Rebuttal on Comparison of Methods for Determining Myrosinase Activity

Sir: In their letter Wilkinson and co-workers essentially make three points, to which we reply as follows.

(1) *Linearity of the enzymatic reaction*: In their first paper Wilkinson et al. (1984) show a linear response of the assay up to an enzyme concentration of ca. 8 U (U expressed in nmol/min) only. At the end of this paper, they state that "the rate of hydrolysis of sinigrin against protein concentration showed non-linearity ... This observation is being further investigated". We repeated their experiment and confirmed the nonlinearity. It is easy to show, as we did, that this feature, which of course greatly impairs the reliability of the assay, is simply due to an insufficient concentration of the auxiliary enzyme system (HK-G6PDH) in the MYR-(HK-G6PDH) coupled-enzyme assay (SCEA) so that the reaction rate of (HK-G6PDH) rather than MYR (myrosinase) becomes rate limiting. This finding is not "speculation" on our part but is the result of a direct experimental observation (Palmieri et al., 1987) coupled with a simple stoichiometric calculation based on eq II-76 of Segel (1975).

(2) *Preequilibration of the reaction mixture*: It is well-known that all coupled-enzyme reactions have a lag phase (e.g. Fig. II-29 in Segel (1975)). In our case the lag was a few minutes (observed also with our PCA coupled-enzyme method) in both the presence and absence of ascorbate. From this, two observations can be made: First, it is operationally improper, and in fact senseless, to measure the reaction rate immediately after time zero. One should measure the rate after the lag phase; since we are under zero-order conditions, it does not matter if the first few minutes are lost. Second, we are unable to understand how Wilkinson and co-workers are able to make meaningful measurements "over the initial 20 second period..." when, moreover, the HK-G6PDH units of their proposed assay (SCEA) are much less than those indicated also by Kunst et al. (1984) for glucose kinetic determination.

(3) *Ascorbate*: We made our point clearly enough in our paper (Palmieri et al., 1987). We only add that, generally speaking, an assay should work without an activator whenever possible. Furthermore, in this specific case the system is already very complex and the activation mechanism is still poorly understood (although it has been much studied in the past decade). Actually the systematic use of ascorbate is not only unnecessary, but even dangerous, since the enzyme activity might be spuriously modified in an unknown manner. For example, we have recently shown with the DSA method that myrosinases from different sources are activated differently by ascorbate (Iori et al., 1987). It is perhaps convenient to use ascorbate when the activity is particularly low, as in the crude extracts of cruciferous stems and leaves used by Wilkinson et al. (1984), but it is certainly not a necessary feature of general myrosinase assay.

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

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Comments on Photohydrolysis of Ethylene Dibromide

Sir: A paper in this journal (Castro and Belser, 1985) described the photochemical hydrolysis of ethylene dibromide and gave figures for the rate constants of two conversion steps, ethylene bromide to 2-bromoethanol and 2-bromoethanol to ethylene oxide. However, while photochemical conversion processes may be expected under certain circumstances to exhibit pseudo-first-order kinetics, the observed rate constant and half-life will both depend on the experimental parameters, in particular on the flux of light entering the reaction vessel that is effective in causing the photoreactions. Thus, neither can be regarded as a fundamental measurement of the efficiency of the reaction.

Let us first consider the hypothetical conversions

$$B \xrightarrow{h\nu} C \qquad C \xrightarrow{h\nu} D$$

where the eventual product D is totally transparent to the photolyzing light, which for simplicity we will assume to be monochromatic. If within the path length l of the reaction vessel, the absorbance, at the wavelength of the photolyzing light, due to both B and C, i.e. $A = (\epsilon_B[B] + \epsilon_C[C])l$, is much less than unity, then the fraction of the incident light of this wavelength absorbed within the cell is given by (1). Of this, some is absorbed by B and some

$$f = 1 - 10^{-A} \tag{1}$$

by C. The fraction of the total incident light absorbed by B is given by (2). Similarly the fraction of the incident

$$\begin{aligned} f_B &= \frac{[B]\epsilon_B}{[B]\epsilon_B + [C]\epsilon_C}(1 - 10^{-A}) \\ &= \frac{[B]\epsilon_B}{[B]\epsilon_B + [C]\epsilon_C}(1 - e^{-2.303A}) \\ &= \frac{[B]\epsilon_B}{[B]\epsilon_B + [C]\epsilon_C}(1 - 1 + 2.303A - \ldots) \\ &\approx \frac{[B]\epsilon_B}{[B]\epsilon_B + [C]\epsilon_C}2.303(\epsilon_B[B] + \epsilon_C[C])l \\ &= 2.303\epsilon_B[B]l \end{aligned} \tag{2}$$

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photolyzing light absorbed by C is given by (3).

