International Scholars Journals

Frontiers of Agriculture and Food Technology Vol. 2 (1), pp. 001-005, January, 2012. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Review

Physicochemical features of rhodanese: A review

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Accepted 12 November, 2011

Rhodanese is a multifunctional, mitochondrial, sulphur transferase that catalyses the detoxification of cyanide by sulphuration in a double displacement (ping pong) mechanistic reaction. It is widely distributed occurring in varieties of plants and animals, where it activity is modulated by a number of factors including differences in species, organs, sex, age and diet. The enzyme is a single polypeptide chain of 289 amino acids with molecular weight of up to 37,000. The active site of rhodanese contains a tryptophanyl residue in close proximity with an essential sulphahydryl group. Many methods for assaying rhodanese have been reported, the most prominent being the one based on the colorimetric estimation of thiocyanate formed from the reaction of cyanide and thiosulphate, catalysed by rhodanese.

Key words: Rhodanese, cyanide, sulphur transferase.

INTRODUCTION

Many plants and plant products used as food in tropical countries contains cyanogenic glycosides. These plants include cassava, linseed, beans and peas, which are known to contain linamarin coexisting with lotaustralin. Millet, sorghum, tropical grass and maize are sources of dhurin. Amygdalin is found in plums, cherries, pears, apple and apricots. These compounds are also present in plants such as rice, unripe sugar cane, several species of nuts and certain species of yam (Osuntokun, 1981; Oke, 1979). Most of these plants and their products are staple foods in the tropics. Upon hydrolysis these compounds yield cyanide, a sugar and a ketone or aldehyde. Cyanide is a potent cytotoxic agent that kills the cell by inhibiting cytochrome oxidase of the mitochondrial electron transport chain. When ingested, cyanide activates the body own mechanisms of detoxification, resulting in the transformation of cyanide into a less toxic compound, thiocyanate.

The principal detoxification pathway of cyanide is that catalysed by a liver mitochondrial enzyme, rhodanese (Cyanide: Thiosulphate Sulphur Transferase; E.C.2.8.1.1). Rhodanese is widely distributed in both plants and animal species. Two forms of rhodanese, have been demonstrated. These forms are dephospho – and phospho– rhodanese. They were identical with respect to kinetic parameters, amino acid composition amino terminal amino acid, sulphahydryl content, tryptic maps and molecular weights. Both forms utilize β-mercaptopyruvate equally but at 1% efficiency of thiosulphate (Blumenthal and Heinrikson, 1971). The phosphorylation is catalysed by a cAMP – dependent protein kinase utiizing ATP (Ogata et al., 1989). Similarly, 4 stable forms of the enzyme designated I, II, III, and IV, having the same molecular weights and primary structures with respect to amino acid composition and peptide map were separated from bovine liver. It was speculated that they are conformational isomers originating from the form that predominate in the mitochondrial extract (Cannella et al., 1981) . This paper reviews the physiological roles, structural features, mechanism of action and assay methods of rhodanese.

PHYSIOLOGICAL FUNCTION

The physiological role of rhodanese (E.C.2.8.1.1. cyanide: thiosulphate sulphur transferase) in animal tissues and perhaps in plants is controversial; particularly its function in the detoxification of acute cyanide exposure (Delvin et al., 1989; Sylvester and Sander, 1990). Rhodanese is a sulphur transferase that

catalyses, *in vitro*, the formation of thiocyanate from cyanide and thiosulphate or other suitable sulphur donors. *In vivo* the enzyme is, however multifunctional (Smith and Urbanska, 1986).

$$S_2O_3^{2-} + CN^- \xrightarrow{\text{rhodanese}} SCN^- + SO_3^{2-}$$

It is generally believed that the major function of rhodanese is cyanide detoxification (Smith and Urbanska, 1986; Buzaleh et al., 1990). This function is more prominent in mammals where highly cytotoxic cyanide is converted to a less toxic thiosulphate and excreted through the kidney (Cagianut et al., 1984; Keith et al., 1989; Bourdoux et al., 1980). In plants, a close relationship exists between rhodanese activity and cyanogenesis, which suggest that the enzyme provides a mechanism for cyanide detoxification in cyanogenic plants (Smith and Urbanska, 1986).

The capacity of *Bacillus stearothermophilus to* detoxify cyanide could be greatly increased when mutants containing 5 to 6 times rhodanese activity of normal cells were used (Atkinson, 1971).

