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AMBIGUITIES IN THE ENZYMOLOGY OF SULFUR-CONTAINING COMPOUNDS

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Abstract - The specificiations of enzymes towards sulphur-containing amino-acids and some of their degradation products and derivatives are discussed.

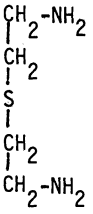
In the course of this meeting we have occasionally met reactions involving a variety of sulfur-containing compounds carried out by systems or enzymes also used for different purposes. In this final lecture we should like to point out how this phenomenon is much more general than believed, thus indicating the occurrence of a real ambiguity in the enzymology of sulfur-containing compounds. This fact came to the attention of one of us early in 1956, when cystamine (I) and lanthionamine (II) were assayed as substrates for diamine oxidase (1). It was known at that time that diamine oxidase was an enzyme of broad specificity, being able to use as substrates diamines having different carbon chain lengths and also diamines with one of the amino groups substituted with an imidazole or a guanido group (2). However it was not known whether a substantial change of the chemical composition would have impaired the enzymatic attack on the substrate. It was therefore a surprise to find an enzyme unable to recognize the substitution of a methylene carbon of the substrate with one or two atoms of sulfur. Diamine oxidase uses not only I and II as substrates but also lanthionamine sulfone, although at a lower rate than I and II (1). The interest of this finding was increased by the observation that the rate of the oxidation of I and II was in the range of that of the traditional substrates and that the oxidation was followed by the cleavage of the intermediate cystaldimine (IV) leading to the formation of compounds of biological interest like thiocysteamine (VI), hypotaurine (VII), thiotaurine (VIII) and taurine (IX) (3-7). It is evident that diamine oxidase uses as reacting points the nitrogen-containing groups while the composition of the carbon chain between them could be radically modified to the point of being partially substituted with sulfur atoms without impairing the enzymatic attack.

Cysteinesulfinic acid (XI) and cyteic acid (XII) are also known to take profit from a number of enzymes used for other purposes. In this case the group producing the ambiguity is the sulfinic or the sulfonic group which simulate the carboxyl group of the carbon analogs glutamic and aspartic acids. XI and XII are able to substitute glutamic and aspartic acids in the common transaminase reactions (8-11). The possible occurrence of a specific transaminase for XI (12) is yet to be substantiated by the isolation of the pure enzyme. We had the opportunity to use XI as substrate for mitochondrial aspartate amino transferase purified up to homogeneity thus confirming that this enzyme actually transaminates XI irrespective of the possible occurrence of a specific enzyme. Other sulfur containing substrates for this transaminase are: (XIII to XV), cysteine-S-sulfonate (13), alanine thiosulfonate (14), and serine-O-sulfate (15). It is remarkable that while the enzyme uses XV as substrate, it is inactive towards serine-O-phosphate. The fate of the transamination products is quite different. In fact, while pyruvate-β-sulfonate produced by the transamination of cysteic acid is a stable compound (9), the transamination products of the other substrates release: sulfite XI and thiosulfate XIII and XIV. The behaviour of serine sulfate (XV) is quite interesting and merits consideration. This compound is substrate of purified aspartate amino transferase, however, when incubated in the absence of the keto acid acceptor it is α,β-eliminated yielding ammonia, sulfate and pyruvate. Aminoacrylate, which is produced in the course of this reaction as an intermediate inactivates the enzyme by reacting with sulfhydryl and other groups of the active site. This type of inhibition, fre-

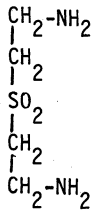
quently called suicide reaction (15), occurs also with other substrates and other enzymes (16). Another point of interest is that thiosulfate (17) and other S-containing compounds add spontaneously to the intermediate aminoacrylate thus removing the inhibition. Incidentally this procedure not only represents a way to protect these enzymes from their suicidal inclinations but is also an alternative way to produce a number of sulfur containing compounds. Thus cysteine-S-sulfonate (and consequently cysteine upon the reductive removal of sulfite) is produced in the presence of thiosulfate (17), thialysine in the presence of cysteamine (16).

Purified bacterial aspartate β-decarboxylase has been found to use also XI in a manner very similar to that observed in the case of aspartate. The final products in the case of XI are alanine and sulfite (18). To our knowledge XIII, XIV and XV have not been assayed as possible substrates for this enzyme and we will not be surprised if they should function too.

