CORRESPONDENCE

Comments on Comparison of Methods for Determining Myrosinase Activity

Sir: In a recent issue of the *Journal of Agriculture and Food Chemistry*, Palmieri et al. (1987) offered criticism of a spectrophotometric coupled enzyme assay (SCEA) developed by us (Wilkinson et al., 1984a) for the analysis of myrosinase (thioglucoside glucohydrolase, EC 3.2.3.1). Our objective was to develop a method applicable to a range of tissues and glucosinolate substrates. We subsequently demonstrated the application of the SCEA method to the analysis of different tissue types from cruciferous vegetables (Wilkinson et al., 1984b).

In their paper Palmieri et al. (1987) dispute our previous findings claiming the SCEA to be both insensitive and unreliable. The basis of this claim was an apparent low and nonlinear myrosinase activity observed in comparison with three other methods tested. The authors speculated that an accumulation of glucose and an insufficient concentration of magnesium were the probable cause.

The published SCEA protocol is based on the well-known coupled enzyme procedure for the determination of glucose, and the protocols cited by Kunst et al. (1984) indicate a range of coupling enzyme and magnesium concentrations may be used depending on the glucose concentration range to be measured. During the development of the SCEA protocol, we demonstrated the rapid reduction of NADP and quantitative determination of added glucose up to 0.2 mmol L^{-1}, and as we demonstrated linear myrosinase activity up to 0.16 mmol L^{-1} min^{-1} (Wilkinson, 1984a), it is our view that glucose does not accumulate in the assay. Furthermore, from an examination of the data published by Palmieri et al. (1987) we note that the published SCEA protocol was amended by allowing the reaction mixture to preequilibriate for minutes prior to measuring the rate of NADP reduction.

It is our view that the low and nonlinear myrosinase activity reported by the Italian workers is a consequence of this very modification of the published procedure because in our experience it is necessary to measure the initial rate of NADP reduction immediately following the addition of the glucosinolate substrate. For this reason measurements were made over the initial 20-s period following substrate addition, although this would obviously depend upon the measured activity.

Finally, Palmieri et al. (1987) adversely comment on the addition of ascrobate in the SCEA. It is, however, well-known that myrosinase isoenzymes are activated by ascorbate and the mechanisms involved in such activation have been investigated (Tsuruo and Hata, 1967, 1968; Ohtsuru and Hata, 1972, 1973a,b, 1975, 1979). It has also been demonstrated that the degree by which myrosinase isoenzymes are activated differs with ascorbate concentration and enzyme source [data briefly reviewed by Wilkinson et al. (1984b)]. Thus, purified isoenzymes showing ascorbate-independent myrosinase activity have been shown to be activated at least 4-fold by ascorbate (Bjorkmann and Lonnerdal, 1973; Palmieri et al., 1987).

Thus, measurement of total myrosinase activity in the absence of ascorbate will both underestimate the ascorbate-independent component and eliminate the contribution from the more important ascorbate-dependent isoenzymes. It is our contention that for measurement of myrosinase activity to be meaningful, it should be conducted at or near ascorbate concentrations promoting maximal activity. In this regard the DSA assay, proposed by Palmieri et al. (1982), is incapable of detecting ascorbate-dependent activity (by virtue of the high absorbance of ascorbate at 227 nm).

If carried out according to the published protocol, the SCEA method enables both ascorbate-dependent and -independent myrosinase activities to be determined in both crude and purified plant extracts. We acknowledge that for some workers the cost of the assay is a prime factor, and for these the pH-stat assay is particularly appropriate, extensive dialysis procedures not being required if crude extracts are desalted with a Sephadex PD-10 column.

Registry No. Myrosinase, 9025-38-1; L-ascorbic acid, 50-81-7.

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