The ultimate goal of this research is to develop chemiluminescent (CL) abzymes and a technology for their use in metal ion detection. Four objectives must be reached in sequential order: 1st, a molecule (hapten) which resembles in space requirements an intermediate in a CL reaction must be synthesized; 2nd, a hapten protein conjugate (antigen) must be prepared and used to obtain monoclonal antibodies (Mab's); 3rd, one or more of the Mab's must catalyze the given CL reaction; and 4th, the hapten must be modifiable such that the CL Mab's become specific complexers of metal ions. Accomplishment of the 1st objective would provide the first Mab mimic of bioluminescence. Attempts to synthesize a hapten to generate CL flavoabzymes will be discussed as will progress in the synthesis of a hapten to be used to prepare abzymes to catalyze the CL reaction of \( \text{H}_2\text{O}_2 \) with oxalate esters.
FINAL REPORT ON CONTRACT
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CONTRACT TITLE: The Synthesis of Chemiluminescent Flavoabzymes and Their Use in Metal Ion Detection

PERIOD OF PERFORMANCE: 15 March 1990 to 15 September 1992
(This includes a six month extension without additional funds)

RESEARCH OBJECTIVE: The ultimate goal of this research was to develop chemiluminescent (CL) abzymes and a technology for their use in metal ion detection. To this time catalysis of a CL reaction by an antibody is unknown.

PROGRESS REPORT:

Three approaches have been used. We first involved ourselves in the synthesis of a hapten suitable to the generation of monoclonal flavoabzymes which mimic the bacterial luciferase flavoenzyme bioluminescence. The objective of the second approach was to employ a known fluorescent fluorescein-Mab containing an engineered metal ion binding site adjacent to the fluorescein binding site. Our plan here was to design a flavin that could replace the fluorescein to directly provide a CL flavoabzyme with the desired metal ion binding site. The third thrust has been to synthesize a hapten which would make it possible to generate Mab’s which stabilize a key intermediate in the CL reaction of oxalate diesters with hydrogen peroxide.

The 1st phase of the study involved approaches to the synthesis of a molecule (structure A) which has the space requirements of a 4a-benzyl hydroperoxide adduct of a 4a,5-dihydroflavin (Structure B) and also has a "trail" (R) that can be attached to the carrier kehole limpet hemocyanin protein to form an antigen.
The first attempted synthetic sequence (Scheme I) involved Ph$_2$(tert-Bu)SiCl silylation of the primary ribityl hydroxyl group of riboflavin followed by methylation of the remaining three secondary ribityl hydroxyl groups using (AgO + MeI)/DMF. The silylation reaction of Scheme I was successful (85%); however, we were not able to carry out the methylation reaction, recovering N(3)-methyl silylated riboflavin and products of partial methylation. We then turned to the use of N(3)-methyl-N(10)-hydroxyethyl lumiflavin (C) as a starting material (Scheme II). A clean procedure for the N(5)-alkylation of lumiflavin (Fl$_{ox}$) is through the formation of an imine with 1,5-dihydrolumiflavin (FlH$_2$) followed by reduction (Scheme III; S. Ball; T. C. Bruice, *J. Am. Chem. Soc.*, 1981, 103, 5494). With the idea to adapt this general procedure, the aldehyde [(S)-PhCH$_2$CH(OH)CHO] was prepared (20%) and the α-hydroxyl group benzylated. Blocking the α-hydroxyl group is required since the α-hydroxyaldehyde (S)-PhCH$_2$CH(OH)CHO exists entirely as the unreactive dioxane (D). Succinylation of the hydroxyethyl function at N(10) of the product of Scheme IV would provide the hapten target molecule. Unfortunately the oxidative ring closure (last reaction of Scheme IV) did not provide the desired product but resulted in N(5) dealkylation.
Scheme 1

1. 1.1 eq Ph₂(t-Bu)SiCl
   2.2 eq imidazole
   DMF / 0°

2. AgO, CH₃I
   DMF / 0°

3. (t-Bu)₄N⁺F⁻
   THF / 25°

4. Oxidation

5. Protection

6. N-Alkylation,
   Deprotection,
   and Coupling

5a: R = -t-Butyl
5b: R = -NH(CH₂)₃COO(t-Butyl)

6a: R = -H
6b: R = -NH(CH₂)₃COOH

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[Diagram with chemical structures and text annotations]
Scheme II

1. $\text{H}_2\text{IO}_6 / 2\text{N H}_2\text{SO}_4, O^0, \text{dark}$

\[
\begin{align*}
\text{HO} & \quad \text{CHO} \\
\text{HO} & \quad \text{OH}
\end{align*}
\]

