This Week's Citation Classic®

Racker E. Spectrophotometric measurements of the enzymatic formation of fumaric and *cis*-aconitic acids. *Biochim. Biophys. Acta* 4:211-4, 1950.
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In this paper a simple spectrophotometric assay for measuring fumarase and aconitase activities was described. [The *SCI®* indicates that this paper has been cited in over 755 publications.]

Assay of Fumarase and Aconitase

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In the late 1940s, I was working on glycolysis and glyceraldehyde-3-phosphate dehydrogenase. I was particularly interested in a possible role of glutathione. At the time the assay for this peptide was to measure the conversion of methylglyoxal to lactate catalyzed by "glyoxalase" in a glutathione-dependent reaction. The procedure was tedious and not very sensitive. Looking at the structure of methylglyoxal, it struck me that I should be able to observe the disappearance of the double bond in a spectrophotometer at 240 nm and follow kinetically its conversion to lactate dependent on the presence of glutathione.

The truth is that I had acquired a Beckman DU-spectrophotometer that had just come on the market, and I had fallen in love with it. I designed a rapid spectrophotometric assay for hexokinase and phosphofructokinase[1] measuring the disappearance of the absorption band of reduced NAD at 340 nm in a coupled assay with α glycerophosphate dehydrogenase. Using the same method, I discovered several new enzymes such as transketolase[2] and aldehyde dehydrogenase.[3] I described the equilibrium constant of the NAD-dependent oxidation of alcohol to aldehyde by alcohol dehydrogenase at a very alkaline pH and its use for measuring NAD in a widely quoted paper.[4]

I was thrilled by the rapidity and accuracy of these measurements. I therefore tried my luck with glyoxalase. When I added a dilute extract of baker's yeast to a buffer containing methylglyoxal, I watched with amazement when on addition of glutathione, the absorption at 240 nm, instead of disappearing, increased rapidly. This observation led to the discovery of lactoylglutathione, which I believe was the first biological thiolester intermediate (just before the discovery of acetyl CoA). Glyoxalase I was the enzyme responsible for the formation of lactoylglutathione; glyoxalase II was responsible for its degradation to lactate and glutathione.[5] It was luck that the yeast extract contained a huge excess of glyoxalase I so that a quantitative assay for glutathione was soon developed. The discovery of a thiolester intermediate led to the proposal of the mechanism of the oxidation of glyceraldehyde-3-phosphate via a thiolester catalyzed by glyceraldehyde-3-phosphate dehydrogenase,[5] which was later demonstrated to be correct.[6]

The moral of the story is—if you are tired of a tedious assay, invent a new one. I measured glutathione in minutes rather than in hours and fell deeper in love with the DU-spectrophotometer. It was so bad that for a time I was sitting at it all day thinking how else I could use it. Looking at various intermediates of carbohydrate metabolism having a double bond, I found fumarate, which was formed from malate by fumarase, and *cis*-aconitate, which was formed from citrate or isocitrate by aconitase. The assay measuring the formation of the double bond of fumarate from malate in the presence of fumarase at 240 nm worked beautifully, with zero order kinetics. I published the procedure in the *Classic* paper, and it is still the method of choice for measuring fumarase. Am I particularly proud of this achievement? I think not. Did I have fun devising the procedure? Definitely yes. The moral for students? If you fall in love with an instrument or a method (e.g., cloning), by all means use it. But stop in time, before biochemistry becomes a tool for its use.

1. **Racker E.** Spectrophotometric measurement of hexokinase and phosphohexokinase activity. *J. Biol. Chem.* 167:843-54, 1947. (Cited 440 times.)
2. ————. Enzymatic formation and breakdown of pentose phosphate. *Fed. Proc.* 7:180, 1948. (Cited 55 times.)
3. ————. Aldehyde dehydrogenase, a diphosphopyridine, nucleotide-linked enzyme. *J. Biol. Chem.* 177:883-92, 1949. (Cited 215 times.)
4. ————. Crystalline alcohol dehydrogenase from baker's yeast. *J. Biol. Chem.* 184:313-9, 1950. (Cited 695 times.)
5. ————. The mechanism of action of glyoxalase. *J. Biol. Chem.* 190:685-96, 1951. (Cited 370 times.)
6. **Racker E & Krimsky I.** The mechanism of oxidation of aldehydes by glyceraldehyde-3-phosphate dehydrogenase. *J. Biol. Chem.* 198:731-43, 1952. (Cited 255 times.)

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