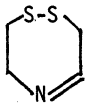
Cystamine I



Lanthionamine II

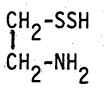


Lanthionamine sulfone III

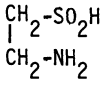


Cystaldimine IV

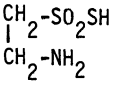
Cysteamine V



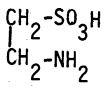
Thiocysteamine VI



Hypotaurine VII



Thiotaurine VIII

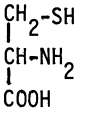


Taurine IX

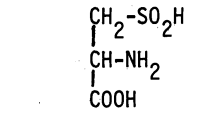
Another point of interest related to the enzymatic similarity of the sulfur analogs of aspartic and glutamic acids is an observation made some years ago but not published. Living rats injected with XI or XII excrete in the urine an amount of glutamic and aspartic acids much higher than under normal conditions. This finding indicates a competition at the level of the kidney reabsorbing system between XI or XII and glutamic or aspartic acid. If so it is evident that the ambiguity occurs also at the level of the transport systems.

Still debated is the question of the occurrence in animal tissues of decarboxylases specific for the decarboxylation of XI and XII distinct from glutamate decarboxylase. A number of reports lend support to the occurrence of a single enzyme operating the decarboxylation of XI and XII, although at different rates (19-21). Some disagreement, however, has appeared at this regard (22). To our knowledge the possibility that glutamate decarboxylase could operate also the decarboxylation of XI and XII has not been eliminated. Actually we have been surprised to find deleted out of the official Enzyme Nomenclature the name of cysteinesulfinic acid decarboxylase, formerly listed under the number 4.1.1.29, and now listed as a side property of glutamate decarboxylase registered under the number 4.1.1.15 (23). Decarboxylation of glutamate and that of XI and XII in rat brain have actually been reported to behave similarly under a set of various conditions (24),

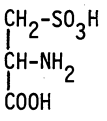
on the other hand the variation of the ratio of the decarboxylation rate of glutamate compared with that of XI of rat brain with age has been claimed as an indication of the occurrence of different enzymes (25). The occurrence of different forms of glutamate decarboxylase in various tissues (26) complicates the solution of this problem. Bacterial glutamate decarboxylase has been found inactive on XI, XII and XVIII while it is very active on homocysteinesulfinic acid (XVII). The inactive compounds however exhibit a strong competitive inhibition on the decarboxylation of XVII (24). This finding cannot be used to understand the mechanism of the animal decarboxylase, because taurine is not an important metabolite in the bacterial kingdom and bacteria could have developed enzymes with different finalities. A preferential activity toward XVII compared with XI is also exhibited by the beef liver glutamate dehydrogenase (27). XVII seems therefore a better simulator of glutamic acid than the lower homolog (XI). Another sulfur containing simulator of glutamate for the dehydrogenase is carboxymethylcysteine (XIX). While the product of the oxidation of XVII is the



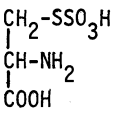
Cysteine X



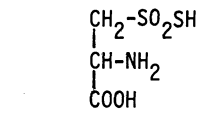
Cysteinesulfinic acid XI



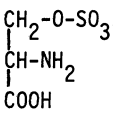
Cysteic acid XII



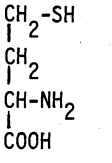
Cysteine-S-sulfonate XIII



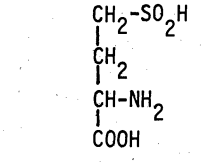
Alanine thiosulfonate XIV



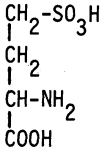
Serine-O-sulfate XV



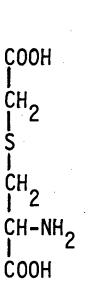
Homocysteine XVI



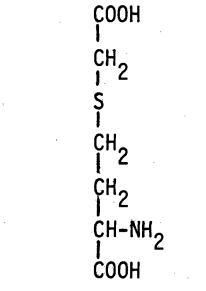
Homocysteine sulfinic acid XVII



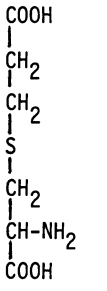
Homocysteic acid XVIII



Carboxymethyl cysteine XIX



Carboxymethyl homocysteine XX

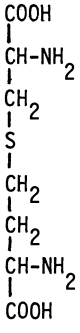


Carboxyethyl cysteine XXI

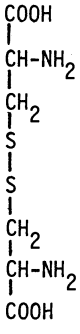
respective sulfinyl keto acid, in the case of XIX the occurrence of a sulfur atom in β promotes further reactions and thioglycolic acid has been detected among the final products (28). Snake venom L-aminoacid oxidase is inactive with the L forms of aspartic and glutamic acid while it uses aminoadipic and aminopimelic acids as substrates. It is in-

teresting to point out here that the sulfur containing dicarboxylic acids XIX, XX and XXI are good substrates for this enzyme thus mimicking more the higher homologues of glutamic acid than glutamic acid itself (29).

