CHARACTERIZATION OF THE ENZYMATIC TAIL PROTEIN OF BACTERIOPHAGE P22

U. ERIKSSON and A. A. LINDBERG
Department of Bacteriology, National Bacteriological Laboratory. S-105 21 Stockholm

and

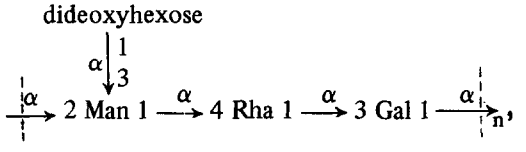
H. JÖRNVALL and H. VON BAHR-LINDSTRÖM
Institute of Chemistry I, Karolinska Institutet, S-104 01 Stockholm 60, Sweden

Received 1 November 1976

1. Introduction

The first step in infection of bacteria by bacteriophages involves recognition of receptor structures on the bacterial surface. In the outer membrane of the cell envelope of *Salmonella* and other gram negative bacteria, a lipopolysaccharide is a structural component. It is composed of a chain often made up of repeating oligosaccharide units, covalently linked to a common carbohydrate core which in turn is attached to a lipid [1].

Bacteriophage P22 infects *Salmonella* bacteria having the repeating unit



in the lipopolysaccharide, i.e. *Salmonella* serogroups A, B and D. The interaction between phage P22 and the lipopolysaccharide receptor results in cleavage of glycosidic bonds between the rhamnose and galactose components. This endorhamnosidase activity presumably facilitates the approach of the phage particle to the cell membrane and a hypothetical second receptor, after which injection of the phage nucleic acid might occur. The glycosidase activity resides in the tail part of the phage, since isolated tail parts have been shown to have the same enzymatic properties as whole phage particles [2]. Hydrolytic actions on carbohydrate receptors have been frequently found in studies of the early stage of infection by bacteriophages [3]. Some properties of the enzymatic tail protein of bacteriophage P22 were recently published [4]. In the present work chemical data on this protein are reported.

2. Materials and methods

The tail protein was purified as previously described [2] after infection of a non-permissive strain of *Salmonella typhimurium* with the phage mutant P22c_1, amN114, H101. The enzyme was reduced with dithiothreitol (0.4 mg/mg protein) and carboxymethylated with ^{14}C-labelled iodoacetate (1.5 mg/mg protein) in urea [5]. The modified protein was digested with TPCK-trypsin (Worthington Co) and peptides were mapped on paper with different steps of high-voltage electrophoresis and chromatography in 1-butanol/acetic acid/pyridine/water (15:3:10:12 by vol.) as previously described [6]. Total compositions were determined with a Beckman 121M amino acid analyser after hydrolysis in 6 N HCl/0.5% mercaptoethanol for 20, 48 and 72 h at 110°C in evacuated tubes. Values for serine and threonine were extrapolated to zero time. The amounts of valine and isoleucine were taken from the values after 72 h hydrolysis. End-groups were identified by the dansyl method and dansylated amino acids detected by thin-layer chromatography in four systems [5]. For sequential degradation of the intact protein,

the dansyl-Edman method was used [7]. C-terminal residues were determined by dansylation of released amino acids after digestions in 0.5% NH_4HCO_3 for 4 h at 37°C, with carboxypeptidase A or B (Sigma Chem. Comp.) in ratio of 1–2% by weight of the protein. Alternatively, hydrazinolysis at 110°C for 6 h in sealed tubes followed by dansylation was performed.

Peptides were detected by staining with Cd-ninhydrin [8] or reagents specific for tyrosine, arginine, tryptophan and histidine, respectively [9]. Electrophoresis in polyacrylamide gel (20%) with 0.1% SDS was carried out as described [10] with marker proteins of cytochrome *c*, myoglobin, carbanhydrase and bovine serum albumin.

3. Results

3.1. *SDS–polyacrylamide gel electrophoresis*

Analysis by polyacrylamide gel electrophoresis of the pure protein after or without reduction with mercaptoethanol showed only one component. The molecular weight of the corresponding subunit equals 16 000 in both cases, as estimated from the relative mobility of the marker proteins. In some preparations, however, multiple bands were noticed at positions which may correspond to undissociated material.

