same major electrophoretic component *M* [1, 4, 5] identified as the product of gene 23 [1, 7].

3) All these variants have at least one antigenic site in common: tubular capsoids and τ-particles share at least one other site, not present on capsids, and, conversely, capsids carry at least one other site not found on tubular forms and τ-particles [4, 5].

As predictable from the results of Sarabhai et al. [8] on the colinearity of protein 23 with its gene, amber mutants in this gene lead to incomplete peptide chains with correspondingly increased electrophoretic mobility [9, 1].

In addition to the variant forms mentioned above, *P*23 can also be found in an other apparently unorganized form, the "lumps" which adhere inside to the cell envelopes. They appear when *P*31 is inactive because of a mutation [9, 10]. The proteins of these envelope-bound particles consist mainly of *P*23 as shown by urea gel electrophoresis ([9] and our fig. 3d). Lumps are also produced by the mutants in gene 23, whether *P*31 is active or not [9]. The amount of labelled protein sticking to the envelopes is directly proportional to the length of the peptide chain.

Since in SDS gel electrophoresis the electrophoretic mobility of polypeptides appears to depend on molecular weight [11, 12], we used this technique to analyze the phage head proteins. In this preliminary note, we show that in SDS gels, the main capsid subunit protein exists in two different electrophoretic mobilities according to the variant from which it derived. Tubular forms and lumps give rise to a form of relative mobility M_p = 0.23, M.W. 61,000, capsids to M_c = 0.28, M.W. 49,000 (table and fig. 1).

Capsids were obtained in an industrial fermentor (1,500 l, courtesy Sandoz S.A., Basel) from mutant 255 in gene 10 according to procedures previously described [1]. In this mutant, tail assembly is blocked at a very early step [13] so as to produce tailless heads. Since these particles are fragile, it is very easy to empty them artificially (freeze-thawing and DNAase) to produce empty capsids which can then be purified. The "polyheads" were produced by mutant *N*50 in gene 20 and partially purified by differential centrifugation [6, 14]. Lumps were produced by mutant *N*54 in gene 31; after chloroform induced lysis, the envelope fraction (with the lumps adhering) was sedimented and washed once.

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Table
Relative mobilities in 10% SDS gels.

Exp. No.	Lumps M_p 100° SDS	Polyheads M_p 65° SDS	Polyheads M_p 100° SDS	Polyheads Urea-Alkali M_p 20° SDS	Capsids M_c 100° SDS	Capsids Urea-Alkali M_c 20° SDS	Full Heads M_c 100° SDS	Pepsin Standard
22					0.28 0.28			0.4 0.4
23			0.24					0.4
25		0.23	0.25 0.25		0.28 0.28			0.4
26	0.23 0.23		0.23 0.23		0.28 0.28 0.28			0.4 0.4 0.4
28		0.22	0.23 0.23 0.23	0.23	0.28 0.28	0.28	0.27	0.4 0.4 0.4
29			0.23 0.23				0.26 0.27 0.27 0.27	0.4 0.4 0.4 0.4
30					0.28		0.28 0.28 0.28	0.4 0.4 0.4
mean	0.23	0.225	0.235	0.23	0.28	0.28	0.273	0.4

Dissociation into protein subunits was accomplished by one of the following procedures: (1) 0.1% SDS either at 37° for 10 to 30 min, 65° for 10 min or 100° for one min, (2) 6 M guanidine hydrochloride, (3) 8 M urea and 10 min at pH 13 and 45°. In all cases, 0.1 M mercaptoethanol was added, usually after previous N_2 bubbling. The protein solutions thus obtained were dialyzed against the buffer solution needed for urea or SDS gel electrophoresis. It was found that samples containing urea could be applied directly to SDS columns.

For the SDS gels, we used 10% acrylamide with 0.2% Bis; the procedure of Maizel [15] was followed throughout.

The urea gels were 7.5% acrylamide according to the previously used procedure [1].

Molecular weight determinations in SDS gels were made by using as calibrating proteins: Bovine serum albumin, *l*-amino acid oxidase, pyruvate kinase, glutamine dehydrogenase, ovalbumin, pepsin, chymotrypsinogen, lysozyme and ribonuclease A [16, 17]. The calibration curve is shown in fig. 1.

We rarely ran all proteins in a single SDS gel because sometimes, probably due to overloading, inconsistent mobilities were observed. Therefore, we used instead permuted combinations of proteins run in several tubes, at the same time. The relative mobilities (in respect to pepsin = 0.4) are summarized in the table. The reproducibility of the measurements was always well within 5% with the exception of the low molecular weight substances like lysozyme and ribonuclease A (see fig. 1).

The result to be emphasized here is that M_p derived from lumps or polyheads has a mean mobility of 0.23 while M_c derived from capsids is more than 20% greater with a mobility of 0.28. These two subunits were obviously run simultaneously in the same gel as seen in fig. 2. The molecular weight of M_c is 49,000 and that of M_p is 61,000.

