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Alteration in the electrophoretic mobility of OmpC due to variations in the ammonium persulfate concentration in sodium dodecyl sulfate-polyacrylamide gel electrophoresis

Studies on *Salmonella typhi* and *Salmonella typhimurium* outer membrane proteins have shown that the relative position of OmpC porin in sodium dodecyl sulfate-polyacrylamide gel electrophoresis undergoes an important shift when the concentration of ammonium persulfate in the running gel is increased from 6 to 12 mM.The apparent molecular mass at these concentrations was estimated to be 34 and 40 kDa, respectively. Under similar electrophoretic conditions the apparent molecular mass estimated for OmpF was 37.6 and 38.2 kDa. Therefore, OmpC moves from a leading position to a position behind OmpF. For *Escherichia coli* OmpC the shift observed is less pronounced than that occurring in *Salmonellae*.

In the study and characterization of outer membrane proteins (OMP) from Gram-negative bacteria, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), according to Laemmli [1], is probably the discontinuous gel system most widely used. By this technique a constant weight ratio of SDS is bound to a protein previously reduced with β-mercaptoethanol [2]. Thus, the separation of proteins becomes largely independent of tertiary structures, dependent solely in the subunit molecular weight (MW) [2].

Nevertheless, the electrophoretic mobility of some proteins is affected by conditions not related to SDS and thiol reagent concentrations [3]. This type of anomalous behavior is exhibited by OmpA, named the heat modifiable protein because the unheated form, which retains much of its native structure in SDS, has a different mobility (29kDa) than the heat-denatured form (33kDa) [4]. The migration of OmpA in SDS-PAGE is also affected by the addition into the sample buffer of lipopolysaccharide or reagents such as $MgCl_2$, $MnCl_2$, or NaCl [5]. Under similar electrophoretic conditions the mobilities of other major OMP, such as OmpC and OmpF porins, are not affected [5].

In the present study, we demonstrate that OmpC porin from *Salmonella typhi* Ty2, *Salmonella typhimurium* LT2, and *Escherichia coli* K12 undergoes an important shift in its relative migration in slab SDS-PAGE when the amount of ammonium persulfate (APS) varied, within a limited range of concentrations, in the separating or running gel. This effect was not obtained when APS concentration was modified in the stacking gel.

OMP preparations were obtained according toSchnaitman [6]. Bacterial culture grown in 5 mL of nutrient broth were sonicated and centrifuged at 10000 *g* for 5 min in a microfuge at 4°C. The supernatant obtained was centrifuged at 13000 *g* for 45 min at 4°C. The pellet was resuspendend in buffer, 10 mM Tris-HCl, 10 mM $MgCl_2$, 2% Triton X–100, pH 8.0, and incubated for 30 min at 37°C. After centrifugation at 13000 *g* for 45 min at 4°C the pellet was resuspended in 100 mM Tris-HCl, pH 8.0, 2% SDS. The OMP preparations were mixed with the sample buffer of Laemmli, containing a final concentration of 0.0625 M Tris-HCl, pH 6.8, 2% SDS, 10% glycerol, 0.001% Bromophenol Blue, and 5% β-mercaptoethanol. The samples were boiled for 5 min at 100°C and subjected to SDS-PAGE as described by Laemmli [1].

Slab gels were 0.8 mm thick and 12 cm long. To start the polymerization, APS was varied from 3–24 mM final concentration in the separating gel. In some experiments a split gel system was used to separate (lengthwise) two slab gels, 11 cm long, prepared with different amounts of APS, both sides having the same stacking gel on top and sharing the same running buffer. The two running gels were kept apart by a Teflon spacer, 1 cm wide, 12 cm long, and 0.8 mm thick. Electrophoresis was run at a constant current of 20 mA. The gels were fixed by gentle shaking during 30 min in a solution containing 10% trichloroacetic acid (TCA), and 50% 2-isopropylalcohol, and stained overnight in 10% TCA, 40% 2-isopropylalcohol, and 0.2% Coomassie Brilliant Blue R-250. Destaining was accomplished in 10% acetic acid. Apparent molecular weights were estimated using a prestained SDS molecular weight standard mixture, SDS-7B (Sigma Chemical Co.), in a 10% polyacrylamide gel. Standard marker proteins were: triose phosphate isomerase (26.6 kDa); lactic dehydrogenase (36.5 kDa); fumarase (48.5 kDa); pyruvate kinase (58 kDa) and fructose-6-phosphate kinase (84 kDa). β-Galactosidase (116 kDa) and α_2-macroglobulin (180 kDa) were not considered for these estimations.

