Cleavage of the haem-protein link by acid methylethylketone

Resolution of the haem and protein components of the majority of haemoproteins has been achieved by the acid–acetone method[1-5], in which the protein is precipitated as a solid phase and is collected by filtration or centrifugation. This method, while valuable as a preparative technique, has the disadvantage that some denaturation of the protein usually results from the high concentration of acetone necessary to ensure complete precipitation of all the protein. This makes the method difficult to apply quantitatively, especially to dilute solutions of haemoproteins.

The substitution for acetone of methylethylketone, which is only partially miscible with water, enables the haem and protein to be rapidly and completely separated as two liquid phases. Thus the exposure of the protein to a high ketone concentration, and the troublesome step of redissolving the precipitated protein are avoided. In addition, filtration or centrifugation are unnecessary, and the method is quantitative with micromolar haemoprotein solutions.

A typical application of this method, using a separating funnel, is as follows: To the ice-cold, salt-free haemoprotein solution containing sufficient 0.1 *N* HCl to give pH 2, is added an equal volume of ice-cold methylethylketone, and the mixture is shaken for a short time. On standing in the cold, separation takes place into a ketonic supernatant containing all the haem, and a lower aqueous layer containing all the protein, which is dialysed against water to remove the dissolved ketone.

Because little manipulation of the material is involved, the method is quick and moreover quantitative with extremely dilute solutions. The volumes of the two phases containing the components are also conveniently small. It has been applied with success to myoglobin, haemoglobin, horse-radish peroxidase, and catalase. In the case of haemoglobin, conversion of the iron to the ferric state with ferricyanide[6] was necessary to achieve a haem-free protein preparation. The protein produced by this method was stable when stored at 0°, and could be completely recombined with haem. The efficiency of recombination was measured by the method of fluorescence quenching by energy transfer[7] to haem, using haemoproteins conjugated with a fluorescent naphthalene dye, and by absorption spectral changes.

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