# Electron Spin Catalysis by Xenon in an Enzyme

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Spin−orbit coupling (SOC) afforded by xenon has the ability to increase electron spin intersystem crossing rates in chemical and enzymatic reactions with radical pair intermediates and excited electronic states.[1] Protein crystallographers have used Xe to create heavy atom derivatives of proteins to acquire diffraction phase parameters, but apart from its anesthetic properties, Xe has generally been regarded as an unreactive noble gas that cannot participate in biological reactions.[2] In this communication, we demonstrate the usefulness of Xe as a mechanistic probe of radical pair intermediates in enzymatic reactions and call attention to the ability of Xe to change electron spin intersystem crossing rates and possibly electron-transfer rates in biological systems.[3]

Substrates and solvents containing bromine and iodine atoms are used in mechanistic studies of photochemical and radical reactions to enhance the electron spin intersystem crossing rate in photoexcited states and in reactive radical pairs.[4] The heavy atom (HA) can be directly attached to the substrate undergoing reaction (internal HA effect),[4a−c] or it can be part of the solvent matrix (external HA effect). In either case, the HA imparts its effect through SOC that arises from high atomic number (*z*) and a nearly filled valence electron shell.[4a−c] Br and I, with approximate SOC values of 2460 and 5069 $cm^{-1}$, are not innocuous heavy atoms, as they can enable alternate reaction pathways through the elimination of $Br^-$ and $I^-$ from substrate, or the halogenated solvent can trap a reactive radical intermediate.[1f,5]

Xenon offers a nonreactive alternative to introduce an external atom with high atomic number, but Xe has a filled octet and does not meet the HA criterion of a partially filled valance shell. Nevertheless, Xe has occasionally been used as a HA perturbant in chemical reactions, but it is most frequently introduced as a liquid or frozen Xe matrix.[1a−c] In previous work, we showed the HA character of Xe atoms can be increased by adsorption in a zeolite.[1f] Noncovalent electronic interactions in the Xe-zeolite adsorbate deform the filled valence shell of the Noble gas. Xe ($Kr5s^25p^6$) is isoelectronic with the monovalent cesium cation $Cs^+$ ($Kr5s^25p^6$) and it is useful to think of the upper limit of the SOC value for these species as the SOC value of 6080 $cm^{-1}$ for the first excited state of Xe.[1f]

In this communication, we demonstrate the ability of Xe to increase the kinetic parameter $V_{max}/K_m$ of coenzyme $B_{12}$-dependent ethanolamine ammonia lyase (EAL; E.C. 4.3.1.7). This enzyme was chosen for study because it has a kinetically significant radical pair (RP) that undergoes magnetic field dependent recombination.[6] The enzyme requires adenosylcobalamin and catalyzes a 1,2 shift of the amine group to produce acetaldehyde and ammonia as products. Homolysis of the Co−C bond of 5′-deoxyadenosylcob(III)alamin yields the 5′-deoxyadenosyl radical and cob(II)alamin as an enzyme-bound RP that originates in the singlet electronic spin state (Figure 1).[6a,7] The RP partitions between forward catalytic throughput and recombination to regenerate 5′-deoxyadenosylcob(III)alamin. Hyperfine coupling from Co(II) promotes intersystem crossing to convert a portion of the initially formed singlet RP to the triplet RP.[7,8] Both the singlet and triplet RP can go forward through the next step in the catalytic cycle, but only the singlet RP can undergo nonproductive recombination. An externally applied magnetic field in the range 500−2000 G *decreases* the rate of intersystem crossing and thereby decreases the conversion of the singlet RP to the triplet RP. Thus, a magnetic field *decreases* the kinetic parameter $V_{max}/K_m$. Spin−orbit coupling imparted by Xe is expected to have the opposite effect on the enzyme-bound RP, thereby increasing the rate of intersystem crossing and increasing the conversion of the singlet RP to the triplet RP. Thus, Xe is expected to *increase* the kinetic parameter $V_{max}/K_m$.