During studies with *S. typhi* Ty2 OMP, an alteration in the electrophoretic mobility of OmpC was detected when the concentration of APS in the running gel was doubled from 6.0 to 12.0 mM. The concentration of APS described in the procedure of Laemmli is 3.3 mM. The effect of APS on *S. typhi* Ty2 and *S. typhimurium* LT2 OMP was demonstrated by selecting 3.3 and 24.0 mM final concentrations in the running gel. These concentrations are referred to as low and high salt conditions, respectively. A purified *S. typhi* Ty2 OMP preparation composed mainly of OmpC and OmpF proteins is shown (Fig. 1, lane A and A′). At the low salt condition, OmpC is represented by a band migrating towards the anode ahead of OmpF (lane A). However, at the high salt condition, OmpC appears to be moving to a retarded position compared to OmpF (lane A′). The change in migra-

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Abbreviations: SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; **APS,** ammonium persulfate; **OMP,** outer membrane proteins

tion of OmpC can be more clearly observed for *S. typhi* 1895 OMP (Fig. 1, lane B and B′). Strain 1895 is an OmF⁻ mutant, isolated in our laboratory [7], only exhibiting two major OMP: OmpC porin and OmpA. It is observed that the protein band corresponding to OmpC runs close to OmpA at low salt (lane B). However, at high salt (lane B′), OmpC is displaced away from OmpA. Using *S. typhimurium* OMP (Fig. 1, lane C and C′) the relative electrophoretic mobility of OmpC is altered from low to high salt condition. OmpC and OmpD porins appear to be comigrating at the low salt condition, but at high salt they are observed as two distinct bands. OmpD is a porin that so far has not been described among *S. typhi* OMP [8].

To demonstrate that the effect on OmpC is not restricted to *Salmonellae*, *E. coli* K12 OMP were also studied (Fig. 2, lanes B and B′). In this case, to facilitate the observation of any change in the migration of porins, OMP from two *E. coli* mutants MC4104 (*ompC*: : Tn5) lacking OmpC (lane A and A′), and MC4105 (*ompF*: : Tn5) lacking OmpF (lane C and C′) were employed. Both mutants are derived from *E. coli* MC4100 [9], and were obtained from M. Inouye. From low to high salt condition, OmpC moves from a position running in front of OmpF to one that it is positioned just behind OmpF. The apparent mass of *E. coli* K12 major OMP were also estimated (Table 1).

When NaCl was added into the separating gel at 34.2 mM (0.2%) in the presence of 3.3 mM APS, the shift in the electrophoretic mobility of OmpC was also observed (result not shown). This amount of NaCl has been employed by other laboratories to improve sharpness of low molecular mass protein bands [10, 11]. In contrast, the electrophoretic behavior of OmpC was not altered when NaCl was included in the sample and/or in the running buffers.