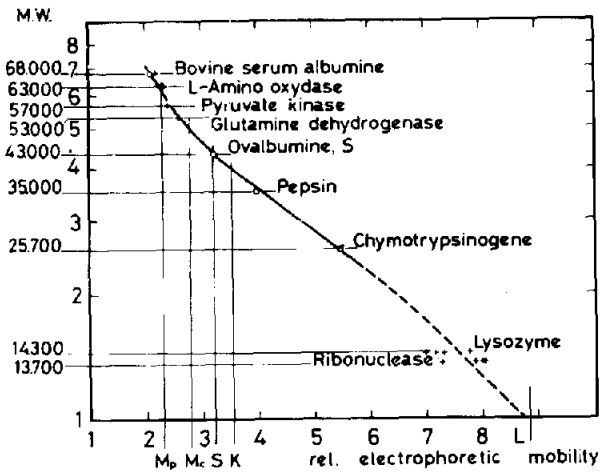

Fig. 1. Calibration curve of molecular weight versus electrophoretic mobility in 10% acrylamide SDS gels. Many independent determinations were made. ○: at least 5 superposable values; +: individual values; x: determinations of M_p, M_c, K and S. S is derived from contaminating polysheaths and corresponds to $P18$.

The difference is observed even when the proteins are denatured by guanidine hydrochloride and mercaptoethanol [18] prior to SDS treatment. Different conditions of SDS denaturation did not explain the difference (table).

The different proteins were rechecked by urea electrophoresis (fig. 3). All the previous results [1] were confirmed and all the M-bands were identically located. We had observed previously, when using reduced amounts of protein, that the band M frequently appeared as a doublet. Doublets are also visible in the case of capsids, where M_c but no M_p is present (fig. 3a). Hence, the two lines of the doublet do not represent M_c and M_p.

The mobility variants M_p and M_c according to all previous evidence must be derived from the same gene product $P23$. Our experiments make it unlikely that they are due to differences in the degree of denaturation. The following hypotheses remain: (1) M_c is derived from the original gene product M_p by digesting or cutting 20% of the peptide chain. This loss might be compensated by a loss of charge, such that in urea gels, no electrophoretic difference is apparent. (2) The gene product is modified not by cutting, but by altering side chains in such a fashion that SDS binding and aggregation are strongly modified. (3) M_p contains a small amount of another chemical substance not of proteineous nature, which is strongly linked to $P23$ and uncharged. This linkage would resist pH 13 in urea and have a very strong affinity for SDS. Thus it would not alter the mobility in urea gel, but, by a heavy uptake of SDS, explain the 20% reduced mobility in SDS-gels.

We favour the first explanation. Since the action of several more additional gene products ($P20$, $P40$, $P21$, $P24$) is needed for the assembly of normal capsids than for the tubular form, the results suggest that one of these gene-products may have proteolitic activity and leads to the excision of 20% of the native $P23$ either prior to or during assembly of capsids. Experiments to prove this hypothesis are under way.

Besides M ($P23$), capsids contain two additional components K and L as seen easily by urea gel electrophoresis (fig. 3). The identification of the corresponding lines in SDS gel electrophoresis was made in our laboratory by L.Black using highly purified tailless, but full heads [16]. From our calibration curve, we obtain for K a molecular weight of 40,000 and for L approximately 8,000 to 11,000. (The gel concentration used is not adequate for these low molecular weights.) By equilibrium sedimentation in guanidine hydrochloride, Larcom and Bendet [19, 20] determined molecular weights of the mixture of protein subunits obtained through dissociation of highly purified capsids. They found two molecular weight classes (46,000 and 11,000). In a mixture of proteins like M_c (49,000) and K (40,000) individual values could not be distinguished by this method; it is most likely therefore, that their value of 46,000 corresponds to an average.

Definitive results about molecular weights and, in consequence, about the postulate of a 20% excision, will only be obtained when we have succeeded in isolating and purifying each of these proteins. It is easy to explain now why all our attempts based on separation by size (gel filtration with 50% acetic acid, urea or guanidine hydrochloride) have failed [21].

