

Liver cytosolic fraction (FII) preparation:

Livers from young male Sprague-Dawley rats were extensively perfused *in situ* with ice-cold Hepes buffer (50 mM, pH 7.6), containing 0.15 M KCl, 0.25 M sucrose, 1 mM DTT and 0.5 mM ATP. The perfused liver (10-15 g) was diced and homogenized in a Dounce glass homogenizer with an equal volume of a homogenization buffer (50 mM Hepes buffer, pH 7.6, containing 0.25 M sucrose, 1 mM EDTA, 0.5 mM ATP and freshly added pepstatin (2 μ M), bestatin (1 μ M), leupeptin (10 μ M), aprotinin (0.04 unit/mL), soybean trypsin inhibitor (1 μ g/mL), TLCK (1 mM) and E-64 (50 μ M). The liver homogenate was sedimented at 10,000g for 20 min at 4°C, the supernatant collected and resedimented by ultracentrifugation at 100,000g for 90 min at 4°C. The supernatant (rat liver cytosol) so obtained was stored at -80°C until further use. For FII preparation, the liver cytosol was thawed on ice, and mixed with 3 volumes of 50% DE-52 slurry preequilibrated with a buffer consisting of 15 mM KPb, pH 7.0, 20% glycerol, 0.1 mM EDTA and 1 mM DTT, with shaking at 4°C for 2 h. The mixture was then packed in a column at a speed of 2 mL/min. The column was washed with 2.5 column volumes of washing buffer (15 mM KPb, pH 7.0, 20% glycerol, 0.1 mM EDTA, 1 mM DTT and 0.5 mM ATP) at a speed of 2 mL/min until no further protein eluted out as monitored with an on-line UV detector at 280 nm wavelength. The packed column was further washed with 2 column volumes of the above washing buffer containing 20 mM KCl. The bound proteins were eluted with 3 column volumes of an elution buffer consisting of 50 mM Tris-HCl, pH 7.2, 0.5 M KCl, 20% glycerol, 0.1 mM EDTA, 1 mM DTT at 2 mL/min. The protein peak fractions with absorbance at 280 nm were pooled and adjusted to a final concentration of 0.1 M Tris, pH 8.0, 1 mM DTT. Solid ammonium sulfate was slowly and gradually added to the pooled proteins to 80% saturation, and the mixture stirred at 4°C for 1 h. The protein precipitate was collected by centrifugation at 8,000g for 20 min, resuspended in one tenth of the original volume of storage buffer consisting of 50 mM Tris-HCl, pH 7.5, 20% glycerol and 1 mM DTT. The dissolved proteins were dialyzed against 100 volumes of the same buffer, and ATP and DTT added to final concentrations of 0.5 mM and

1 mM, respectively, before storage in aliquots at -80°C. Before use, the protein concentration of each aliquot was determined by the BCA assay, and the protein constituents analyzed by SDS-PAGE.