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A mimic of the pyruvate dehydrogenase complex

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ABSTRACT

Pyruvic acid undergo decarboxylation catalyzed by a hydrophobic thiazolium salt and reacts with a hydrophobic analog of lipoic acid to form a hydrophobic acylthioester that reacts with aniline to form acetanilide in water, but only in the presence of a hydrophobically modified polyaziridine that acts to gather the reactants just as the enzyme complex does.

A complex with several copies of three different enzymes converts pyruvic acid to acetyl coenzyme A, starting by using thiamine pyrophosphate bound to enzyme 1 to decarboxylate the pyruvate to an enamine intermediate **1**. Then lipoic acid, as an amide **2** on a lysine of enzyme 2, dips that long lipoamide into enzyme 1 to let its disulfide unit react with the enamine and form the amide **3** of *S*-acetyldihydrolipoic acid. This long chain then binds back into enzyme 2 where it transfers the acetyl group to coenzyme A (Scheme 1).[1–7] Then the resulting dihydrolipoic acid amide **4** dips into enzyme 3 where it is oxidized by FAD and NAD^+ to form NADH and regenerate the lipoic acid amide. This oxidative pathway generates the acetyl coenzyme A needed for many biochemical processes. A similar enzyme complex converts α-ketoglutaric acid to succinyl CoA. We decided to attempt to mimic such a sequence, not with a mixture of enzymes but instead with a single polymer to mimic them all by binding the substrates and intermediates so as to promote their reaction (Scheme 2). Based on our previous work with polymers of modified polyethylenimine as enzyme mimics,[8–11] we used hydrophobic binding into a hydrophilic water-soluble polymer **5** that had been modified to incorporate hydrophobic sidechains.

The commercial polyethylenimine from Aldrich with M_n = 10,000 and M_w = 25,000 was partially alkylated with dodecyl bromide on 6.6% of the amine groups and then reductively methylated on the remaining N–H groups, forming **5** as we have described previously.[11] We also used the thiazolium salt **6** that we had described previously, in our studies of benzoin condensations catalyzed by hydrophobic binding into modified polyethylenimines.[11] We synthesized a derivative of lipoic acid with a hydrophobic tail **10** so that it could also bind to the polymer. Without that hydrophobic tail simple lipoic acid did not react with the thiazolium intermediate **9** in Scheme 2. Finally, we trapped the acyldihydrolipoic acid intermediate **12** with aniline, forming either acetanilide from pyruvic acid or benzoylanilide from benzaldehyde.

We incubated a mixture of sodium pyruvate (120 mM), thiazolium salt **6** (6 mM), lipoamide **10** (44 mM), aniline (132 mM), and polymer **5** (6 mM) in 10% (v/v) DMSO and aqueous buffer (0.5 M Na_2HPO_4, pH 8.0) at 40 °C under argon for 3.5 days. Then the organic materials were isolated by extraction with EtOAc and the acetanilide product was isolated and weighed. The yield was 4%, obviously not reflecting a good preparative method. However, no acetanilide was formed under these conditions if the polymer **5** was omitted. We also replaced the pyruvate with benzaldehyde, in a reaction with thiazolium salt **6** (2 mM), lipoamide **10** (44 mM), aniline (44 mM), and polymer **5** (6 mM) with benzaldehyde (40 mM) added last in the same degassed DMSO/buffer solution under argon at 40 °C for 1.5 days. The product benzanilide was isolated by extraction and formed with a yield of 5%. No benzanilide was formed if the polymer **5** was omitted, or if the lipoamide **10** was replaced by simple lipoic acid.

The mechanism of Scheme 1 for the enzymatic reaction is clear, in particular that the intermediate (cf. **11**) from reaction of the enamine intermediate **1** with the lipoic acid amide **2** partitions so as to liberate the thiazolium ylide/carbene hybrid and leave the acetyl group on the dihydrolipoic acid species in **3**, since that species then reaches into another enzyme where the coenzyme A is waiting for the acetyl group. However, in our model system there is another possible path different from that shown in Scheme 2. If intermediate **11** lost the thiolate group then a 2-acylthiazolium ion species **14** would be formed. We have generated such an intermediate in previous work and shown that it is a powerful acylating agent that

