The Purity of Two Commercial Hemeproteins

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Rapidly increasing numbers of proteins and other biological substances are becoming commercially available. Large-scale, commercial isolation is rational, and prices compete with the expenses for small-scale isolation in the research laboratory. The use of commercial preparations is generally accepted, and seemingly necessary, in many situations for optimizing experimenter time. There exists, however, some risk that uncritical use of commercial preparations may eventuate artifactual data and confusing results. In a survey of four randomly chosen but well known biochemical journals from 1982 we found twenty-two publication on myoglobin (Mb) and horseradish peroxidase (HRP), excluding numerous immunological studies. In several of these, the authors used commercial material without further purification, and two publications did not report any history of the hemeprotein used. We wish to report some observations on two commercial hemeprotein preparations.

Materials and methods. Sperm whale Mb and HRP were obtained as lyophilized powders from a commercial supplier and stored at −20 °C until use, for no more than 2 months. Cation-exchangers cellulose CM-52 and Sephadex CM-50 were bought from Whatman (Maidstone, Kent, U.K.) and Pharmacia (Uppsala, Sweden), respectively. Acrylamide and bisacrylamide for gel electrophoresis were purchased from LKB (Stockholm, Sweden), and purified with ion-exchange resin before use. Chemicals used for buffers were all analytic grade or better. Deuterium oxide (99.5 %) used for ^{1}H NMR spectroscopy was obtained from Worthington (Malvern, PA, USA).

Electronic spectra were recorded on a Beckman DU-7 spectrophotometer. Electropherograms [1] were analyzed on a Beckman Acta III spectrophotometer equipped with a gel scanner, at the rate of 0.2 cm/min. ^{1}H NMR spectra were recorded on a Bruker WM-250 spectrometer using residual HDO as internal reference (S=4.60) at 35 °C. About 10000 scans, collected at one second intervals using 90° pulses, were Fourier transformed and subjected to a base-flattening routine.

Results and comments. A typical batch of metMb gave clear, stable solutions in 10 mM sodium phosphate, pH 6.0. The material consistently yielded four fractions by ion-exchange chromatography [2] on Sephadex CM-50. MetMb was the dominating protein in all fractions, as judged by optical spectroscopy. The relative amounts of the four fractions were 7, 8, 20 and 60 %, I through IV. The proton NMR spectra of the cyanide form of the two main fractions (Fig. 1) reveal unique and distinct environments around the heme group in these two proteins. The two major fractions of metMb are clearly spectroscopically unique.

A batch of lyophilized HRP considered to be of typical quality was dissolved in 50 mM phosphate, pH 7.0. It gave a slight turbidity, which reappeared after centrifugation. Gel electrophoresis of the solution, reasonably clear after several centrifugations, showed the presence of at least twelve protein fractions, at least two of which contained heme (Fig. 2). Chromatography on Sephadex CM-50 in 10 mM sodium acetate, pH 4.7, also gave multiple components.[3] Commercial HRP is not a unique substance, but a mixture of heme- and non-hemeproteins. A potent non-peroxidase glycoproteins immunogen was detected in commercial HRP.[4] After chromatography HRP as well as Mb gave clear, stable solution.

It recently happened that colleagues from two laboratories of recognized reputation complained about the tendency of commercial HRP to give turbid and "unstable" solutions. Having heard that, and after noticing that commercial preparations sometimes had been used without pretreatment appropriate to the situation, we want to draw the attention to this potential source of confusing results. Mb and HRP are reproducibly heterogeneous, as judged by electrophoretic, chromatographic and proton nuclear magnetic resonance (NMR) spectroscopic techniques. Investigators should not assume commercial hemeproteins as provided to be homogeneous preparations.

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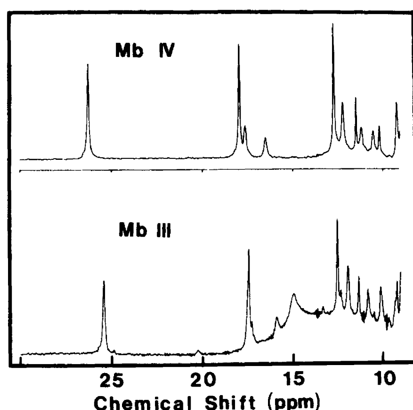

Fig. 1. ^{1}H NMR spectra of two major myoglobin fractions, III and IV. The three largest singlets are porphyrin ring methyl substituents, and are numbered according to Hoard.[5]

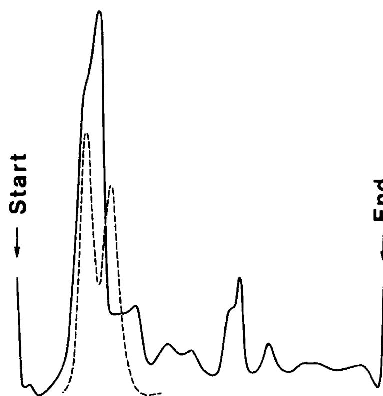

Fig. 2. Electropherogram of horseradish peroxidase on discontinuous polyacrylamide (7.5 % crosslinkage) gel in 0.375 M acetate, pH 4.3. Samples of 30 (–) and 10 (---) μl, were layered onto prepolymerized 9 cm. length tube gels, run for five hours at 200 V, then stained with comassie blue. The two largest fractions contained heme, as judged visually.

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