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Letter to the Editor

Lowry's method of protein estimation: some more insights

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In the thought provoking-letter published in the April 1992 issue of this journal, R. A. Brown has rightly pointed out our lack of knowledge in the reaction mechanism of Lowry's method of protein estimation (Lowry et al 1951). Though the necessity of learning the theoretical background of such a widely used laboratory technique need hardly be emphasized, surprisingly it is often ignored. We have carried out a thorough literature survey on the mechanism of the Lowry reaction. This letter deals with a brief discussion on this aspect.

This method involves the pretreatment of protein with alkaline copper sulphate in the presence of tartarate followed by the addition of Folin's phenol reagent.

In a dipeptide copper complex, the copper atom is held in a coplanar tridentate chelate involving the free amino nitrogen, the peptide bond nitrogen and the free carboxyl group. The additional presence of a side-chain nitrogen, as in asparagine and histidine, helps in the formation of a more stable, quadridentate chelate. In a tripeptide copper complex, the quandridentate chelate can be formed with the two available peptide bonds without the participation of a functional group. The chelated protein then reduces the Folin's reagent, a mixture of phosphomolybdic-tungstic acids (Creighton 1984).

This reduction results in the loss of one, two or three oxygen atoms from the tungstates and molybdates to produce several reduced species with a characteristic blue colour (λ_{max} 745–750 nm). The ability to form a stable chelate determines the rate of electron removal, i.e. the reducing potential of the protein.

The Folin reagent has a half-life of 8 s at the alkaline pH of the reaction (pH = 10) and therefore rapid electron transfer from the protein is crucial for ultimate colour yield.

Thus it is evident that the whole peptide backbone is involved in the colour formation when pretreated with copper. Only the chromogenic amino acids such as tyrosine, tryptophan, cysteine and to a lesser extent histidine can contribute to the colour yield without copper-pretreatment (Chou & Goldstein 1960). D-Amino acids behave in an identical manner to their L-counterparts. Pretreatment with copper does not affect the colour formation by tyrosine or tryptophan, but colour formation is drastically reduced for cysteine and peptide containing cysteine because the sulphydryl group is blocked by copper ion. It is interesting to note that penicillin, which contains a dipeptide of cysteine and valine with a blocked amino group, yielded more colour than the corresponding free dipeptide; however, when it is cleaved by β-lactamase to give two amino acids linked through sulphur of cysteine, the colour yield is high. Desthiopenicillin did not give any colour and penicillamine (ββ-dimethylcysteine) yielded less colour than cysteine confirming the involvement of the sulphydryl group in colour production.

All dipeptides are chromogenic in the presence of copper and the response is enhanced in the presence of side-chain amino or carboxyl groups. Particularly chromogenic are dipeptides containing histidine, arginine or glutamic acids residues. Tripeptides are highly chromogenic even in the absence of functional side chains.

Sequences of the type Val- Glu- Ala and Val- Glu- Ala- Leu are chromogenic although their contribution to the total colour yield is small. Thus a number of features contribute to the total colour yield giving the technique a broad specificity.

It is noteworthy that the colour formation is susceptible to interference by potassium ions (Vallejo & Lagunas 1970), magnesium ions (Kuno & Kihara 1967), EDTA (Neurath 1966), Tris (Kuno & Kihara 1967), thiol reagents (Vallejo & Lagunas 1970) and carbohydrates (Lo & Stelson 1972). Aromatic amino acids, uric acid, guanine and xanthine also interfere in the Folin phenol method. As a remedy Bennet (1967) recommended precipitation of protein with trichloroacetic acid and redissolving the precipitate before colour formation. For protein estimation of cell membrane components Parnaik et al (1983) recommended addition of sodium dodecylsulphate just before the addition of Folin's reagent.

References

Bennet, T. P. (1967) Membrane filtration for determining protein in the presence of interfering substances. Nature 213: 1131–1132

Brown, R. A. (1992) An appreciation of the Folin-Lowry protein assay. J. Pharm. Pharmacol. 44: 369

Chou, S. C., Goldstein, A. (1960) Chromogenic groupings in Lowry protein determination. Biochem. J. 75: 109–115

Creighton, T. E. (1984) Chemical nature of polypeptides. In: Proteins. W. H. Freeman and Co., New York, pp 1–60

Kuno, H., Kihara, H. K. (1967) Simple microassay of protein with membrane filter. Nature 215: 974–975

Lo, C., Stelson, H. (1972) Interference by polysucrose in protein determination by the Lowry method. Anal. Biochem. 45: 331–336

Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J. (1951) Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265–275

Neurath, A. R. (1966) Interference of sodium ethylenediaminetetraacetate in the determination of proteins and its elimination. Experientia 22: 290

Parnaik, V. K., Srivastava, P. K., Das, M. R. (1983) Inhibition of avian myeloblastosis virus reverse transcriptase by an RNA-binding protein from plasma membranes of normal and tumor cells. J. Biosci. 5: 107–114

Vallejo, C. G., Lagunas, R. (1970) Interference by sulfhydryl, disulfide reagents and potassium ions on protein determination : Lowry's method. Anal. Biochem. 36: 207–212

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