and precipitated with 70% ammonium sulphate as described by Sipos and Merkel[5]. The precipitated trypsin was dissolved in 10^{-3} M HCl, dialysed against 10^{-3} M HCl and then lyophilized. Protein was estimated by the method of Lowry *et al.*[6] SDS–PAGE was carried out as described by Fairbanks *et al.*[7]

The method of Schroeder and Shaw[4] for purification of β-trypsin using SE-Sephadex column involves equilibration of the column with 0.1 M Tris-HCl, pH 7.1, 20 mM Ca^{2+} and subsequent elution with the same buffer. When identical conditions of column equilibration and elution were attempted with SP-Sephadex column, different forms of trypsin present in commercial trypsin were eluted as a single peak corresponding to the position of inert trypsin. This indicated that trypsin preparation did not retard under such chromatographic conditions. As the molarity of tris was reduced, the different components of trypsin preparation exhibited retardation and the best separation was achieved with 0.01 M tris. This profile is shown in Figure 1 where peak A, B and C correspond to inactive material, α-trypsin and β-trypsin respectively. Identification of peaks B and C was made possible by SDS–PAGE profile. Since α-trypsin consists of two chains linked by disulphide bond, it is expected to show two bands in SDS–PAGE (if trypsin sample is treated with β-mercaptoethanol before electrophoresis) in contrast to β-trypsin which should emerge as single band. When pooled fractions from peak B (No. 88–96) and from peak C (No. 105–122) were subjected to SDS–PAGE, it exhibited two and one bands respectively (Figure 2). This confirmed that peak B and C corresponded to α-trypsin and β-trypsin respectively and β-trypsin was the major component in this lot of commercial trypsin.