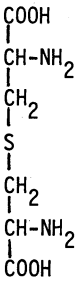
Cystathionases is an enzyme using as substrate not only cystathionine but also a number of compounds structurally related to cystathionine like cystine (XXIII), lanthionine (XXIV) and djenkolic acid (XXV) (30-32). It also cleaves homoserine (31), thus acting on a sulfur free compound. This appears therefore as a reverse ambiguity, i.e., an enzyme involved in the cleavage of a number of sulfur compounds acting on a sulfur free substrate. However the Km for homoserine is so low compared with that for the other substrates that this appears the physiological substrate. A point to stress is that this enzyme, although of broad specificity, is very poorly active on homocysteine, which is very similar to homoserine, while it cleaves cysteine into pyruvate, NH_3 and H_2S (33).This intriguing finding was explained when it was observed that the substrate for this reaction is not cysteine but cystine present in the samples of cysteine. Cystine is



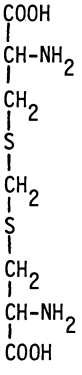
Cystathionine XXII



Cystine XXIII

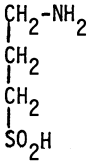


Lanthionine XXIV

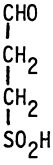


Djenkolic acid XXV

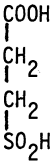
cleaved by cystathionase into ammonia, pyruvate and thiocysteine, the latter being than reconverted into cystine by the excess of cysteine (34, 35). It is evident that sulfhydryl and hydroxyl groups are not enzymically similar. It is very interesting to remark at this point how sulfur in the form of sulfhydryl groups is subject to variable degree of specificity. In fact sulfhydryls are non-specifically oxidized to the disulfide level by a number of metal ions, by metal containing proteins and by other catalysts, provided they have the appropriate redox potential. In the contrary the oxidation of cysteine and cysteamine to the respective sulfinates requires two distinct oxygenases, although the reaction is formally very similar (36, 37). On the other hand the oxidation of dihydrolipoic acid requires also a specific dehydrogenase.



Homohypotaurine XXVI



Semialdehyde XXVII



Succinic monosulfinic analog XXVIII

Cystathionine β-synthetase has a broad specificity with respect to the sulfur compound to be added to the serine moiety and also with respect to serine. Serine can be replaced by O-acetylserine, cyanoalanine, chloroalanine and even by cysteine, while cysteamine, mercaptoethanol, thioglycolic acid, sulfide, cysteine and other thiol compounds could replace homocysteine (38, 39). This β-replacement is so fast that it has been used to prepare a number of cysteine-S-derivatives by simply passing the appropriate reagents through a column of a bacterial synthetase bound to a rigid support (40). The table reported in the next page lists some of the substrates used by this enzyme.

The products of the decarboxylation of homocysteine-sulfinic acid and homocysteic acid are respectively homohypotaurine (XXVI) and homotaurine.

CYSTATHIONINE-β-SYNTHASE
(Serine sulfhydrase)
EC 4.2.1.22

Main reaction: Serine + Homocysteine $\longrightarrow$ Cystathionine

Serine can be replaced by:	Homocysteine can be replaced by:	S-containing products detected by using appropriate substrates:
O-acetylserine	H_2S	Cysteine
β-Cl-alanine	Mercaptoethanol	OH-ethyl cysteine
β-CN-alanine	Cysteamine	Aminoethyl cysteine
Cysteine	Cysteine	Lanthionine
Various alkyl-S-cysteines	Methyl-mercaptan Various mercaptans	Methylcysteine S-alkyl cysteins

General reaction: β-X-Ala + HS-R $\longrightarrow$ Cys-R + XH

where X = OH, SH, CN, CH_3COO, S-R

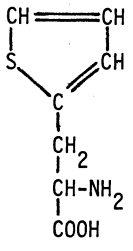
Proposed new name: β-X-Ala sulfurtransferase

The biological significance of these two compounds is not yet known nor whether they are produced from homocysteine in the animal body. However, their similarity to GABA has stimulated a number of investigations on the relationship of these compounds at the enzymatic level. The system GABA transaminase succinic semialdehyde dehydrogenase, which converts GABA into succinic acid has been found operative also in converting XXVI into the monosulfinic analog of succinic acid XXVIII with a mechanism identical to that known for GABA (41). The physiological role of this reaction is unknown at present.