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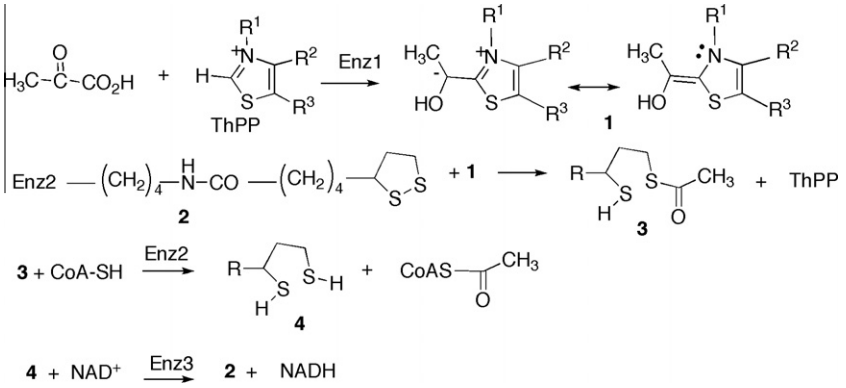
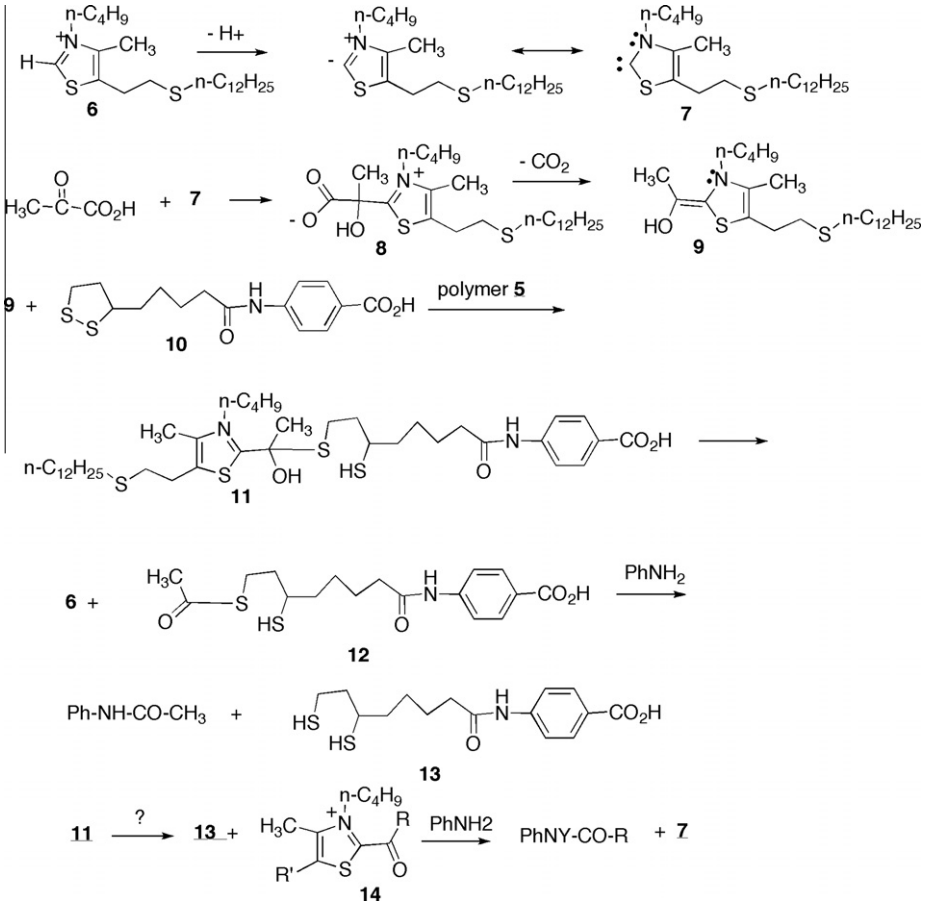
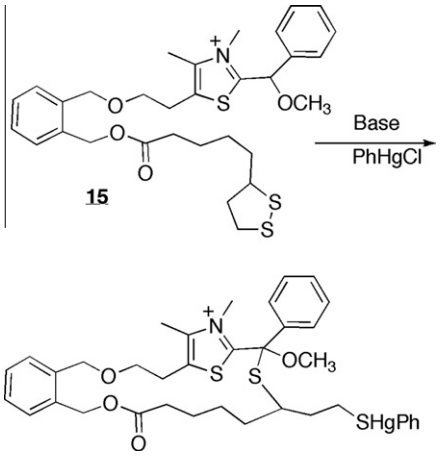


Scheme 1.



Scheme 2.

could acetylate the aniline.[12] With this alternate scheme involving a different way for the tetrahedral intermediate to fragment, the lipoic acid component would be acting simply as an oxidizing agent, not forming the acyldihydrolipoic acid species. We generated species **14** by replacing the lipoic amide species **10** in our reactions with potassium ferricyanide, the oxidant we had used previously to generate such an acylthiazolium salt,[12] and found that it also reacted with aniline to form acetanilide. The system with lipoamide **10** replaced by potassium ferricyanide also converted benzaldehyde to benzanilide, while if neither **10** nor the ferricyanide was present at a sufficient concentration the product was benzoin, the process we had described in our previous publication.[11] Thus further studies are needed to establish which mechanism is used by the lipoic amide in our model system. The most striking difference we saw was that there was some acetanilide and benzamide formed with the ferricyanide oxidations even when the polymer was omitted. Thus an important role of the polymer **5** in the lipoamide processes is to bind **10** into the same cavity in which intermediate **9** is present, while ferricyanide ion neither needs nor obtains such binding.



In 1996 Jordan et al. reported a successful intramolecular model in which a disulfide group in compound **15** reacted with a surrogate for the intermediate **1** to form a carbon–sulfur bond with assistance by phenylmercury chloride.[13] However, they found that an analogous intermolecular process was not successful. This is consistent with our own findings. Only with the polymer **5** present did we observe intermolecular reactions. Thus our success came from the role that polymer **5** plays in gathering the components of the reaction into the non-polar region of the polymer by mutual hydrophobic binding, in a mimic of part of the role that the enzymes play in the biological system.

Acknowledgement

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