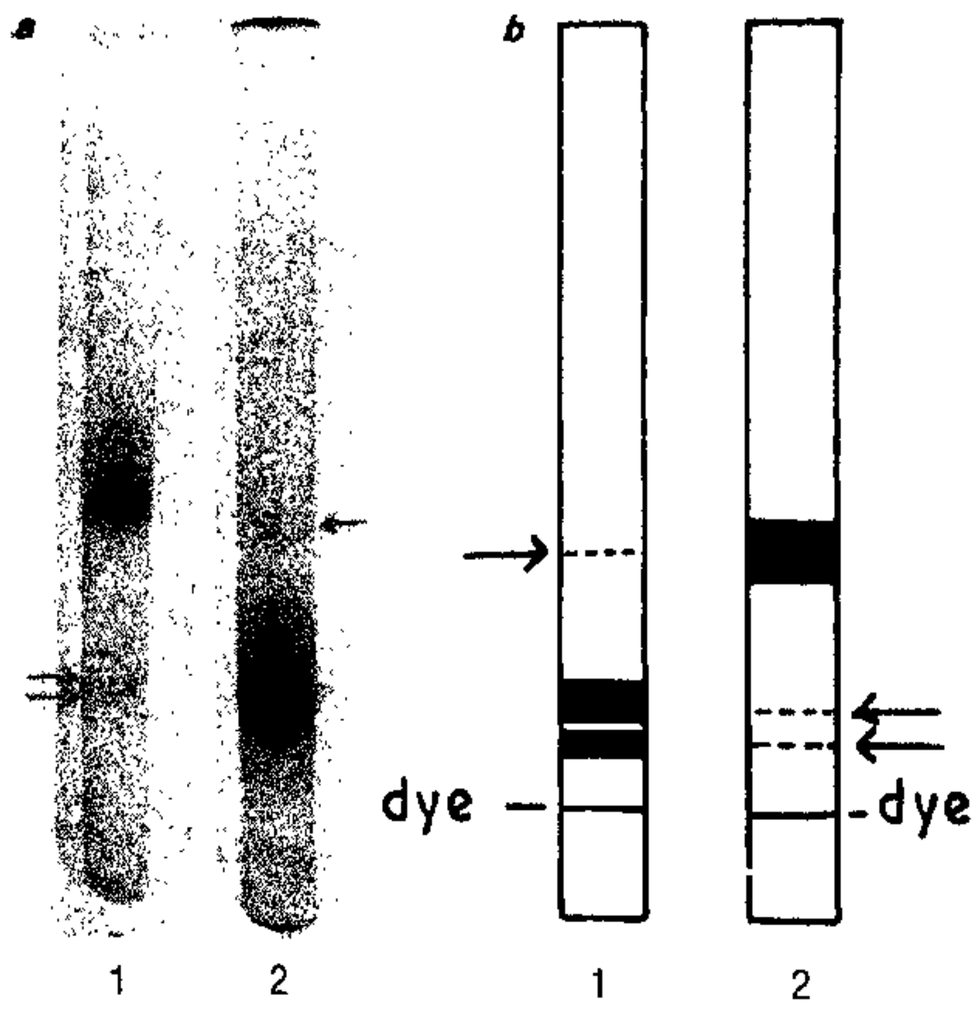

Figure 2. *a*, SDS–PAGE pattern of trypsin; *b*, diagram of pattern in *a*. Lanes 1 are trypsin from peak B of figure 1, lanes 2 are trypsin from peak C. Faint bands were also seen at indicated arrow position when trypsin was loaded in higher amount.

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In vitro packaging system for bacteriophage F0 DNA

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A packaging system for bacteriophage F0 (host *Salmonella typhimurium*) has been developed using purified components, viz. mature F0 DNA, F0 proheads, the products of gene 6 (group 6) and 9 (group 9). The reaction required magnesium ions, ATP and polyvinyl alcohol (or polyethylene glycol). The system can use DNAs from other Salmonella phages as well (e.g. DNAs from phage P22, MB78, KB1 and 9NA). Spermidine can enhance the packaging reaction but its presence is not indispensable. The packaging reaction exhibited a sigmoidal relationship with respect to the concentration of ATP with the concentration for half-maximal activity about 20 μM.

BACTERIOPHAGE F0 (Felix-01) is not a very well characterized phage of *Salmonella typhimurium*[1]. It synthesizes many proteins, basically divided into capsid and noncapsid proteins, of which the products of gene 6 and gene 9, called group 6 and group 9 respectively, are required for DNA packaging (unpublished observation from this laboratory). These two proteins are noncapsid proteins with mol wt of 28,000 (group 6) and 34,000 (group 9). During head assembly of this phage, DNA is packaged into the prohead cavity and this process requires magnesium ions, ATP and polyvinyl alcohol and the products of gene 6 and 9. To understand mechanisms involved in packaging, it is important to construct a packaging system composed of defined factors. I have developed such a system for Salmonella phages. As it is true for other host packaging systems[2–4], several host factors are also required for phage assembly along with some phage proteins. This report describes a defined system for the packaging of F0 DNA.

Phage F0 and *S. typhimurium* strains were obtained from K. E. Sanderson, University of Calgary, Canada. Their maintenance and growth conditions have been described previously[5-8].

DEAE-cellulose (DE-52), ammonium sulphate, phenyl-Sepharose CL-4B, Sephadex G-75, ethylene glycol, hydroxyl apetite were obtained from Pharmacia Co., USA. All other chemicals were purchased from Sigma Chemicals, USA.

For preparation of proheads, one litre culture of *S. typhimurium* was grown to 2×10^8 cells per ml in minimal medium[7] at 37°C and infected with phage F0 at a multiplicity of infection of 10. Forty min after infection, cells were centrifuged at 5,000 *g* for 10 min at 4°C and suspended in 10 ml of buffer A (150 mM NaCl, 15 mM sodium citrate and 2.5 mM EDTA). After the addition of lysozyme (freshly made from lyophilized enzyme, final concentration 100 μg/ml prepared in 10 mM Tris-HCl, pH 7.5), the mixture was kept on ice for 30 min. Cells were lysed by the addition of 25% Triton-X 100 and after 10 min, the lysate was mixed with equal volume of 4 M NaCl and stirred for 15 min. The suspension was then centrifuged at 10,000 *g* for 30 min at 4°C. The supernatant was collected and centrifuged at 80,000 *g* for 90 min at 4°C. The pellet was suspended in buffer B (0.5 M NaCl, 10 mM Tris-HCl, pH 7.5, 1 mM magnesium chloride) and washed by two cycles of differential centrifugations as described earlier[4]. Proheads were finally purified by centrifugation through a 5–25% sucrose gradient in buffer B at 25,000 *g* for 3 h at 4°C. The fractions containing the proheads were dialysed against buffer B and centrifuged by high speed centrifugation. The concentration of prohead suspensions was determined and represented as phage equivalent units per ml, assuming that the protein content of the prohead is equal to that of a phage particle[5].