3.2. *N-terminal analysis*

Lysine was found to be the only N-terminal amino acid. Sequential degradations also revealed single residues for the next four steps and the N-terminal sequence was established to be Lys–Glx–Thr–Ala–Ala– .

3.3. *C-terminal analysis*

Digestions with carboxypeptidases A and B followed by detection of released amino acids by dansylation, revealed the presence of tyrosine, lysine, valine and alanine (in the order of decreasing amounts) as visually estimated from thin-layer chromatography. No amino acid could be detected by hydrazinolysis.

3.4. *Amino acid composition*

Results of hydrolysis of the carboxymethylated enzyme are given in table 1. The data show no obvious characteristic for this protein, but acidic residues are common, while for example glycine and branched chain aliphatic residues are less frequent. In some preparations of tail protein the total compositions showed a larger excess of glycine and leucine, thus resembling the amino acid composition given previously [4]. These preparations, however, were not quite reproducible and contained the tail protein in an apparently lower yield. Significant variations in yields between different batches have also been reported for another phage bound glycosidase [11].

Table 1
Amino acid composition of the carboxymethylated P22 tail protein

	Mol %	Nearest integer/ 16 000 mol. wt
Cys (Cm)	6.5	10
Asx	12.7	19
Thr	8.0	12
Ser	11.0	17
Glx	10.2	15
Pro	3.6	5
Gly	4.0	6
Ala	9.4	14
Val	7.1	11
Met	1.7	3
Ile	2.5	4
Leu	1.9	3
Tyr	4.4	7
Phe	2.4	4
Lys	7.9	12
His	3.3	5
Arg	3.4	5

Values for serine, threonine, valine and isoleucine have been estimated as mentioned in the text. No tryptophan was detected after specific staining of tryptic peptides.

3.5. *Peptide mapping*

A sample of 50 nmol of the carboxymethylated protein was digested with TPCK-trypsin. Peptides produced were first separated by high-voltage electrophoresis at pH 6.5. The second dimension was chromatography for basic peptides, electrophoresis at pH 3.5 for acidic peptides and electrophoresis at pH 1.9 for neutral peptides. The latter were finally further resolved by an extra dimension chromatography [6]. Peptide maps were stained with ninhydrin, and the presence of 12 neutral, 5 basic and 5 acidic peptides was revealed as shown in fig.1. These results

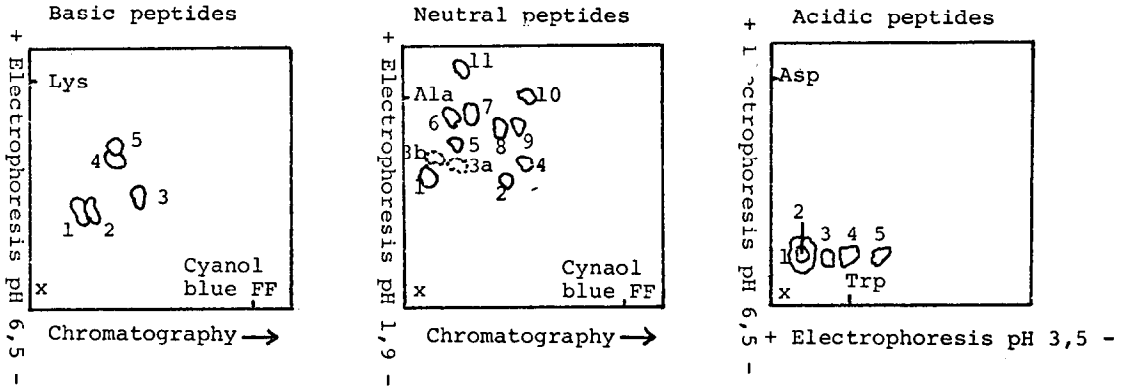


Fig.1. Maps of tryptic peptides of the P22 tail protein. (x) Indicates the origin. Numbers identify the peptides in table 2.

together with those of other staining methods are shown in table 2. Radioactive peptides were quantitatively estimated by liquid scintillation counting of corresponding paper areas after localization by autoradiography.