(88 %)

2. $\text{NaBH}_4 / 0.1 \text{ N NaOH, dark}$

\[
\begin{align*}
\text{CH}_2\text{OH} & \quad \text{CHO} \\
\text{CH}_2\text{OH}
\end{align*}
\]

(86 %)

3. $\text{CH}_3\text{I, K}_2\text{CO}_3 / \text{DMF, 25 °}$

C

Scheme III

1. $\text{H}_2, \text{HCl, Pd/C (10 %), EtOH}$

\[
\begin{align*}
\text{N} & \quad \text{N} \\
\text{N} & \quad \text{N}
\end{align*}
\]

2. $\text{CH}_3\text{CHO}$

\[
\begin{align*}
\text{N} & \quad \text{N} \\
\text{N} & \quad \text{N}
\end{align*}
\]

3. $\text{H}_2, \text{Pd/C (10 %)}$

\[
\begin{align*}
\text{N} & \quad \text{N} \\
\text{N} & \quad \text{N}
\end{align*}
\]
Scheme IV

1. H₂, HCl, Pd/C (10%) / EtOH

2. (s)-PhCH₂CH(Obz)CHO

3. H₂, Pd/C (10%)

4. NaNO₂ in 0.1 N AcOH / CH₃CN
Scheme V

1. NaS$_2$O$_4$, K$_2$CO$_3$ / DMF
2. PhCH$_2$CH(OH)CH$_2$I

3. AcOH to pH 5
4. NaNO$_2$ in 0.1 N AcOH / CH$_3$CN

Scheme VI

1. NaS$_2$O$_4$, K$_2$CO$_3$ / DMF
2. ICH$_2$CH$_2$OH

3. AcOH to pH 5
4. NaNO$_2$ in 0.1 N AcOH / CH$_3$CN
The Lerner group at Scripps had redesigned a fluorescein binding MAb such that a metal binding pocket resides next to the bound fluorescein dye (Iverson, B.L.; Iverson, S.A.; Roberts, V.A.; Getzoff, E.D.; Tainer, J.A.; Benkovic, S.J.; Lerner, R.A. Science 249, 659 (1990)). They found that metal binding quenched the fluorescence of the MAb bound fluorescein. It struck us that a splendid alternate approach would be to use this MAb to complex a flavin in place of the fluorescein dye. Using the assigned three-dimensional structure of the MAb and computer docking experiments along with AM1 calculations, we determined that 7-fluoro-8-hydroxy-isoalloxazine would be an excellent flavin replacement for fluorescein. The synthesis of 7-fluoro-8-hydroxyisoalloxazine was initiated. We put this approach aside when the cell-line for production of this particular MAb was lost.

Our 3rd approach, involves the synthesis of an antigen to elicit an antibody which would stabilize a key intermediate in the reaction of oxalate diesters with hydrogen peroxide. This much studied CL reaction involves the formation and decomposition of a dioxetane (Scheme VI; R.E. Milofsky, J.W. Birks, K.A. Chem. Soc. 113, 9715 (1991)). The synthetic sequence which we have followed in the preparation of an antigen is shown in the Scheme VII. This synthetic approach has been successful and sufficient quantities of 10 have been sent to Professor Stephen Benkovic who will oversee the preparation of Mab's.

INVENTIONS. None

PUBLICATIONS. None but we do anticipate a publication
Scheme VII

PhCH$_2$OH + Br(CH$_2$)$_3$OH $\xrightarrow{\text{NaH/DMF}}$ PhCH$_2$O(CH$_2$)$_3$OH

1 + 2 $\xrightarrow{110 \, ^\circ \text{C} \text{ Overnight}}$ 50% yield

PhCH$_2$O(CH$_2$)$_3$OH

3 + 4 $\xrightarrow{-78 \, ^\circ \text{C} \text{ yield 75%}}$

5 $\xrightarrow{\text{CH}_3\text{Li/THF}}$ PhCH$_2$O(CH$_2$)$_3$O

6 $\xrightarrow{\text{CF}_3\text{C(O)(O)CCF}_3 \text{ -78 } ^\circ \text{C/THF}}$

7 $\xrightarrow{2 \text{ M NH}_4\text{Cl} \text{ Yield 70%}}$

8 $\xrightarrow{\text{CH}_3$/ Heat} PhCH$_2$O(CH$_2$)$_3$O

9 $\xrightarrow{\text{H}_2$/Pd/C 1 At.}