COMMUNICATION TO THE EDITOR

A Convenient Assay for Ribonuclease[1]

To The Editor:

Ribonuclease has been commonly assayed by reaction with ribonucleic acid and subsequent measurement of the increased absorption at 260 nm of the resulting polynucleotides. At the end of the reaction, the excess substrate, which also absorbs at 260 nm, is precipitated by the addition of uranium acetate (Brawerman and Chargaff 1954, Tuve and Anfinsen 1960), lanthanum nitrate (Van et al 1982), or hydrochloric acid (Yokoyama et al 1982); the low-molecular-weight polynucleotides remain in solution.

The low absorbance of the deoxyribonucleic acid substrate has been used to advantage in the measurement of deoxyribonuclease. As the substrate is hydrolyzed, the increased absorbance at 260 nm due to polynucleotides relative to a substrate blank can be measured without precipitation and removal of the substrate (Liao 1977).

We have found that ribonuclease can be measured conveniently without precipitation of substrate by a method similar to that of Liao (1977) for deoxyribonuclease.

[1]Mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

The substrate solution is 0.1 mg of RNA (Sigma Chemical Co., St. Louis, MO) in 1 ml of 0.1 *M* acetate, pH 5.0. A unit of RNase can be defined as the activity that yields one absorption unit at 260 nm per 10 min per milliliter of reaction solution at 30°C. Specific activity for an enzyme preparation can be expressed as units per microgram of protein.

Nine-tenths milliliter of substrate solution (0.11 mg of RNA per milliliter) and a suitable quantity of enzyme in 100 μl of the 0.1 *M* acetate buffer are allowed to react in 1-ml quartz cuvettes (1-cm light path) at 30° C in the thermally controlled cell compartment of a Gilford model 260 spectrophotometer (Gilford Instrument Co., Oberlin, OH). Appropriate substrate and enzyme blanks are included in separate cuvettes in the cuvette holder. Absorptions at 260 nm are measured at 2-min intervals for 10 min. A change in absorption of 0.010–0.110 over the 10-min period is appropriate for the substrate concentration used.

For this procedure, a UV spectrophotometer is required in which the substrate blank can be adjusted to zero absorbance. Most good-quality instruments that have been available for the last 10 years have this capability. If a thermally controlled cell compartment is not available, the reaction can be performed in the spectrophotometer cuvettes kept at 30°C in a water bath or in another constant-temperature device. Readings can then be taken after the 10-min incubation period.

Table I and Fig. 1 show representative values for a ribonuclease

TABLE I
Replication of Ribonuclease Assay

Enzyme Quantity Protein (μg)	ΔA_{260}/10 min in Replicate								Standard Deviation	Variance $\times 10^{-7}$
	1	2	3	4	5	6	7	Mean		
1.45 ± 0.03	0.013	0.013	0.014	0.014	0.013	0.014	···	0.0135	0.00055	2.5
2.90 ± 0.03	0.026	0.027	0.027	0.028	0.028	0.030	0.029	0.0279	0.0013	16.0
4.35 ± 0.15	0.040	0.037	0.042	0.041	0.041	0.041	···	0.0403	0.0018	26.0
5.80 ± 0.15	0.055	0.054	0.056	0.055	0.053	0.055	0.054	0.0546	0.00098	8.2
7.25 ± 0.15	0.065	0.063	0.066	0.065	0.067	0.066	···	0.0653	0.0014	16.0
8.70 ± 0.15	0.082	0.083	0.081	0.081	0.081	0.082	0.081	0.0816	0.00079	5.3
10.15 ± 0.15	0.089	0.091	0.089	0.090	0.091	0.090	···	0.0900	0.00089	6.7
11.60 ± 0.15	0.102	0.103	0.104	0.103	0.103	0.105	0.106	0.1037	0.0014	16.0