The distribution of rhodanese in both adult and larvae insects is not restricted to those species that encounter exogenous cyanide through feeding on cyanogenic plants (Beesley et al., 1985). This is an indication that cyanide detoxification may not be the primary role of this enzyme in insects. In insects, it was proposed that the enzyme might be involved in a more important role of sulphur transfer for protein synthesis. In squid (*Loligo peali),* it is more likely to be involved in the formation of C-S bond of isethionate, which is present to the concentration o f 150 mM in its giant axon (Hoskin and Kordik, 1977). In *Rhodopseudomonas spheroids* rhodanese catalyse the formation of cysteine from cysteine trisulphide (Dexifra et al., 1975).

Rhodanese in its phosphorylated and dephosphorylated forms has been reported to function as a converter enzyme that interact with mitochondrial membrane bound iron–sulphur centers of the mitochondrial electron transport chain where it modulate the rate of respiration (Ogata and Volina, 1990). There is an indication of a possible role of rhodanese in providing labile sulphide necessary for the synthesis of ferredoxin in the chloroplast of spinach, parsley, cabbage, and red turnips (Tomati, 1972). It also catalyses the formation of iron -sulphur centers in *Escherichia coli*, and a physiological role of the enzyme in aerobic metabolism in this organism was suggested (Keith and Volina, 1987).

Rhodanese was also reported to reconstitute spinach ferredoxin (Pagani et al., 1984); restore durum wheat leaves cyanide inactivated NADH:nitrate reductase activity and if added before cyanide treatment, it protects the enzyme (Tomati et al., 1976). It also restore, partially, the activity of NADH dehydogenase (Pagani and Galante, 1983). It was also fund to increase the activity of malate dehydrogenase (Agro et al., 1976). Restoration of MgATP and chelator inactivated nitrogenase of *Klebsiella pneumoniae* has been reported (Pagani et al., 1987). A possible role of the enzyme in modulating S-amino levulinate synthetase activity has also been reported (Vazquez et al., 1987).

In *Thiobacillus intermedius,* the mechanism of oxidation of thiosulphate to sulphate seems to involve the action of rhodanese (Charles, 1969). At pH 8.8 beef liver rhodanese however catalyses the reduction of thiosulphate to sulphite (Koj, 1968). Its activity was also related to the oxidation of thiosulphate and elemental sulphur to sulphate by the fungus, *Rhizopus* oryzae (Ray et al., 1990).

STRUCTURE

Electron density map of bovine liver rhodanese shows, in conjunction with gel electrophoresis that, rhodanese consists of a single polypeptide chain with molecular weight of 32,000 to 33,000 (Crawford and Horowitz, 1976; Bergsma et al., 1975). The map revealed clear double domain structure having similar conformation with few structural differences. Each of these domains has four-stranded parallel β-structure, with one helix running anti parallel to the β-sheet (Bergsma et al., 1975). Smith et al. (1974) however reported a dimmer with molecular weight of 37,000. These workers found that each monomer has six short helixes and three strands parallel twisted pleated sheet. The carbonyl terminus of one monomer and the amino terminus of the other were found to be in close contact, forming a salt bridge.

AMINO ACID COMPOSITION

Using sequential Edman degradation of over lapping peptides obtained by selected chemical and enzymatic cleavages of rhodanese purified from chicken liver revealed that the primary structure of the enzyme contain 289 amino acids. 212 of these amino acids matched with those obtained from bovine liver. The differences were mainly due to conservative substitutions(Kohanski and Heinrikson, 1990).

Between 5 and 11 tryptophan per 26 alanine residues in bovine liver rhodanese was reported (Baillie and Horowitz, 1976). In a more intensive study, involving combination of amino acid analysis, solvent perturbation difference spectroscopy, specific residue modification and direct UV spectra analysis, it was found that the enzyme contain 10 tyrosine, 8 tryptophan and 16 phenylalanine per 26 alanine residues(Baillie and Horowitz, 1976).

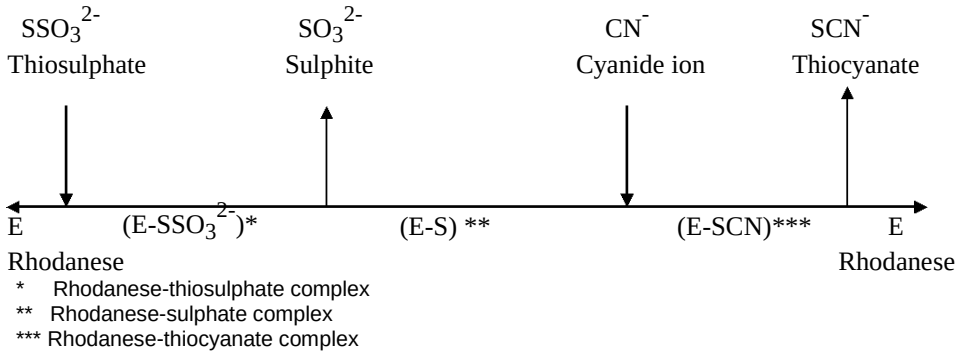

Figure 1. Mechanism of rhodanese action.

