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ISOLATION AND PARTIAL CHARACTERIZATION OF THE M_r 100 kD PROTEIN FROM *RICKETTSIA PROWAZEKII* STRAINS OF DIFFERENT VIRULENCE

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Summary. - The major M_r 100 kD protein (protein I) from the standard virulent *R. prowazekii* strain Breinl, from the nonvirulent strain E and its virulent revertant EVir were isolated by chromatography and characterized. Purified protein I from the three strains of different virulence and origin had the same physico-chemical and antigenic properties, protected guinea pigs against infection with the virulent strain Breinl and induced the production of antibodies, which neutralized the toxic and haemolytic activities of *R. prowazekii.* The amino acid composition of protein I as determined for the three above mentioned strains was similar. Modified residues of Lys, Asn, and/or Gln were found in protein I. Protein I from virulent strains Breinl and EVir differed from that of nonpathogenic strain E by the quantity of N^{ϵ}-Me-Lys and N^{ϵ}-Me_3-Lys, but all had the same total amount of Lys and its derivatives. It may be suggested that a difference may exist in the processing of the protein I of nonpathogenic strain E and of virulent strains of *R. prowazekii.*

Key words: Rickettsia prowazekii; strains with different virulence; main protein I; amino acid composition

Introduction

Rickettsia prowazekii, the causative agent of typhus fever, is an obligatory intracellular parazite. The nature of its pathogenicity is not understood. We attempted to solve this problem by analysing the main rickettsia surface antigens, which participate in the interaction with the eukaryotic cells. The M_r= 100-120 kD protein (protein I) of *R. prowazekii* is believed to represent the

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main surface antigen and protective immunogen (Weiss *et al.*, 1987). The present investigation was devoted to isolation and characterization of protein I from a standard virulent strain Breinl, nonvirulent strain E and its virulent revertant strain EVir.

Materials and Methods

R. prowazekii strains E, EVir, Breinl were used. The passaging schedule of these strains is indicated in Table 1. *R. prowazekii* were cultivated in chick embryo (CE) yolk sacs and purified from the host cell components using Verografin (SPOFA, Č.S.F.R.) density gradient centrifugation (Aniskovich *et al.*, 1989). Polyspecific rabbit sera were obtained against each of the three strains as described above. To obtain a monospecific protein I antiserum, a band of protein I was cut from the polyacrylamide gel (PAG) following electrophoresis of *R. prowazekii* cells and homogenized in 0.05 mol/l phosphate buffer (pH 7.5). A rabbit was immunized subcutaneously with 10, 100, 200, and 400 μg protein I on days 1, 14, 28, and 56, respectively. After bleeding on the day 70 the serum ELISA titre was 1:50 000.

The protein was determined according to Lowry *et al.*, (1951). PAGE was performed as described by Laemmli (1970). The gel was stained with Coomassie R-250 or silver reagent („Bio-Rad", U.S.A.). To identify the lipopolysaccharide components (LPS) the gel was treated with sodium periodate. The used modification of western immunoblotting was described earlier (Eremeeva *et al.*, 1989).

Protein I purification consisted of Tris-soluble antigen isolation according to Dasch (1981), precipitation of protein fraction with ammonium sulphate, combination of gel filtration, and ion exchange chromatography on FPLS system (Pharmacia-LKB). The separated components were tested by the ELISA-method with monoclonal antibodies against protein I (Drobyshevskaya *et al.*, 1990). The amino acid composition was determined on an amino acid analyzer LC 5001 „Biotronic" as previously described (Rodionov, 1988).

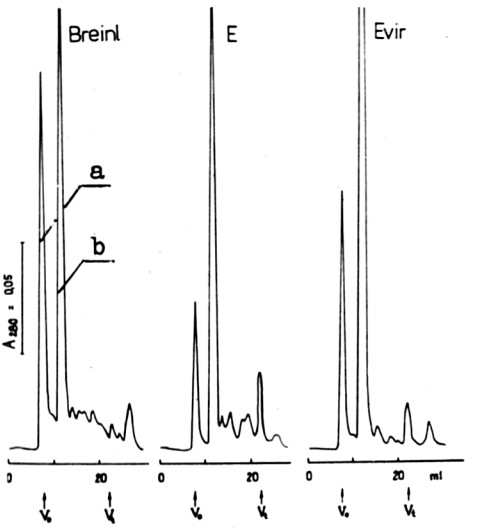

Fig. 1
Gel chromatography of Tris-soluble antigens from *R. prowazekii* on Superose 12TM HR 10/30 column using 50 mmol/l sodium phosphate buffer (pH 7.5), containing 0.15 mol/l NaCl
Flow rate - 0.5 ml/min. V_0 and V_t - free and full column, respectively. *A* and *b* - peaks contained the protein I.

