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Pseudomonas Cytochrome *c* Peroxidase

IX. Molecular Weight of the Enzyme in Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis

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Cytochrome *c* peroxidase has been purified from *Pseudomonas aeruginosa* in a homogeneous form.[1,2] Minimum molecular weights of 21 600 and 24 400 were calculated for the enzyme from its iron and heme *c* content, respectively.[3] A molecular weight of 53 500 was obtained on the basis of the sedimentation and diffusion coefficients determined on the analytical ultracentrifuge and the experimentally measured partial specific volume.[3] This value indicates that the enzyme contains two heme groups per molecule. The molecular weights calculated for two iron atoms and two heme groups are 43 200 and 48 800, respectively. Further studies were necessary to establish the molecular weight of the enzyme and to clarify whether it consists of one or two polypeptide chains. The enzyme was therefore studied by electrophoresis in polyacrylamide gel in the presence of the ionic detergent sodium dodecyl sulfate (SDS) which dissociates oligomeric proteins to protomers. Under these conditions, the electrophoretic mobilities of proteins are related to the molecular weight of the protomer polypeptide chain.[4]

Experimental. Pseudomonas cytochrome *c* peroxidase (PsCCP) was prepared from the acetone-dried cells of *P. aeruginosa* as previously described.[1,2] The preparation was homogeneous in disc electrophoresis (performed according to Maurer,[5] pH 8.6, 7 % gel; staining according to Weber and Osborne [4]). SDS-polyacrylamide gel electrophoresis was carried out in 10 % gel as described by Weber and Osborne.[4] When preparing the samples for SDS-electrophoresis, the protein solutions were heated at 100°C for 5 min before incubation and dialysis to prevent possible proteolysis during these steps.[6] Bovine serum albumin (Fraction V, Armour), ovalbumin (Grade V, Sigma), pepsine (crystallized, Sigma) and horse

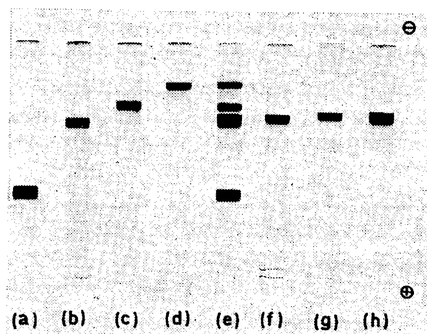

Fig. 1. SDS-polyacrylamide gel electrophoresis of *Pseudomonas* cytochrome *c* peroxidase and the molecular weight marker proteins in 10 % gel, according to Weber and Osborne.[4] (a) Horse heart cytochrome *c*, M.W. 12 400; (b) pepsin, M.W. 35 000; (c) ovalbumin, M.W. 45 000; (d) serum albumin, M.W. 67 000 (the marker proteins were treated with 1 % SDS and 1 % β-mercaptoethanol); (e) marker proteins *plus* PsCCP, treated with 1 % SDS and 1 % β-mercaptoethanol; (f) PsCCP, treated with 1 % SDS and 1 % β-mercaptoethanol; (g) PsCCP, treated with 1 % SDS; and (h) succinylated PsCCP, treated with 1 % SDS and 1 % β-mercaptoethanol.

heart cytochrome *c* (Type III, Sigma) were used as molecular weight markers. The gels were photographed according to the instructions of Oliver and Chalkley.[7] Succinylation of PsCCP with succinic anhydride was performed as described by Hass.[8]

Results and discussion. PsCCP was treated with SDS in the absence and presence of β-mercaptoethanol and studied by SDS-electrophoresis (Fig. 1). The relative mobility of PsCCP was found to be independent of the presence of β-mercaptoethanol, so indicating the protomer nature of PsCCP. Additional evidence for this was obtained by succinylation of PsCCP, another technique used for the dissociation of oligomeric proteins into their subunits.[8] Fig. 2 shows the quantitative succinylation

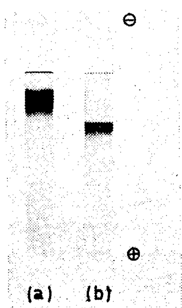

Fig. 2. Disc electrophoresis of (a) *Pseudomonas* cytochrome *c* peroxidase; and (b) succinylated *Pseudomonas* cytochrome *c* peroxidase in 7 % gel, pH 8.6, according to Maurer.[5] The gels were equal in length and diameter.

of PsCCP as studied by disc electrophoresis. The succinylated PsCCP was treated with SDS and β-mercaptoethanol and analyzed in SDS-electrophoresis (Fig. 1). It moved as a single band with an insignificantly lower relative mobility than the PsCCP of the previous experiments. An apparent mean molecular weight of $40\,000 \pm 1\,500$ for the polypeptide chain was obtained on the basis of its relative mobility and a standard curve drawn by plotting the logarithm of the known protomer weights of the marker proteins as a function of their respective relative mobilities (Fig. 3). The protomer molecular weight of PsCCP obtained by SDS-electrophoresis agrees

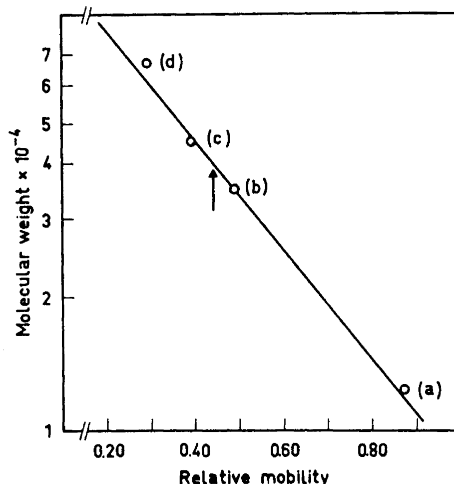

Fig. 3. Determination of the molecular weight of *Pseudomonas* cytochrome *c* peroxidase in SDS-electrophoresis according to Weber and Osborne.[4] The markers are the same as in Fig. 1. The arrow indicates the relative mobility of PsCCP. The extrapolated value for PsCCP is 40 000.

well with those calculated from the chemical analyses assuming the presence of two heme groups per molecule. This shows that the two heme groups are linked to a single polypeptide chain.

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