ACTIVE SITE

The catalytic site of rhodanese is located in the bottom of the crevice formed by the two domains of the enzyme (Koloczek and Vanderkool,1987). It has been shown that sulphahydryl (SH) groups are important in the rhodanese catalysis (Wang and Volini, 1968; Keith and Volini, 1987). There is evidence suggesting the presence of a tryptophanyl residue, which is in close proximity with the essential sulphahydryl group in the active site of the enzyme (Wang and Volini, 1968). It was proposed that this SH group is the site of substrate – sulphur binding in the obligatory enzyme – sulphur intermediate (Keith and Volini, 1987). Solution studies indicated that the active site SH group could form an intramolecular disulphide bond with another SH group in the protein. These groups are however located in such a way that the disulphide bond formation is very unlikely (Wang and Volini, 1968). Instead the active site SH group can participate in hydrophobic interaction (Horowitz and Westley, 1970). The sulphur – donor substrates, thiosulphate and ethanethiosulphonate utilize the same site in rhodanese but produce different sulphur – substituted enzymes (Jarabak and Westley, 1974).

MECHANISM OF ACTION

A number of solution studies of the enzyme have been interpreted as indicating that the protein has structural flexibility and that reversible conformational changes accompany catalysis. This is important in the rate limiting binding step (Horowitz and Criscimagna, 1983).

Rhodanese form covalent substituted – enzyme intermediate during catalysis. It functions by double displacement mechanism with formation of a covalent enzyme – sulphur intermediate (Keith and Volini, 1987). This mechanism involves binding of thiosulphate to a metal ion in the enzyme. In this complex, there is election shift away from the planetary sulphur atom of the thiosulphate with resultant stretching and weakening of the S – S bond, making it more susceptible to attack by a strong enzymic nucleophile which affects the cleavage (Leininger and Westley, 1968). The enzyme substrate [ES] complex, differing in reactivity depending on the nature of the sulphur donor substrate (Jarabak and Westley, 1974), is formed by discharging sulphite ion from enzyme-thiosulphate complex. The acceptor substrate, cyanide ion then combines with the E – S intermediate to form the second product, thiocyanate ion, there by regenerating the free enzyme (Volini and Wang, 1978). The enzyme thus functions through the ping-pong mechanism (Vazquez et al., 1987). The scheme above (Figure 1) explains the mechanism of action of cyanide:thiosulphate sulphur transferase (rhodanese)

ASSAY METHODS FOR RHODANESE

Many methods have been reported for the assay of rhodanese activity in various extracts. The most prominent is the one based on the colorimetric determination of thiocyanate. In this case thiocyanate produced by the action of the enzyme on cyanide and thiosulphate, is reacted with ferric nitrate reagent to produce a red coloured complex – ferric thiocyanate which can be estimated colorimetrically at 460nm (Sorbo,1955; Baskin and Kirby, 1990; Markku et al., 1999). This method is suitable for measuring the activity of rhodanese preparation of any degree of purity (Sorbo, 1955).

This method is however not suitable for estimated of the enzyme in some tissue and organisms. Scott and Wright (1980) thus reported a modified method for human hemolyzates in which a protein-precipitating agent is included. Determination of the activity of rhodanese in soil samples involves incubating the buffered substrates and toluene. The method is rapid, sensitive and precise (Tabatabai and Singh, 1976). The assay methods used for extracts from animal tissues are not applicable in plant homogenate (Lieberei and Selmar, 1990) and micro-organisms (Singleton and Smith, 1988). This is due to concomitant occurrence of both enzymic [biological] and non enzymic [non biological] thiocyante prodution. The

non-enzymic formation of thiocyanate strongly affects the assay procedure.

A histochemical method was described in which the reducing effect of sulphite ion resulting from the enzyme reaction was used (Tanka and Gatai, 1983). In a similar work, Canenella reported a colorimetric method based on the continuous determination of sulphite (Cannella et al., 1984).

A method for the assay of rhodanese activity which uses pH-STAT apparatus with cyanide sensitive electrode to monitor the rate of the enzyme catalysed reaction under controlled conditions has been reported (Cannella et al., 1975). A standard kinetic analysis of bovine kidney enzyme, including pH and temperature dependence is similar to those obtained by the classic spectrophotometric method. A simple method for the rapid precise determination of the enzyme in polyacrylamide gel after electrophoresis was also developed (Guilbaut et al., 1971). The optimum activity of the enzyme has been recorded at various pH and temperature depending on the source.

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