Protective activity of the purified proteins was tested in guinea pigs. Animals were immunized subcutaneously with 10 μg (1:1 with incomplete Freund's adjuvant „Difco", U.S.A) and subsequently with 2 μg of the protein without adjuvant within the 90 days interval. On days 21 and 45 following the second immunization the guinea pigs were infected i.p. with 10^3 ID_{50} suspension of *R. prowazekii* strain Breinl. The rickettsial toxin and haemolysin neutralization tests were performed as described (Balayeva and Zubok, 1963).

Results and Discussion

We found previously that the Tris-soluble fractions of *R. prowazekii* contained several other proteins and nucleic acids in addition to protein I (Eremeeva *et al.*, 1989). To isolate the protein I we separated this protein from nucleic acids by precipitation with ammonium sulphate at 55 % saturation. The protein fraction was subjected to gel filtration on Superose 12TM column after dialysis against 0.05 mol/l sodium phosphate buffer (pH 7.5), containing 0.15 mol/l NaCl (Fig. 1). The chromatographic patterns of each of the three strains were similar and revealed two main peaks designated *a* and *b*. They contained

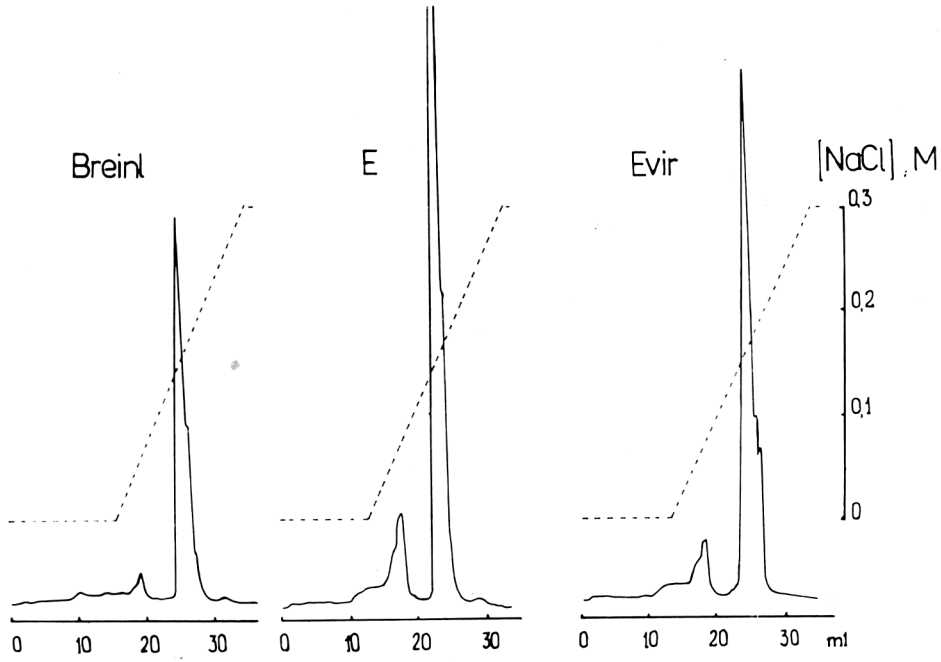

Fig. 2
Purification of protein I fractions, isolated by gel chromatography on Mono Q TM HR 5/5 column. Linear NaCl gradient in 20 mmol/l bis-Tris (pH 6.0)
The NaCl concentration gradient is shown by a dotted line. Flow rate - 0.5 ml/min.

the components which reacted in ELISA with monoclonal antibodies against protein I. The SDS-PAGE patterns of peak *a* revealed a 30 kD polypeptide band after Coomassie blue staining and LPS-components after silver staining. The peak *b* contained mainly protein I. The fraction *b* was further purified by ion exchange chromatography on Mono Q column in a linear NaCl gradient. The purified protein I was eluted with 0.15 mol/l NaCl (Fig. 2).