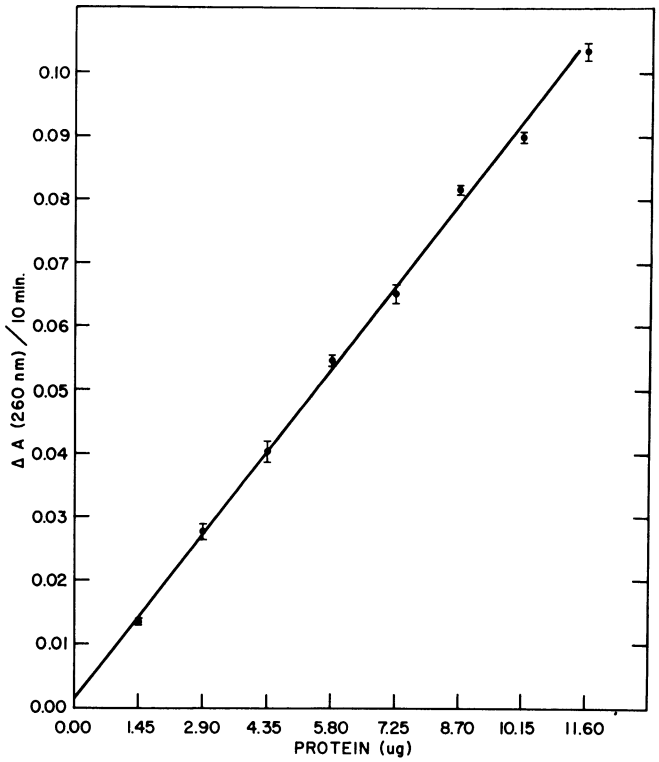

Fig. 1. Relationship between quantity of enzyme and change in absorbance at 260 nm. Plotted points show ± one standard deviation.

preparation from malt roots (Prentice 1983). Protein in enzyme solutions was determined by measurement of absorption at 280 and 260 nm according to the method of Warburg and Christion as described by Layne (1957). The correlation coefficient (Fig. 1) for enzyme quantity and change in absorption was 0.99. Similar results have been obtained with a commercial ribonuclease preparation (Sigma Chemical Co., St. Louis, MO; type X11-A powder).

The sensitivity of the assay (Δ_{260} per 10 min per microgram of protein) for our highly purified enzyme from malt roots is 10. Low levels of ribonuclease in crude preparations can be easily detected by this method, as indicated in Fig. 1, where the specific activity (Δ_{260} per 10 min per microgram of protein) is only 0.009.

We have used this method for following the purification of ribonuclease from tissues of germinated barley. It is applicable for measuring overall ribonuclease activity in malts from numerous barley varieties. The method is particularly useful when chromatographic fractions are evaluated for activity. The precipitation method with the centrifuging step is not practicable when numerous assays are to be done.

N. PRENTICE
S. HEISEL
U.S. Department of Agriculture
Agricultural Research Service
Cereal Crops Research Unit
501 N. Walnut St.
Madison, WI 53705

LITERATURE CITED

BRAWERMAN, G., and CHARGAFF, E. 1954. On a deoxyribonuclease from germinating barley. J. Biol. Chem. 210:445.

LAYNE, E. 1957. Spectrophotometric and turbidimetric methods of measuring proteins. Page 447 in: Methods of Enzymology, Vol. 3. S. P. Colowick and N. O. Kaplan, eds. Academic Press, New York.

LIAO, T.-H. 1977. Isolation and characterization of multiple forms of malt deoxyribonuclease. Phytochemistry 16:1469.

PRENTICE, N. 1983. Comparison of malts for nuclease and nucleobase potentials. J. Am. Soc. Brew. Chem. 41:133.

TUVE, T. W., and ANFINSEN, C. B. 1960. Preparation and properties of spinach ribonuclease. J. Biol. Chem. 235:3437.

VAN, V., KIMOTO, K., and MURAKAMI, K. 1982. Purification and some properties of two ribonuclease from *Euphausia superba*. Agric. Biol. Chem. 46:691.

YOKOYAMA, Z., MIYAMOTO, M., and HIRANO, K. 1982. Purification and properties of acid ribonuclease in rice bran. Agric. Biol. Chem. 46:247.

[Received October 17, 1983. Accepted December 13, 1983]