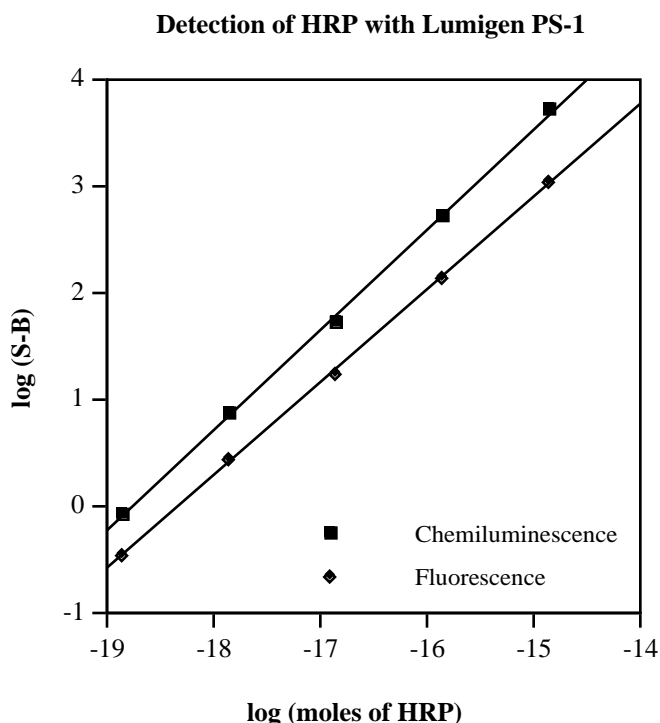


Fluorescent/Chemiluminescent Substrates for Peroxidase Detection

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Horseradish peroxidase (HRP) is widely used as an enzyme label for medical diagnostics and research applications. The availability of substrates for colorimetric, fluorimetric and chemiluminescent assays provide numerous detection options. Chemiluminescent detection reagents for HRP in current use include those based on the oxidation of a hydrazide such as luminol and acridan substrates such as Lumigen PS-1 (1) and Lumigen PS-3 (2) and also contain an enhancer compound to promote the catalytic action of the peroxidase. Reaction of Lumigen PS-1 with HRP in homogeneous solution causes intense chemiluminescence which reaches peak intensity in about 5 min. The Lumigen PS-3 reagent is designed for use in rapid ultrasensitive detection of western and Southern blotted analytes. A unique feature of these acridan-based reagents is the generation of a fluorescent species on reaction with peroxidase, a property which has been termed chemifluorescence. These reagents thus represent the first dual-use substrates enabling both chemiluminescent and fluorescent detection.



We have developed several additional acridan-based substrates for HRP which are capable of subattomole detection sensitivity. By varying several structural parameters within the class of compounds we have produced substrates which either produce fluorescence alone or both chemiluminescence and fluorescence. The properties and kinetic behavior of the fluorescent emitters is under present investigation.

References

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