The eluted protein I from the strains E, EVir, Breinl was chromatographically and electrophoretically homogenous (Fig. 3 and Fig. 4) and did not contain traces of LPS. The purified protein from the strains E, EVir, Breinl had

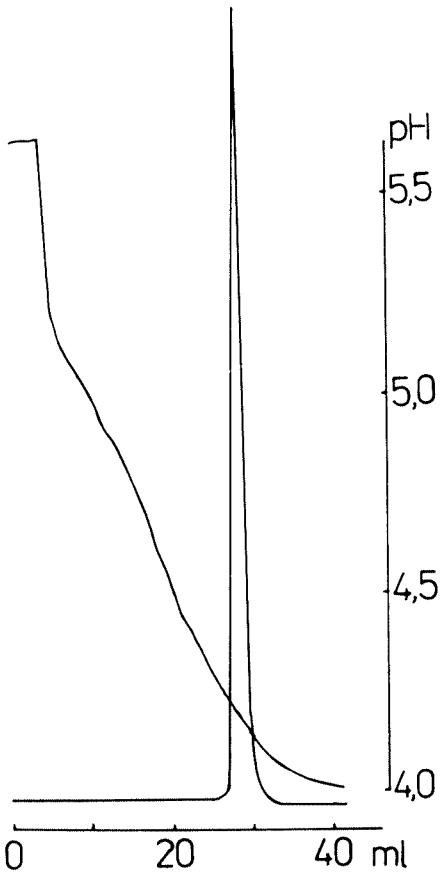

Fig. 3
Determination of the purity the isolated protein I sample from the strain Breinl by chromatofocusing on Mono P TM HR 5/20 column 25 mmol/l piperazine-HCl (pH 7.5) used as starting buffer and poly-buffer 74 (pH 4.0) as elution buffer Flow rate - 0.5 ml/min. The chromatograms of the isolated protein I samples prepared from strains E and EVir were identical.

Fig. 4
Native-PAGE of protein I from strains Breinl, E, EVir in a linear gradient of 7.5 % to 20 % monomers

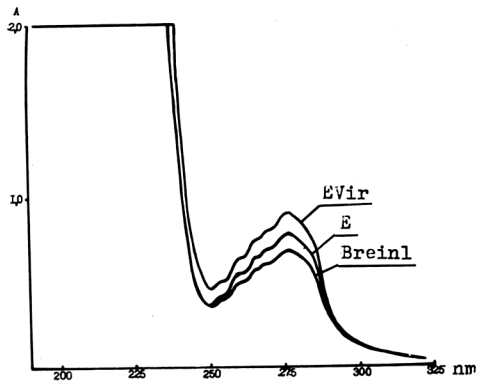

Fig. 5
Ultraviolet absorption spectrum of protein I measured on a Unicam SP.800 („Beckman") spectrophotometer
R. prowazekii strain Breinl protein - 1.02 mg/ml, E protein - 1.14 mg/ml, EVir protein - 1.35 mg/ml.

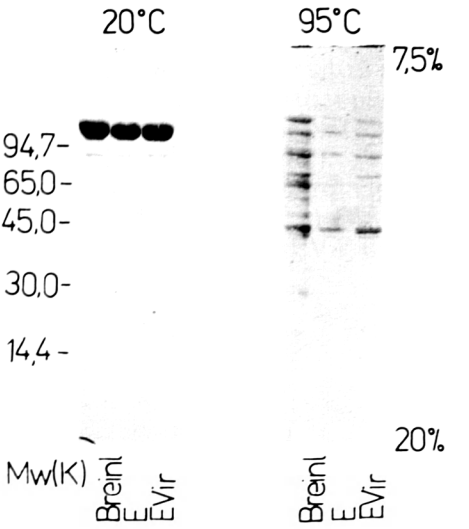

Fig. 6
SDS-PAGE analysis of protein I strains Breinl, E, EVir in a linear gradient of 7.5 % to 20 % monomers
Samples were solubilized in 2 % SDS for 2 hr.

the same M_r=100 kD, pI=4.22, a very special absorption spectrum and identical patterns in native- and SDS-PAGE (Figs. 4, 5, 6). Several SDS-PAGE components M_r 14–100 kD were found after heating each of the three protein I samples at 95 °C for 2 hr. Their finding did not depend on the addition of protease inhibitor PMSF. The protein samples incubated at room temperature contained only a single 100 kD component (Fig. 6). Therefore, we believe that protein I has a subunit composition. At the same time it can be also supposed that the appearance of low molecular components after protein I heating is the result of the cleavage of Asp-Pro-bonds (Rittenhouse and Marcus, 1984).

The isolated proteins also retained the similar antigenicity and immunogenicity, as demonstrated in immunoblotting crossreactions with strainspecific serum and with monoclonal antibody against protein I from the strain Breinl. The latter fact indicated that three proteins from the strains with different virulence and origin contain at least a single common epitope.

The main protein I from strains E, EVir, Breinl was established to possess the protective activity retained after purification, while expressing the similar physico-chemical and immunological properties. The twofold immunization with each of the purified proteins protected the guinea pigs against infection with 10^3 ID_{50} of the virulent strain Breinl cell suspension. In addition, the monospecific sera against the purified protein I contained antibodies which neutralized *in vitro* $3x10^7$ metabolically active cells of *R. prowazekii* as calculated by method of Walker and Winkler (1979), and $2LD_{50}$ of toxic *R. prowazekii* suspension of each of the three strains.

