Messenger RNA and Ribosomes in Protein Synthesis

Edited by C. F. PHELPS and H. R. V. ARNSTEIN

The Biochemical Society's Forty-Seventh Symposium, held in London in December 1981, assembled some of the leading workers in this area of biochemistry. The subjects for discussion were chosen for their timeliness and distinctiveness, and included accounts of ribosome and messenger RNA structure and function, initiation factors, caps and ribonucleoproteins, as well as consideration of the processes leading to the distribution of newly synthesized proteins within the cell. The papers presented are now published in this volume.

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Tetramethylbenzidine

A reported noncancerous analog of benzidine

For many years benzidine has been used as a sensitive and specific reagent for the detection of blood. However, its extreme carcinogenicity has curtailed its use in recent years. In fact, in 1974 the Occupational Safety and Health Administration banned its manufacture and use in the United States.³

One of the early hypotheses for the carcinogenicity of aromatic amines involved ortho hydroxylation.¹ Thus, it seemed that 3,3',5,5'-tetramethylbenzidine (TMB) (in which ortho hydroxylation is impossible) might be an effective and safe substitute for benzidine.¹ Indeed, it was found that subcutaneous injection of TMB into rats produced no tumors specifically attributable to it, in doses greater than those in which benzidine or o-tolidine cause a high yield of neoplasms.⁴ The Salmonella/microsome test (Ames test)⁵ showed TMB to be nonmutagenic,⁶,⁷ suggesting that it is noncarcinogenic.

Garner et al.⁸ have evaluated the use of TMB as a presumptive test for blood in forensic work. In various concentrations of glacial acetic acid, TMB reacted with blood in the presence of H₂O₂ to form a colored product. Comparative studies with benzidine showed TMB to be equally sensitive in blood detection.

Standerfer and Vanderjagt⁹ found that methods employing TMB for plasma hemoglobin assay compared well with those using benzidine in accuracy, precision and sensitivity. Iron porphyrins, which can exhibit a peroxidase-like action, were detected at very low levels on paper chromatograms with TMB as visualizing agent.¹⁰ The peroxidase activity of the heme protein cytochrome P-450 was determined by sodium doxeyl sulfate-polyacrylamide gel electrophoresis with TMB staining.¹¹ In this method TMB-H₂O₂ stain is superior to benzidine-H₂O₂. The blue-stained bands are distinct and the color is stable.¹² The activity was also detected in immunoprecipitates in Ouchterlony double-diffusion plates.¹³

In the area of neurohistochemistry, TMB proved more sensitive than 3,3'-diaminobenzidine in demonstrating retrograde and anterograde axonal transport of horseradish peroxidase (HRP) in rat brain by light microscopy.¹⁴ TMB also gave results comparable to those obtained with benzidine dihydrochloride in the demonstration of retrograde transport of HRP.¹⁵

We also offer TMB dihydrochloride which, in contrast to the water-insoluble free base, dissolves slowly in water or in citrate buffer.¹⁶ TMB dihydrochloride appears to be suitable for the quantitative determination of hemoglobin in solutions, and for the identification and localization of myeloperoxidase in cells.¹⁷

TMB and TMB dihydrochloride are rapidly gaining acceptance as reliable and safe substitutes for benzidine and its carcinogenic derivatives.

References:

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