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Breslow R. On the mechanism of thiamine action. IV. Evidence from studies on model systems. *J. Amer. Chem. Soc.* **80**:3719-26, 1958. [Department of Chemistry. Columbia University. New York. NY]

It was known that thiamine pyrophosphate was a required coenzyme for a number of biochemical processes that clearly required some special chemical help, but there was no reasonable proposal of how the elaborate structure of the thiamine molecule could furnish such help. In this paper the function of the thiamine molecule was clarified, and some new ideas and techniques were also introduced. [The *SCI*® indicates that this paper has been cited in more than 445 publications.]

Model Studies Clarify an Enzyme Mechanism

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Mechanistic chemists can generally propose sensible possible pathways for chemical or biochemical reactions. However, as an undergraduate learning biochemistry in the 1950s I saw one baffling exception. Thiamine pyrophosphate (ThPP) is the coenzyme for several important biochemical processes. The intermediates in enzymatic reactions involving ThPP are formally "acyl anions," which are unstable species. The function of ThPP must be to directly bond, so as to convert the anions instead to new stable species, but nothing in the structure of ThPP suggested how it might do this.

Japanese chemists had accidently discovered that treatment of benzaldehyde with a thiazolium salt led to the production of benzoin, but here too there was no idea or evidence about how this catalysis by a thiazolium salt occurs. The benzoin reaction also involves a formal "acyl anion" intermediate, while the most unusual feature of ThPP is the presence of a thiazolium ring. Thus I thought it likely that solving the mechanism of the benzoin thiazolium catalysis would give an insight into the biochemical function of ThPP.

When I came to Columbia as instructor in chemistry, I worked on this benzoin reaction. After other possibilities were systematically excluded it became clear that the catalysis must involve the carbon atom between sulfur and nitrogen of the thiazolium ring. If the C-H bond ionized (for which there was no precedent) the resulting carbon anion should be able to act as catalyst by a reasonable pathway. The test was whether this hydrogen atom would exchange with D_2O. People at Columbia still remember my shout when I dissolved a thiazolium salt in D_2O and saw a new C-D band appear in the infrared. Definitive proof came from NMR studies.

Using a home-built 30 MHz instrument (tuned by inserting a screwdriver into the chassis) in the laboratory of Ben Dailey, I watched a single proton signal in the NMR of thiazolium salts disappear as that proton exchanged with D_2O. From its NMR position it was clearly the proton on the carbon between sulfur and nitrogen; from the rate of exchange I could estimate the acidity of that hydrogen atom. This was the first application of NMR to such a mechanistic problem. I could then propose a pathway for the formation of benzoin catalyzed by thiazolium salts and related pathways for the biochemical processes in which ThPP plays a role.

These proposals were in part confirmed by work reported in this paper and our subsequent work and also later by others who showed that our proposed substrate-ThPP adduct was formed by the enzyme and converted to products. In this and later work I also showed the relevance of the detailed structure of ThPP to its function and pointed out the relationship of the novel carbon anion to an important class of reactive intermediates—carbenes. Much more recent work has explored the chemical properties of these so-called "nucleophilic carbenes."

Chemical models can suggest how biological processes occur, and most of the interest in this paper came from that aspect. However, information can also flow in the other direction. New chemistry, which we have called "biomimetic," can be inspired by biochemistry. Our studies stimulated chemists to invent new synthetic methods, using thiazolium salts as catalysts. Also, we[1] and others[2] have prepared enzyme mimics that incorporate thiazolium salts into synthetic[1] or natural[2] binding sites and perform useful synthetic steps with characteristic enzyme-like selectivity.

There are still other poorly understood biochemical processes waiting to teach us some new chemistry while we solve a biochemical mystery. In the years since our paper, I have had the pleasure of introducing many PhD students and postdoctorals to this field. They are now some of the most active and successful investigators of enzyme models and their mechanisms. While I have received a number of awards and honors for this earliest work and later research, the success of so many of my former coworkers is at least as gratifying.

1. **Breslow R & Kool E.** A γ-cyclodextrin thiazolium salt holoenzyme mimic for the benzoin condensation. *Tetrahedron Lett.* 29:1635-9, 1988.
2. **Suckling C J & Zhu L-M.** Carbon-carbon bond formation mediated by papain chemically modified by thiazolium salts. *Bioorg. Medicinal Chem. Letter.* 3:531-1. 1993.

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