The identity of the properties of the protein I from the strains with different virulence and origin suggests the similarity in their structure. This suggestion

Table 1. The Rickettsia prowazekii strains

Strain	Passage history	Biological characteristics					
		CE ID_{50}/ml	white mice ID_{50}/ml	guinea pigs ID_{50}/ml		days of fever	CF titre*
Breinl	Isolated by Wolbach *et al.* (1922) Passaged in guinea pigs, CE, lice (passage history unknown)+6 passages in CE+5 passages in lice+ 6 passages in CE	$10^{9.5}$	$10^{9.5}$	$10^{9.5}$	10^{-4}	10.7	1:533
					10^{-5}	8.7	1:426
					10^{-6}	9.0	1:480
					10^{-7}	8.0	1:373
					10^{-8}	7.3	1:320
					10^{-9}	1.7	1:120
E	Isolated by Clavero and Perez-Gallardo (1944) as a spontaneous mutant of the virulent strain Madrid I. Obtained from Prof. Wisseman (U.S.A.) in 1969; underwent 270 passages in CE +8 passages in CE+1 passage in tissue culture + 2-5 passages in CE.	$10^{7.9}$	10^{2} $10^{4.1}$	$10^{6.5}$	10^{-2}	3.5	1:40
					10^{-3}	3.0	1:10
					10^{-4}	2.3	1:10
					10^{-5}	2.3	1:10
					10^{-6}	1.7	1:10
					10^{-7}	0	0
EVir	Preparated by 13 passages in white mice lungs from strain E (obtained from the U.S.A. in 1969) by Balayeva (1971).	$10^{8.75}$	$10^{8.5}$	$10^{8.4}$	10^{-4}	10.6	1:530
					10^{-5}	14.0	1:373
					10^{-6}	9.3	1:266
					10^{-7}	6.5	1:160
					10^{-8}	11.0	1:480
					10^{-9}	10.0	1:320

* by micro-CF-test

was confirmed by amino acid analysis of the proteins (Table 2). The amount of the common protein amino acid residues was practically identical. The residues of N^{ε}-Me-Lys, N^{ε}-Me_3-Lys and modified residues of Asn and/or Gln were found in protein I. The peculiarity of the nonpathogenic strain E protein I was that it did not have N^{ε}-Me_3-Lys residues and had larger quantities of N^{ε}-Me_3-lys residues as compared with the proteins from Breinl and EVir strains. However, the common amount of Lys and its derivatives was the same in proteins I from the strains with different virulence. We believe that the precursor proteins and their genes are similar in all three strains regardless of their virulence and genetic origin, but there is a difference in the processing of the proteins of virulent strain Breinl and EVir on one hand and the nonpathogenic strain E on the other hand. The relationship of this difference to biological properties of *R. prowazekii* will be the subject of our further investigation.

Table 2. Amino acid composition of protein I from *R. prowazekii*

Amino residue	Relative content, M/M His		
	strain Breinl	strain E	strain EVir
Asx	19.38±0.47	19.52±0.85	19.50±0.62
Thr	11.46±0.54	11.85±0.53	12.20±0.36
Ser	6.46±0.36	6.58±0.28	6.51±0.31
Glx	6.77±0.35	6.85±0.28	6.76±0.33
Pro	2.54±0.27	2.55±0.31	2.63±0.28
Gly	12.19±0.91	12.50±0.82	21.75±0.96
Ala	10.47±0.51	10.65±0.36	10.68±0.42
Val	5.85±0.23	6.10±0.26	6.06±0.29
Met	0.60±0.03	0.61±0.02	0.62±0.03
Ile	7.47±0.32	7.72±0.18	7.75±0.25
Leu	8.39±0.46	8.59±0.30	8.49±0.35
Tyr	1.21±0.05	1.32±0.03	1.32±0.04
Phe	4.11±0.15	4.19±0.05	4.22±0.09
N^{ε}-Me_3-Lys*	1.18±0.11	0	1.21±0.04
Lys	0.89±0.10	1.11±0.11	1.18±0.12
N^{ε}-Me-Lys*	1.23±0.10	2.69±0.25	0.91±0.06
His	1.0	1.0	1.0
Arg	0.95±0.04	0.94±0.04	0.96±0.03
Other nynhidrinpositive compounds			
Ammonia	8.54±0.47	9.22±0.40	8.83±0.38
Methylamine	1.44±0.09	1.39±0.03	1.31±0.05

* determined by Rodionov (1990).

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