$$f_C = 2.303\epsilon_C[C]l \quad (3)$$

If the flux of photolyzing light is N_i quanta cm^{-2} s^{-1}, then the mean rate of absorption of quanta within the vessel, in quanta per unit volume per unit time, is given by

$$I_a = \frac{N_i(1 - 10^{-A})}{l} \text{ (quanta } cm^{-3} s^{-1})$$

However, we are concerned with the two components of this figure: the rates of absorption of quanta by species B and C, respectively. Subject to the conditions detailed above, these are given by eq 4 and 5. Thus, the instan-

$$I_a^B = 2.303N_i\epsilon_B[B] \text{ (quanta } cm^{-3} s^{-1}) = \frac{2.303N_i\epsilon_B[B]}{10^3N_A} \text{ (einsteins } dm^{-3} s^{-1}) \quad (4)$$

$$I_a^c = \frac{2.303N_i\epsilon_C[C]}{10^3N_A} \text{ (einsteins } dm^{-3} s^{-1}) \quad (5)$$

taneous rate of the photochemical conversion of B to C would be given by the product of I_a^B and the quantum yield, ϕ_1, for this process (eq 6). The solution to this

$$-\frac{d[B]}{dt} = \phi_1 I_a^B = \frac{2.303N_i\epsilon_B\phi_1[B]}{10^3N_A} \quad (6)$$

equation is eq 7, which shows that the concentration of B should decrease exponentially with time from its initial value $[B]_0$. Thus, the concentration of B decreases in the same manner as for a reaction following first-order kinetics. However, the counterpart of the pseudo-first-order rate constant is a function of the light flux, the extinction coefficient, and the quantum yield.

$$[B]_t = [B]_0 e^{-(2.303N_i\epsilon_B\phi_1 t/10^3N_A)} \quad (7)$$

If the quantum yield for the conversion of C to D is ϕ_2, then the rate of this second process is given by (8). Since this is proportional to the concentration of C, it means that this step also would progress as if it followed pseudo-first-order kinetics, with the same qualification as in the preceding paragraph.

$$\phi_2 I_a^C = \frac{2.303N_i\epsilon_C\phi_2[C]}{10^3N_A} \quad (8)$$

With regard to the report by Castro and Belser (1985), it may be pointed out that while a medium-pressure mercury lamp emits strongly at around 313 and 365 nm (Calvert and Pitts, 1966), neither ethylene dibromide nor 2-bromoethanol absorb at those wavelengths. Thus, the only light capable of causing these reactions would have been the relatively small output at around 265 nm and the 254-nm resonance line. In this range, the absorbance of a few centimeters of a 9×10^{-3} M solution is comfortably less than unity, so one would expect the concentration of ethylene dibromide to fall exponentially under the influence of the ultraviolet lamp.

A measurement of the quantum yield of this process thus requires a knowledge of the flux of light of wavelengths effective in causing reaction. The reported experiments with ferrioxalate do not serve this purpose, chiefly because this system is photosensitive well into the visible and so is affected by light that is irrelevant to the photohydrolysis. But in addition it must be pointed out that even 1 cm of 6×10^{-3} M ferrioxalate solution is totally absorbing over this wavelength range. Consequently, the mean rate of light absorption can be shown to be given by (9), which indicates that the concentration of ferrioxalate

$$I_a = N_i/10^3lN_A \text{ (einstein } dm^{-3} s^{-1}) \quad (9)$$

should fall linearly and not exponentially and also that the mean rate of this photoreaction is not in any way a function of the coefficient of absorption, ϵ. Thus, the relative rates of Br^- production from ethylene dibromide and Fe^{2+} production from ferrioxalate bear no simple relationship to the quantum yields of these two reactions. Clearly the factors of 32 and 3.8 quoted (Castro and Belser, 1985) have no fundamental significance and pertain only to the particular experimental conditions of their work.

In summary, it would seem eminently desirable that the measured efficiency of any reaction brought about by the action of light is reported in terms of its quantum yield, which is widely understood as the ratio of the number of molecules reacting to the number of quanta absorbed.

Registry No. Ethylene dibromide, 106-93-4.

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Rebuttal on Photohydrolysis of Ethylene Dibromide

Sir: The theory drawn by Dr. Logan is correct, if rather well-known. It is consistent with our observations.

We are interested in discerning those processes that may be relevant to the transformation of biocides in the environment. Hence, in this work and a more recent one (Castro et al., 1987), we have chosen to irradiate simultaneously over a range of wavelengths that approximate relevant sunlight irradiation at earth. The total radiant energy of the medium-pressure Hanovia lamp we have employed in this and subsequent work is 175.8 W and, according to the manufacturer, is distributed over the following wavelengths (nm): 220–280 (15%), 280–320

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