Phage DNA was isolated as described by Verma *et al.*[6] For packaging a complete reaction mixture (50 μl) contained 60 mM Tris-HCl, pH 7.5, 10% polyvinyl alcohol, 5 mM magnesium chloride, 1 mM 2-mercaptoethanol, 60 mM NaCl, 0.05 mM ATP, 10^9 phage equivalent of mature phage F0 DNA, 5×10^9 proheads, 10 pmol of group 6 and 5 pmol of group 9. After 90 min of incubation at 37°C, the reaction was terminated by the addition of DNase I (5 μg/ml). The suspension was further incubated for 30 min at 37°C and filled heads were obtained. The head acceptor extract was prepared as described earlier[2] and 10 μl of head acceptor extract was mixed with 50 μl of filled head suspension. After incubation at 37°C for 30 min phages were assayed on *S. typhimurium* as described earlier[2].

The conditions for *in vivo* packaging of phage F0 were the same as described earlier except that the concentration of ATP was varied from 2 μM to 300 μM. Initially the relationship between phage production and ATP concentration gives a sigmoidal curve whereas the phage production remains constant from ATP concentration ranging from 40 μM to 150 μM. However, higher concentrations of ATP (i.e. higher than 150 μM) have inhibitory effect on the phage production. In the absence of ATP packaging does not occur. The energy released by hydrolysis of ATP is utilized for the movement of DNA into head and the requirement of ATP varied for different Salmonella phages, viz. F0, MB78, P22, KB1, and 9NA (ref. 9–12) and the reason for this variation in the requirement of ATP is not fully understood. The concentration of ATP for half maximal activity was 20, 15, 25, 25 and 30 μM for phages F0, MB78, P22, KB1 and 9NA respectively. However, a non-hydrolyzable analog, adenosine 5′-*O*-(3-thiotriphosphate) inhibited packaging reaction in all the cases.

Extensive studies with viral specific proteins indicate that two noncapsid proteins, group 6 and group 9, interact with proheads and DNA at the time of packaging (unpublished observations). Along with these proteins some other factors are also required for packaging and a complete account of these factors is given in Table 1. As is clear from Table 1, ATP, polyvinyl alcohol and magnesium are essentially required for packaging along with DNA, proheads and proteins (group 6 and group 9). When all the above components are incubated for 90 min at 37°C with head acceptor extract (containing tail and tail fibre proteins) packaging was observed. The requirement for polyvinyl alcohol can be replaced by polyethylene glycol (mol wt 8000) and like phage T3[2] the stimulatory effect of polyethylene glycol decreases the molecular weight of the polymer. Spermidine is not absolutely required but

Table 1. Requirements of different components for *in vitro* packaging system.

Condition	Phage production (PFU/ml)
Control (complete reaction mixture)	2×10^7
− DNA	5–10
− Prohead	5–10
− ATP	1–5
− Group VP 6	1–5
− Group VP 9	100–150
− Polyvinyl alcohol	1–5
− Spermidine	$3–5 \times 10^5$
− $MgCl_2$ + 10 mM EDTA	$1–2 \times 10^2$
+ 20 μM ATP-γ-S	3×10^3
+ 200 μM ATP-γ-S	10–20
− ATP + 20 μM ATP-γ-S	1–5
+ DNase I (head acceptor extract was added to the reaction mixture at the start of DNA packaging reaction)	1–5
− F0 DNA + MB78 DNA	$5–7 \times 10^5$
− F0 DNA + P22 DNA	6×10^6
− F0 DNA + KB1 DNA	$5–6 \times 10^5$
− F0 DNA + 9NA DNA	$5–6 \times 10^5$
− 2-Mercaptoethanol + 10 mM *N*-ethylmaleimide	$2–5 \times 10^3$