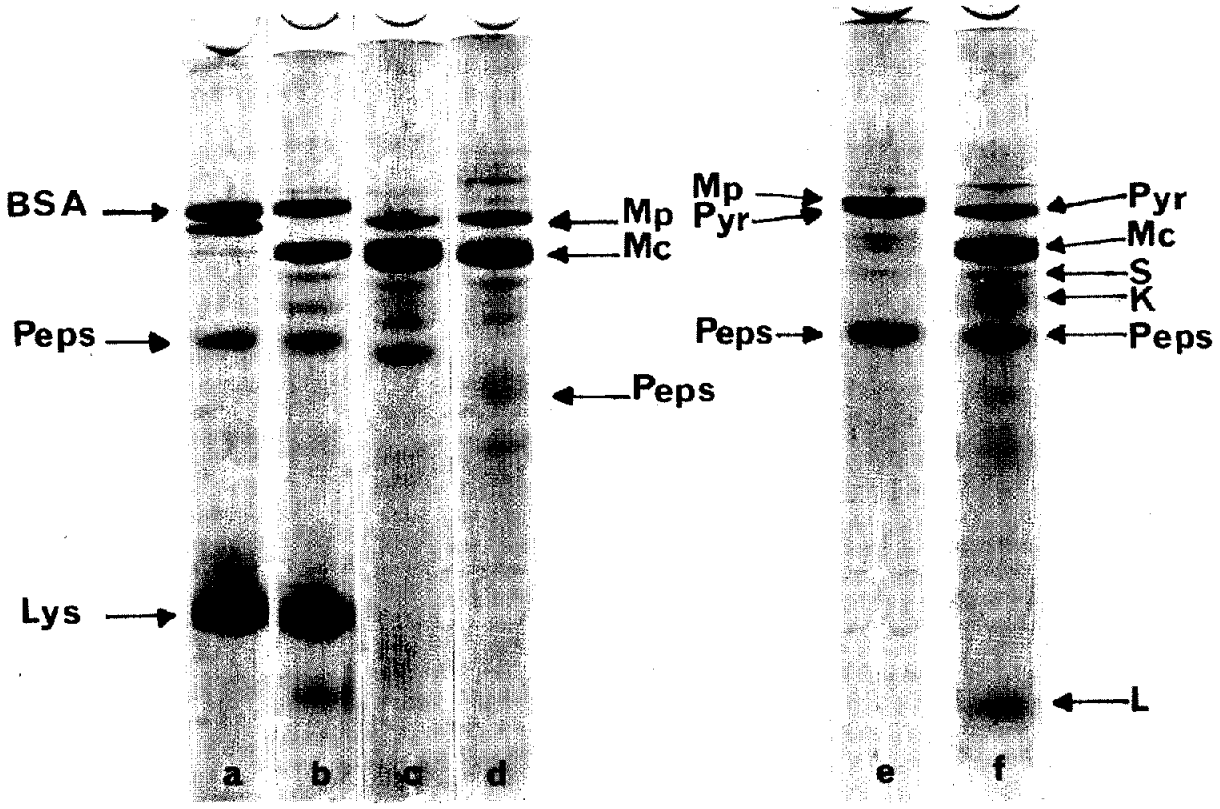

Fig. 2. SDS gel electropherograms of (a) polyheads, (b) capsids, (c) polyheads and capsids, (d) polyheads, capsids and lumps, (e) polyheads, (f) capsids. On the photograph (e) the lines formed by the pyruvate kinase and M_p are barely distinguishable. Reference proteins are: bovine serum albumin (BSA), pyruvate kinase (Pyr), pepsin (Peps), lysozyme (Lys). Different proteins were mixed before they were heated to 100° in 0.1% SDS.

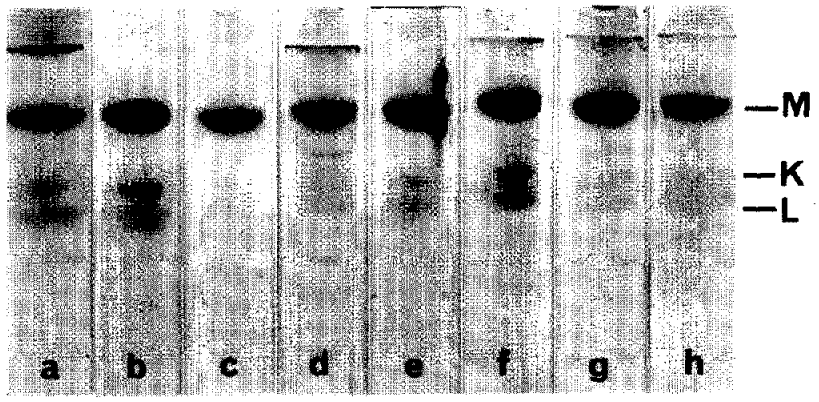

Fig. 3. Urea gel electrophoresis of (a) and (b), capsids, (a) showing the double band, (c) polyheads, (d) lumps, (e) capsids and polyheads, (f) capsids and lumps, (g) polyheads and lumps, (h) capsids, polyheads and lumps.

Acknowledgement

We gratefully acknowledge the critical comments from R.Traut who provided us also with suitable proteins for calibration.

Note added in proof

In the meantime we learnt that Dr. U.Laemmli, M.R.C.-Unit for Molecular Biology, Cambridge, England, and Dr. F.Eiserling, Dept. of Bacteriology, University of California, Los Angeles, have obtained - completely independently - similar results.

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