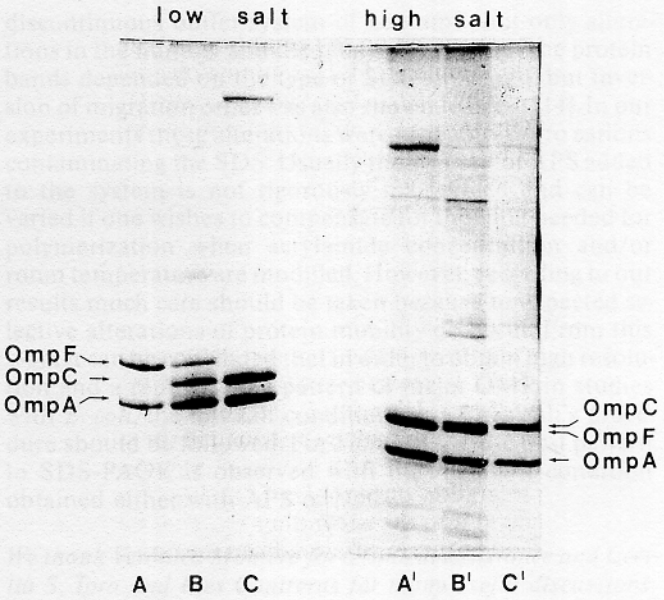

Figure 2. 12.5% split SDS-PAGE of OMP from *E. coli* MC4104 OmpC⁻ (lane A and A′), *E. coli* K12 (lane B and B′) and *E. coli* MC4105 OmpF⁻ (lane C and C′). OMP samples and gel conditions as in Fig. 1.

Table 1. Apparent molecular mass (kDa)[a)]

	OmpC		OmpF		OmpD	
	LS	HS	LS	HS	LS	HS
S. typhi	34.6	40.0	37.6	38.2	–	–
S. typhimurium	34.6	40.0	37.6	38.2	34.6	37.2
E. coli	34.4	38.3	36.0	38.3	–	–

a) The data for apparent molecular mass were obtained by plotting $\log_{10}$ of the molecular mass of standard proteins as a function of the R_f in 10% SDS-PAGE.

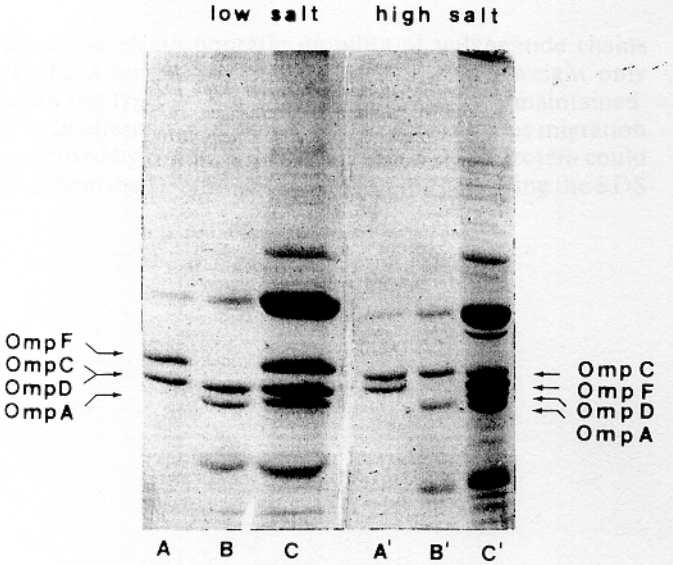

Figure 1. 12.5% split SDS-PAGE of OMP from *S. typhi* Ty2 (lane A and A′), *S. typhi* 1895 OmpF⁻ (lane B and B′) and *S. typhimurium* LT2 (lane C and C′). OMP samples were boiled 5 min at 100°C in the sample buffer of Laemmli before application. The split slab gel system consists of two running gels prepared with different amounts of APS to start polymerization, sharing the same stacking gel on the top. Low salt: 3.3 mM: high salt: 24 mM.