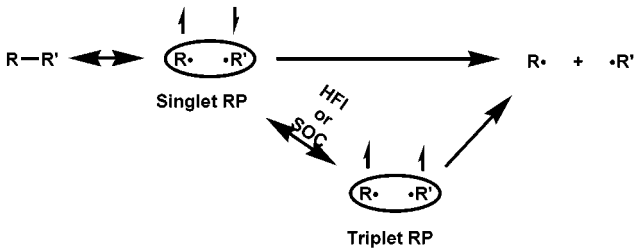


**Figure 1.** Accelerated intersystem crossing (S ↔ T interconversion) in a radical pair via spin−orbit coupling (SOC). Hyperfine interactions (HFI) can also promote S ↔ T mixing, but HFI-induced intersystem crossing can be decreased by an applied magnetic field, whereas the contribution of SOC-induced intersystem crossing is invariant with magnetic field.

Recombinant EAL from *Salmonella typhimurium* was obtained as described previously and the enzyme was assayed spectrophotometrically by coupling acetaldehyde formation to the oxidation of NADH by alcohol dehydrogenase.[6a,9] Perdeuterated ethanolamine was employed as substrate to introduce a kinetic isotope effect and maximize the observed magnetic field effect, as previously described.[6a] Each cuvette with assay solution was charged with Xe or Ar gas at 40 psi.[10] Argon provided a suitable control gas for which no HA effect is expected. A magnetic field of 0 or 1500 G in the assay medium was obtained with a thermostated electromagnet as described previously.[6a,d]

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(10) Perdeuterated ethanolamine was purchased from Cambridge Isotopes, Inc and Xe gas (99.999%) was purchased from Spectra Gases, Inc. All other chemicals were purchased from Sigma, Inc. Quartz cuvettes (1.4 mL; 1 cm path length) with silicone rubber stopper inserts were charged with the assay solution (minus coenzyme). Each cuvette was connected to a gas manifold via 18-gauge cannula. The headspace above each cuvette was evacuated to a pressure of 0.02 Torr for 2 min, followed by charging with Xe gas to a pressure of 40 psi. The cuvette was incubated at 25 °C for 5 min and the evacuate/flush/incubate process was repeated for a total of 3 incubation cycles. Negligible evaporation of solution occurred with this procedure. The enzyme was assayed under an atmosphere of 40 psi or Ar or Xe, as indicated. The enzymatic reaction was initiated by the addition of 5′-deoxyadenosylcobalamin via syringe pipet. The velocity vs [S] data were fitted to the Michaelis−Menten equation, $vel = (V_{max}[S])/(K_m + [S])$ by nonlinear regression.

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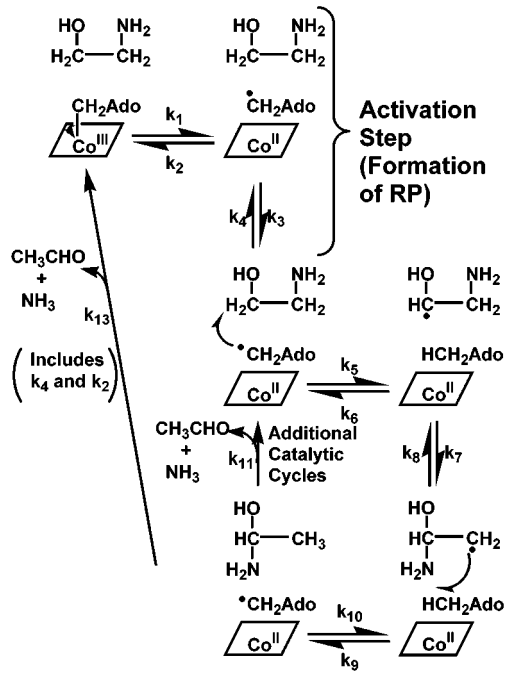


**Figure 2.** Reaction mechanism for ethanolamine ammonia lyase. Homolysis of the Co−C bond produces the 5′-deoxyadenosyl radical and cob(II)alamin in the singlet spin state. Both the singlet and the triplet RP can progress forward through the catalytic cycle, with the 5′-deoxyadenosyl radical abstracting H• from ethanolamine to generate the substrate radical. Only the singlet RP can undergo nonproductive recombination to re-form the Co−C bond. Therefore, an increase in S ↔ T mixing will increase catalytic throughput and increase $V_{max}/K_m$. Under $V_{max}$ conditions, the enzyme is saturated with substrate ethanolamine and subsequent catalytic cycles can occur via $k_{11}$. With saturating substrate, recombination of the 5′-deoxyadenosyl radical:cob(II)alamin radical pair does not have to occur (i.e. $k_{13}$ does not have to operate) and the magnetosensitive RP does not undergo recombination.