All estimates in table 2 are approximate since partial sensitivity of peptide bonds to tryptic hydrolysis may in some cases produce more than one peptide from single regions. For example, such a mutual relationship between peptides is suspected for the two neutral fragments 3a and 3b (cf. fig.1).

No tryptophan could be detected by staining with the Ehrlich method.

4. Discussion

4.1. *Purity of P22 tail protein*

Only one band could be seen on SDS–polyacrylamide gel electrophoresis, indicating a pure material. This was further confirmed by the clear results of N-terminal analysis and Edman degradations, as well as by the peptide mapping, yielding distinct spots in numbers consistent (cf. below) with the total compo sition.

4.2. *Subunit size and homogeneity*

The molecular weight of the undissociated protein has previously been estimated to be 240 000 [2]. The size of the subunits was determined to be 16 000 as deduced from marker proteins. It seems probable that no interchain disulfide bridges occur, since identical molecular weights were estimated from the electrophoretic analysis, independently of the reductive pretreatment. The value obtained corresponds to about 150 amino acids. The nearest interger values for separate amino acids thus estimated are also given in table 1. Those are highly consistent with

Table 2
Summary of mapping of tryptic peptides

Amino acid	Peptides																						Total
	Neutral												Basic					Acidic					
	1	2	3a	3b	4	5	6	7	8	9	10	11	1	2	3	4	5	1	2	3	4	5	22
Cys (Cm)	3	–	1	1	–	–	1	–	–	–	–		–	–	–	–	–	–	1	1	2	1	11
His	1	1	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	1	5
Tyr	1	1	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	1	1	–	–	5
Arg	–	–	–	–	–	–	–	–	–	–	1	1	–	–	–	1	1	–	–	–	1	1	6

Specific residues were detected by staining reagents, except for Cys (Cm) which was estimated by measurements of radioactivity of spots, detected by autoradiography (~20 000 cpm equals one residue). All values given are approximate.

values for all residues detected in the mapping of the tryptic peptides (table 2) and show that larger subunit sizes estimated from SDS–polyacrylamide gel electrophoresis are likely to represent undissociated material.

The present results establish the purity and subunit homogeneity of the preparation and show that the entire protein consists of an aggregate of identical subunits.

Acknowledgement

This work was supported by grants from the Swedish Medical Research Council (projects 13X-3532 and 40X-656).

References

[1] Lüderitz, O., Westphal, O., Staub, A. M. and Nikaido, H. (1971) in: Microbiol. Toxins IV, pp. 145–233, Academic Press, New York and London.
[2] Eriksson, U. and Lindberg, A. A. (1977) J. Gen. Virol. in press.
[3] Lindberg, A. A. (1976) in: Surface Carbohydrates of the Procaryotic Cell (I. W. Sutherland, ed) Academic Press, New York and London.
[4] Iwashita, S. and Kanegasaki, S. (1976) Eur. J. Biochem. 65, 87–94.
[5] Jörnvall, H. (1970) Eur. J. Biochem. 14, 521–534.
[6] Jörnvall, H. (1970) Eur. J. Biochem. 16, 41–49.
[7] Gray, W. R. (1967) Methods Enzymol. 11, 469–475 and (1972) Methods Enzymol. 25, 333–344.
[8] Heilmann, J., Barrollier, J. and Watzke, E. (1957) Hoppe Seyler's Z. Physiol. Chem. 309, 219–220.
[9] Ambler, R. P. (1963) Biochem. J. 89, 349–378.
[10] Maizel, J. V. Jr. (1969) in: Fundamental Techniques in Virology (Habel, K. and Salzman, N. P. eds) pp. 334–362, Academic Press, New York and London.
[11] Bessler, W., Freund-Mölbert, E., Knüfermann, H., Rudolph, C., Thuron, H. and Stirm, S. (1973) Virology 56, 134–151.