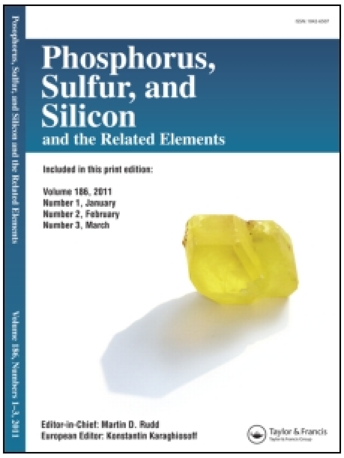


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# Physical-Chemical Properties of Selenium-Containing Inorganic Radicals and their Reactivity with Amino Acids and Enzymes

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# PHYSICAL-CHEMICAL PROPERTIES OF SELENIUM-CONTAINING INORGANIC RADICALS AND THEIR REACTIVITY WITH AMINO ACIDS AND ENZYMES.

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Abstract The physical-chemical properties of some selenium-containing inorganic radicals have been studied by using the pulse radiolysis technique. These inorganic free radicals can be formed readily in irradiated aqueous solutions by scavenging of OH primary radicals with anions such as selenocyanate and the anionic forms of selenium dioxide ($HSeO_3^-/SeO_3^{2-}$). Particular attention has been paid to the spectral and kinetic properties of the radical species involved. The investigations on the reactivity of these radicals with amino acids and enzymes have allowed information both on oxidation mechanism and on the preferential site of attack.



## INTRODUCTION

Radiation induced free radicals react with molecules of biological interest and are involved in the development of many biological and pathological events.[1] Some inorganic oxidizing free radicals are used as selective probes in the identification of the sites of radical attack at essential amino acid residues in proteins.[2] While the primary radicals from water radiolysis, $e^-_{aq}$, OH and H, damage biomolecules in a non-specific way, the secondary radicals can react more selectively and can attack specific functional groups.

Moreover the spectra of the transient products from the reactions of these oxidizing radicals with certain amino acidsare characteristic of the aminoacid involved, but independent of the inorganic free radical. The spectra are useful "finger prints" which allow to determine initial sites of attack of these radicals on enzyme structure and to allow to observe changes on the site of attack with changing conditions such as pH.

This communication is related to the physical-chemical properties of some inorganic selenium radicals[3,4] and to their potential use for probing the structure of proteins and determining the function of specific amino acids.[3,5]

## RESULTS

Inorganic free radicals can be formed in irradiated aqueous solutions by rapid and quantitative scavenging of OH radicals with inorganic anions ($X^-$ or $Y^{n-}$) according to the reactions:

$$X^- + OH^\bullet \rightarrow X^\bullet + OH^- \qquad X^- = Cl^-,\ Br^-,\ I^-,\ N_3^-,\ SCN^-,\ SeCN^-,$$

$$X^\bullet + X^- \rightleftarrows X_2^-$$

$$Y^{n-} + OH^\bullet \rightarrow Y^{(n+1)-} + OH^- \qquad Y^{n-} = CO_3^{2-},\ SO_4^{2-},\ HPO_4^{2-},\ PO_4^{3-},\ SeO_3^{2-}$$

The secondary radicals derived from the pulse radiolysis of selenocyanate and selenium dioxide answer the need for a good selective secondary radical.

In the case of selenium dioxide, the pulse radiolysis data in aqueous solution has shown the presence of three selenite radicals in acid-base equilibrium within well defined pH range[3]:

$$(H_2SeO_3)^+ \overset{pK\ 3.9}{\Longleftrightarrow} HSeO_3 \overset{pK\ 7.4}{\Longleftrightarrow} SeO_3^-$$

Table I summarizes the spectral and kinetic data for selenocyanate and the three different forms of selenite radicals.

TABLE I Absorption maxima, extinction coefficients and decay kinetics values of selenium inorganic radicals

| Radical | pH | $\lambda$ max (nm) | $\varepsilon$ max ($dm^3\,mol^{-1}\,cm^{-1}$) | $2k/\varepsilon$ ($cm\,s^{-1}$) | Ref. |
|---|---|---|---|---|---|
| $(SeCN)_2^-$ | 7 | 440 | 9300 | $2.0\times10^6$ | 3 |
| $(H_2SeO_3)^+$ | 1 | 430 | 930 | $2.1\times10^6$ | 4 |
| $HSeO_3$ | 5-6 | 430 | 600 | $7.5\times10^5$ | 4 |
| $SeO_3^-$ | 9-11 | 430 | 1350 | $4.0\times10^5$ | 4 |

Table II shows the bimolecular rate constant of the selenium inorganic radicals with amino acids.

Table II Rate constants in $dm^3 mol^{-1} s^{-1}$ for the reactions of some selenium inorganic radicals with amino acids.

| Radical | cysteine | cystine | methionine | tyrosine | tryptophan | histidine | Ref. |
|---|---|---|---|---|---|---|---|
| $(SeCN)_2^-$ | $6.8x10^7$ | | $<1.0x10^7$ | $1.0x10^7$ | $1.0x10^7$ | $<1.0x10^7$ | 3 |
| $HSeO_3$ | | | | $1.1x10^9$ | $3.3x10^9$ | | 5 |
| $HSeO_3/SeO_3^-$ | | $3.5x10^7$ | $1.2x10^8$ | $1.1x10^9$ | $3.3x10^9$ | $4.3x10^7$ | 5 |
| $SeO_3^-$ | | | | $1.3x10^9$ | $2.5x10^9$ | $1.6x10^8$ | 5 |

The differences in the redox properties of the radicals reflect their different selectivity and reactivity with amino acids.
For halide or pseudo-halide radicals, the reactivity with amino acids decreases in the order:

$$Cl_2^- > Br_2^- > (SCN)_2^- > I_2^- > (SeCN)_2^-$$

and parallel the oxidizing properties of the radical and of the parent anion.
$(SeCN)_2^-$ reacts rapidly only with cysteine above the limit of measurement; with all the other amino acids the reactivity was negligible.
The selenite radicals react selectively with amino acids, in particular with aromatic, heterocyclic, and sulphur-containing amino acids in the order tryptophan > tyrosine > methionine > histidine, independently of the acid-base structure of the radical. Non sulphur-containing amino acids are rather unreactive. Kinetic and spectroscopic data on the reaction of $(SeCN)_2^-$ and $HSeO_3/SeO_3^-$ with enzymes give useful informations both on the oxidation mechanism and on the preferential site of attack.
In particular the investigations at different pH's on the reactivity of selenite radicals with amino acids and enzymes reflect the different behaviour of the acid-base structures of the radical, the influence of particular ionizable groups in the reacting molecule and the structure

modifications at the level of the proteins.

## CONCLUSIONS

The combination of kinetic and spectroscopic data with the enzyme radiation-inactivation data can give useful information on the composition of the active site of the enzyme.

For example the enzyme radiation-inactivation data indicate that selenocyanate shows a protective effect with those proteins, of which cysteine residues are not essential to the catalytic activity (ribonuclease, α-chymotrypsin, lysozime); on the contrary, the alcoholdeydrogenases, which contain crucial cysteine-SH groups, are more inactivated in the presence of selenocyanate. In the case of selenium dioxide, this increases the yield of radiation-induced inactivation of the enzyme tested (lysozime, α-chymotrypsin,yeast & liver alcoholdeydrogenases ), with the exception of ribonuclease.

In all cases the data are consistent with the present knowledge of the structure of the catalytic site and of the functions involved in the maintenance of the active conformation of the studied enzymes.



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