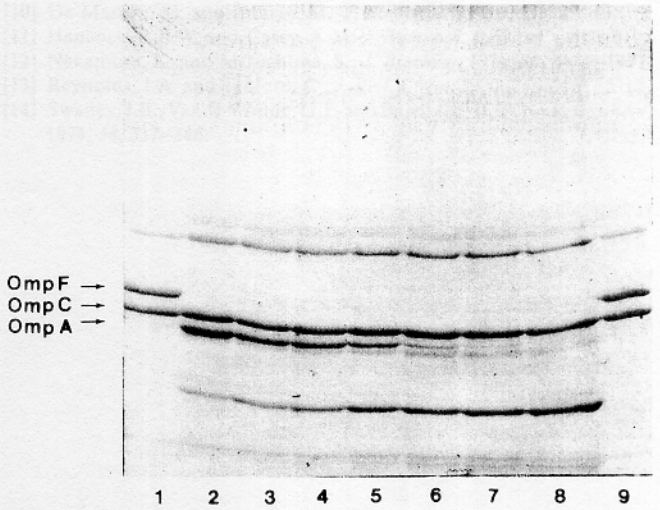

Figure 3. 12.5% SDS-PAGE of OMP from *S. typhi* 1895 suspended in sample buffer of Laemmli, supplemented with different amounts of NaCl. All the samples were boiled 5 min at 100°C before application. Lane (2)–(8) contain 0, 15, 31, 62, 125, 250, and 500 mM NaCl, respectively. Lane (1) and (9) contain purified preparation of *S. typhi* Ty2 porins, lacking OmpA, prepared in the sample buffer of Laemmli without NaCl. The separating gel was prepared with 3.3 mM APS (low salt).

As previously reported, OmpA shows two apparent molecular masses, depending on the condition of solubilization of the protein sample [4, 5]. This anomalous behavior has been explained by recognizing two different stable conformations of OmpA in SDS [12]. The mobility of OmpA was also affected by the addition of NaCl in the sample buffer [5]. We observed that, as the concentration of NaCl increases, a displacement of OmpA occurs (Fig. 3). In other words, the electrophoretic migration of OmpA changed from a position corresponding to the 33–35 kDa form to that of 25–29 kDa. This was detected even though the samples were boiled for 5 min at 100°C. OmpA remains in the native configuration even upon heating of the sample in SDS, provided that NaCl is present during the heat treatment at no less than 125 mM. The critical effect of NaCl on OmpA is produced only when the salt is contained in the sample buffer. In addition, NaCl or APS produced a dramatic alteration in the migration of porins in SDS-PAGE only when the salt is added into the separating gel. Therefore, the effect of NaCl on porins is different from the one preventing the heat denaturation of OmpA in SDS.

When an excess of salt is added into the separating gel the migration of OmpC relative to OmpF is retarded, appearing as a higher molecular weight protein, just the opposite of what is observed at low salt. This is more noticeable in *Salmonellae* than in *E. coli*. It is possible that a high concentration of cations in the running gel will generate a flow of positive charges in an opposite direction to the protein-dodecyl sulfate negatively charged complexes, slowing down the migration of most proteins. In other words, the cation flow impedes the migration of proteins and apparently OmpC is more affected than OmpF and other polypeptides. In the case of two polypeptides with similar molecular masses, such as OmpC and OmpF, the resolution attained during electrophoresis will depend on the form adopted by denaturation. The more unfolded the protein, the more retarded it will be by the cation flow.

Since the electrophoretic mobility of polypeptide chains can be a unique function of the molecular weight only when the typical 1.4 g SDS/g protein ratio is maintained [13], an alternative explanation to the anomalous migration presented by OmpC is that binding of SDS to protein could be diminished by the salt in the running gel. Using the SDS discontinuous buffer system of Laemmli, not only alterations in the number and the relative position of the protein bands depended on the type of SDS employed, but inversion of migration order was also shown to occur [14]. In our experiments these alterations were probably due to cations contaminating the SDS. Usually the amount of APS added to the system is not rigorously maintained and can be varied if one wishes to compensate for the time needed for polymerization when acrylamide concentration and/or room temperature are modified. However, according to our results much care should be taken because unexpected selective alterations of protein mobility do occur. From this work it can be concluded that in order to obtain high resolution and a reproducible pattern of major OMP in studies with *E. coli*, the low salt condition as in Laemmli's procedure should be followed. For *Salmonellae*, the best pattern in SDS-PAGE is observed with the high salt condition obtained either with APS or NaCl.

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