Apparent Molecular Weights of a Heat-Modifiable Protein from the Outer Membrane of *Escherichia coli* in Gels with Different Acrylamide Concentrations

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The apparent molecular weights of the two forms of a heat-modifiable protein from the outer membrane of *Escherichia coli* K-12, estimated in gels with different concentrations of acrylamide, indicate that the protein binds excess amounts of sodium dodecyl sulfate, possibly due to large beta structures before boiling.

The cell envelope of the *Enterobacteriaceae* consists of two membranes and a peptidoglycan layer in between. Methods have been developed to separate the outer membrane from the cytoplasmic membrane (10). By using sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis, it has been shown that the outer membrane of *Escherichia coli* contains a set of several abundant or major proteins (13) accounting for up to 70% of total membrane protein. At least partly, they exhibit two stable forms in SDS solutions.

One of the heat-modifiable proteins, variously referred to as d (6), II* (4), 3a (14) or O-10 (8) (for nomenclature, see reference 2), exhibits an apparent molecular weight of 28,000 to 29,000 after solubilization of the isolated outer membranes in a sample buffer as described by Rosenbusch (12) for 30 min at 37°C (or even room temperature); the second form can be generated by boiling the membranes for solubilization, leading to an apparent molecular weight of 33,000 to 35,000. The behavior of this protein must be explained in terms of two different, stable conformational states in SDS solution (8, 11); metal ions could be involved with such a mechanism (7).

According to Segrest and Jackson (15), in SDS-gels of low concentrations of acrylamide, proteins migrate in the electric field due to a negative charge which results from binding of SDS to the polypeptides; in low-concentrated gels it was furthermore observed that glycoproteins migrate more slowly (15) and proteins complexed with lipids run faster than expected from their molecular weights (5). This means that their apparent molecular weight will be over- or underestimated. In gels of high concentrations of acrylamide, proteins are separated by molecular sieving, i.e., independent of charges.

An investigation of pure proteins showed that SDS binds in amounts approximately proportional to their molecular weights; the molecular weight is determined with great confidence (16), except for proteins containing large regions of beta structure, in which overproportional SDS binding was observed (18). (The outer membrane major proteins are very rich in beta structure [9].) Boiling of such proteins extends beta structure to alpha helix (a process which is possibly reversible after removing SDS [8, 12]) and thereby causes reduction of SDS binding.

In Fig. 1 it is shown that the apparent molecular weight of a heat-modifiable protein of *E. coli* K-12-JF404 (3) after boiling is not dependent on the concentration of acrylamide in the gel, indicating that the protein is a pure protein in the range of the sensitivity of this analytical procedure (15). In sharp contrast, the apparent molecular weight of the 37°C-solubilized form of this protein is strongly dependent on the concentration of acrylamide in the gel: decreasing concentrations lead to decreasing resulting molecular weights.

At concentrations above 15% acrylamide, the apparent molecular weights of the two forms are identical. That means that in gels with high concentrations of acrylamide the protein no longer exhibits heat modification.

At 10% acrylamide (the concentration used in the Lugtenberg system [6]), the molecular weights are in accordance with data published elsewhere. (Data are not presented in the Ferguson plot [1] for an easier correlation of the molecular weights depending on the gel composition.)

This finding leads to the conclusion that the different electrophoretic mobilities of the two forms of the heat-modifiable protein d are not due to enhanced SDS binding to the boiled form.
a suggestion confirmed earlier by the data of Reithmeier and Bragg (11), but that they are the result of excess SDS binding to the 37°C-solubilized form. The molecule with its overproportional charges runs too fast in gels with low concentrations of acrylamide. This excessive binding of detergent to a protein with large parts of beta structure can be reduced by boiling, extending the molecule to the alpha helical form. This alpha helix binds detergent ions only proportional to its amino acid residues. This conformational change is the reason for the heat modification on the molecular level that already had been suggested by Nakamura and Mizushima (8).

To overcome the effect of heat modification in the gel electrophoretic estimation of molecular weights, it is appropriate to separate the proteins by molecular sieving—dependent of charges—in gels of high concentrations of acrylamide.

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**LITERATURE CITED**


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