PRO EXPERIMENTIS

The Interference of Phenols in the Fluorometric Determination of Nucleotides

LOWRY *et al.*[1] described a fluorometric method by which both oxidised and reduced phosphopyridine nucleotides, DPN, TPN, DPNH, and TPNH could be determined in animal tissues. An attempt was made to apply this method to plant tissue in view of the importance of a knowledge of such ratios in plants.

Homogenates of etiolated pea or lettuce seedlings were prepared in tris buffer and analysed by the method of LOWRY. No nucleotides could be detected. In recovery experiments where DPN was added to the homogenates, a loss of DPN of between 30 and 60% was observed. This loss was apparently not due to enzymic destruction since it was observed also when extracts of pea tissue were prepared in ice cold methanol and even after boiling such extracts. It appeared likely that some compounds in the extracts were interfering either with the development of fluorescence of the nucleotides during alkali treatment or were acting as quenching agents. Phenolic substances are widely present in plant tissue and are known to form oxidation products in strong base which might be responsible. A number of such compounds were therefore tested. The compounds were added to the DPN solution either before adding alkali and keeping at 38° C for 1 h or immediately before dilution for reading the intensity of fluorescence. Some of the results obtained are given in the Table. The recovery of DPN is calculated from the expected and observed fluorescence. An Eel fluorimeter having a primary filter transmitting at 365 mμ and a secondary filter with a transmission peak at 465 mμ was used. All tests were made in a range where the fluorescence of DPN and quinine sulphate is linear with concentration.

Effect of various substances on the fluorescence of DPN at a final concentration 1 μg/ml

Compound	Concentration	% Recovery of DPN if compound added	
		Before adding alkali	Before dilution
Catechol	2×10^{-4} *M*	63	62
Chlorogenic Acid	$1{\cdot}1\times10^{-4}$ *M*	100	—
Phloroglucinol	2×10^{-4} *M*	76	77
Hydroquinone	2×10^{-4} *M*	65	61
Dopa	1×10^{-4} *M*	—	63
Caffeic acid	$1{\cdot}1\times10^{-4}$ *M*	—	90
Catechol + Hydroquinone each	2×10^{-4} *M*	49	—
Tyrosine	$1{\cdot}1\times10^{-4}$ *M*	100	—
Resorcinol	2×10^{-4} *M*	100	—
2,4-dihydroxy benzoic acid	$1{\cdot}3\times10^{-4}$ *M*	100	—

It will be seen from this Table that a number of compounds are capable of producing an apparent loss of DPN. This appears to be due to a quenching effect in the case of catechol and phloroglucinol and hydroquinone. Whether quenching occurs or not is clearly related to the structure of the phenolic compound tested. It is particularly worth nothing that even compounds which fluoresce themselves such as DOPA and caffeic acid can cause an apparent reduction of the fluorescence of DPN in strong alkali. The fluorescence is clearly not additive.

It must be concluded that the method of LOWRY *et al.*[1] cannot be applied to any tissue where such phenolic compounds are likely to occur, especially since the amounts of and ratios between various phenols in plant tissues are liable to change markedly during development of the plant.

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Zusammenfassung

Die fluorometrische Bestimmung von Nukleotiden in Pflanzenextrakten wird durch phenolische Stoffe gestört. Nukleotide können mit dieser Methode nicht routinemässig bestimmt werden.

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[1] O. H. LOWRY, N. R. ROBERTS, and J. I. KAPPHANN, J. biol. Chem. *224*, 1047 (1957).

PRO EXPERIMENTIS

Vergleichende röntgenhistoradiographische und interferenzmikroskopische Trockengewichtsbestimmungen[1]

Von ENGSTRÖM und LINDSTRÖM[2] wurde die Röntgenhistoradiographie mit weichen Röntgenstrahlen zur Trokkengewichtsbestimmung an kleinsten biologischen Objekten eingeführt. Sie stellt neben der Interferenzmikroskopie eine wichtige unabhängige Methode für diese Zwecke dar. DAVIES *et al.*[3] untersuchten erstmals vergleichend mit beiden Verfahren verschiedene Objekte und fanden eine gute Übereinstimmung der Messergebnisse.

Wir benutzten in unseren röntgenhistoradiographischen Untersuchungen das Gerät von Philips (CMR 5). Wegen des kleinen ausgeleuchteten Feldes können hier Objekt und Referenzsystem kaum gleichzeitig photographiert werden. Wir mussten deshalb eine Methode entwickeln, die es gestattet, Trockengewichtsbestimmungen ohne Referenzsystem durchzuführen. Dazu wurde die Zeit-Schwärzungskurve für den verwendeten Film (linear bis $D = 0{,}7$ für Kodak Maximum Resolution Plates bei Entwicklung mit Gevaert G 209 A, 6 min, 18°C) bei konstanter Spannung festgelegt und mit einem Referenzsystem ein Trockengewichtsäquivalent bestimmt. (Für Einzelheiten der Methodik siehe MÜLLER *et al.*[4], im Druck.)

Die Objekte (Erythrozyten von *Rana temporaria*, Bullenspermien, Bullenthymuslymphozyten, Plattenepithelien der menschlichen Mundschleimhaut) wurden auf Prä-

[1] Mit Unterstützung der Deutschen Forschungsgemeinschaft.

[2] A. ENGSTRÖM und B. LINDSTRÖM, Nature *163*, 563 (1949); Biochim. biophys. Acta *4*, 351 (1950).

[3] H. G. DAVIES, A. ENGSTRÖM und B. LINDSTRÖM, Nature *172*, 1041 (1953).

[4] D. MÜLLER, W. SANDRITTER und G. SCHWAIGER, Histochemie (im Druck).