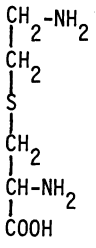
Du Vigneaud was among the first to investigate the biological and biochemical behaviour of an aminoacid having part of the carbon chain replaced by sulfur. Thienylalanine was used as a sulfur analog of phenylalanine (XXIX) and it was found that XXIX was an inhibitor of rat growth by competing with phenylalanine (42). Aminoethylcysteine, the sulfur analog of lysine (thialysine XXX) was prepared a number of years ago (43) and more recently was the selenium analog XXXI (44). Thialysine is so strictly related to lysine that aminoethylation of cysteine residues in proteins is frequently used in order to increase the points of cleavage of proteins by trypsin (45). In our laboratory thialysine was prepared with the aim to test its possible role as an intermediate between cysteine and ethanolamine in the biosynthesis of cysteamine. We did not obtain clear cut results on this point, however we were able to obtain evidence on the ability of the living rat to produce cystamine and taurine from thialysine (46). More recently it has been demonstrated that certain tissues, in particular kidney extracts, are able to decarboxylate lanthionine, producing thialysine which in its turn is metabolized to taurine (47). Whether this could be taken as a possible alternative for the production of taurine through the cysteamine patway by those tissues without the cysteinesulfinic pathway, is now under investigation.

The similarity of thialysine with lysine prompted a series of investigations aimed at establishing how far the delicate mechanism of protein biosynthesis could distinguish the two compounds and whether thialysine could function as an antagonist of lysine in animals and bacteria. In recent studies it was found that thialysine and also selenalysine are used by the protein synthesizing system of E. coli, that of rat liver and that prepared from rabbit reticulocites (48, 49). Both the lysine analogs are in fact activated by aminoacyl-tRNA synthetase, are transferred to $tRNA^{lys}$ and are incorporated into polypeptides. Furthermore both the analogs act as competitive inhibitors of lysine in all these reactions. Thiazolidine carboxylic acid (thiaproline XXXII) and selenazolidine carboxylic acid (selenaproline XXXIII) which are respectively the S containing and the Se containing analogs of proline, behave similarly (50, 51). These results indicate that even the very precise system

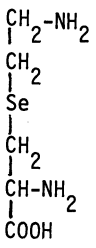
of protein biosynthesis is unable to distinguish between an S or Se atom and a methylene carbon in the substrate. The ability of S and Se substituted amino acids to mimick the carbon containing analogs suggested an attempt to use this kind of competition in those cases where protein biosynthesis is very active. Thus it was found that thialysine inhibits the replication of the Mengovirus in mammalian cell cultures, although the growth of the Vescicular Stomatitis Virus was unaffected (52). An attempt to use selenalysine as an antagonist of lysine in order to depress the growth of cancerous cells, however, has not given promising results up to now.



Thienylalanine XXIX

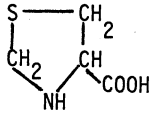


Thialysine XXX

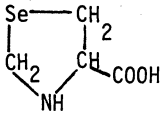


Selenalysine XXXI

The competition between sulfur and selenium in the biological field is a well known phenomenon and sulfur amino acids with sulfur replaced by selenium have been occasionally detected (53). In our laboratory we have found that rhodanese is able to use selenosulfate in the place of thiosulfate producing selenocyanide as the final product (54). As an inter-



Thiaproline XXXII



Selenaproline XXXIII

mediate the seleno-containing enzyme is formed which is the analog of the sulfur charged enzyme when thiosulfate is the substrate. In the case of the selenoenzyme the typical absorption at 330 nm detected in the active site as the result of the persulfide formation (55) is shifted to 375 nm and is even more pronounced, indicating the formation of a perselenosulfide group (54). Whether to the selenorhodanese form could be ascribed the role of the intermediate for the transfer of selenium from inorganic to organic compounds of biological interest in a stimulating approach worth further investigation.

As a conclusion of this short survey on the enzymatic behaviour of sulfur compounds it appears that sulfur and selenium are able, to a certain extent, to replace each other and both to replace carbon, being poorly distinguished by a number of enzymes which, otherwise, exhibit definite specificity towards other properties of the substrate. Another relevant point to remark, which could be the consequence of the first conclusion, is the observation that the large variety of organic and inorganic sulfur compounds occurring in living organisms are produced by a relatively low number of specific enzymes. Many sulfur compounds arise in fact by the action of enzymes used also for reactions not involving sulfur. Since it is frequent that the enzymatic product of a compound containing sulfur is more labile than the product of the original substrate, the product may undergo further non enzymatic changes to other compounds. This fact helps explain why a limited number of specific enzymes are able to produce such large number of sulfur compounds as those found in living organisms.

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