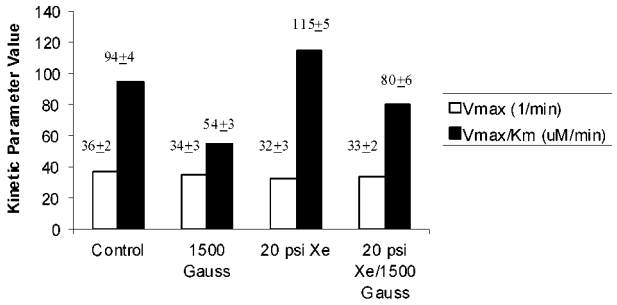


**Figure 3.** Each assay contains 100 mM Hepes, pH 7.5, 5 $\mu$M 5′-deoxyadenosylcobalamin, 0.1 mM NADH, 6 units of alcohol dehydrogenase, EAL, and 1−300 $\mu$M perdeuterated ethanolamine in a total volume of 1.4 mL at 25 °C. Each cuvette was filled with Xe or Ar according to the evacuate/flush/fill procedure described and assayed at 40 psi Xe or Ar.

The dependence of $V_{max}$ and $V_{max}/K_m$ on Xe, Ar, and magnetic field is shown in Figure 3. The kinetic parameter $V_{max}$ is nearly invariant with a range of 32−36 $\mu$M min$^{-1}$ under all experimental conditions of Xe and magnetic field. In contrast, the kinetic parameter $V_{max}/K_m$ *increases* by 22% from 94 ± 4 min$^{-1}$ to 115 ± 5 min$^{-1}$ in the presence of 40 psi Xe, whereas $V_{max}/K_m$ *decreases* by 42% from 94 ± 4 min$^{-1}$ to 54 ± 3 min$^{-1}$ at 1500 G in agreement with previous observations. If Xe were merely binding to EAL in or near the active site, it should decrease $V_{max}/K_m$ and appear to be a competitive inhibitor. If Xe were bound to the enzyme in such a way as to interfere with catalysis or denature the protein, but not interfere with substrate binding, it would decrease $V_{max}$ and appear to be an uncompetitive or noncompetitive inhibitor. The effect of a magnetic field and Xe on $V_{max}/K_m$ but not on $V_{max}$ is consistent with the RP intermediate occurring before the first irreversible step in the kinetic mechanism (cf. Figure 2).[11]

Since both Xe and a magnetic field are postulated to alter the partitioning of the enzyme-bound 5′-deoxyadenosyl radical:cob(II)alamin RP, but with an opposite effect on the rate of electron intersystem crossing, the magnetic field effect on $V_{max}/K_m$ should be diminished in the presence of Xe, as SOC from the noble gas will compete with Co(II)-induced hyperfine coupling to provide a nonmagnetic field dependence. Indeed, the magnitude of the magnetic field effect on $V_{max}/K_m$ is decreased from 94 min$^{-1}$ to 80 min$^{-1}$ in the presence of a 1500 G magnetic field and 40 psi Xe.

Xe has a covalent bond radius of 1.30 Å and an atomic van der Waals radius of 2.2 Å.[12] Therefore, the atomic radius for an adsorbed Xe atom must lie between the limiting values of 1.30 and 2.2 Å. The Xe atom must bind in or near the active site to enhance intersystem crossing through SOC, which is most likely to occur through partial electronic overlap involving the p-orbitals of Xe.[13] Xenon may be especially useful as a tool to demonstrate the kinetic significance of strongly coupled radical pairs with electron−electron separation distances of 3−6 Å and large singlet−triplet energy gaps. In these cases, an external magnetic field is not sufficient to perturb hyperfine coupling and no magnetic field effect is observed, despite having a kinetically significant RP.[1f,14]

The enhancement of electron spin intersystem crossing rates through SOC is sometimes referred to as "electron spin catalysis" when a heavy atom accelerates singlet/triplet interconversion and thereby increases the forward rate of a reaction.[15] Xe may be a convenient route to introduce a heavy atom into a protein to promote electron spin interconversion to study the role of electron spin in other enzymes with a spin-sensitive RP intermediate.[16] X-ray and $^{129}$Xe NMR studies show specific binding sites for Xe in proteins.[2a,17] Supporting these observations, HXeOH and HXeSH adducts have been formed from neutral ground-state compounds at low temperatures.[18]

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