Tetrahedron Letters,Vol.23,No.31,pp 3207-3208,1982 0040-4039/82/313207-02$03.00/0
Printed in Great Britain

ISOLATION AND STRUCTURE OF A NEW SULPHUR-CONTAINING AMINOACID FROM SEA URCHIN EGGS[1]

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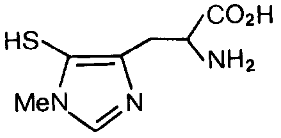
Abstract : Unfertilized sea urchin eggs (*Paracentrotus lividus*) contain, in addition to glutathione, a new low molecular weight thiol, 1-methyl-5-thiol-L-histidine (1) which was isolated and characterized as the disulphide 2 by spectral and chemical evidence.

In 1931 Rapkine[2] first reported experimental evidence suggesting that a cyclic redox process involving SH groups takes place during cell division and growth of the sea urchin egg. Since then this view has been widely accepted as a regulatory mechanism of cell cleavage and differentiation, receiving additional support from subsequent studies[3]. In these, however, attention was mainly focused on the cellular changes of glutathione, regarded as the chief non-protein thiol.

We have now evidence that unfertilized eggs of *Paracentrotus lividus* contain, in addition to glutathione, large amounts of a hitherto unknown thiol aminoacid, identified as 1-methyl-5-thiol-L-histidine (1)



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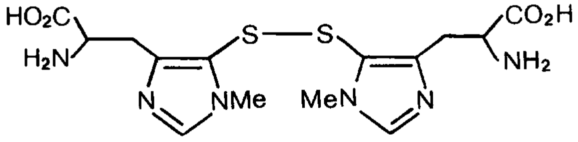

2

As revealed in preliminary experiments, both 1 and the corresponding disulphide 2 were present in aqueous extracts of sea urchin eggs. Therefore an isolation procedure was developed, which involves in the early stage the conversion of the thiol to the more stable disulphide by aerial oxidation of the lipid-free aqueous extracts in the presence of a few crystals of I_2 for 24 h. The mixture is then passed through a column of Dowex 50W-X2 (15x1.1cm) on which 2 is strongly absorbed. After several washingswith water, 0.1N,0.5N, and 1N HCl, the compound is eluted with 2N HCl and is further purified by column chromatography

Sephadex LH-20, with 80% aq EtOH as the eluent (average yield: 0.4mg/gwet eggs). The disulphide, $C_{14}H_{20}N_6O_4S_2$, white prisms[4] from aq EtOH darkening at 202-205°, $]_D^{20}$ = +76°(c=1.2,0.1N HCl) shows characteristic UV spectrum with a broad λ max(0.1N l) at 257nm (ε =6750). On paper electrophoresis at pH 6.5 it moves towards the thode as a single spot detectable with ninhydrin (greyish-purple) but not with uly reagent. Reduction of the disulphide with KBH_4 in aq EtOH gives the corresnding thiol (Ellman's reagent and UV evidence) which on standing in air spontaously or, more smoothly in the presence of a catalytic amount of I_2, is reoxized to the parent disulphide. The ^1H nmr spectrum (D_2O) of 2 shows three signals the sp^3 region at δ3.44(2Hx2,d,J=7.5Hz, β-CH_2),3.94 (3Hx2,s,N-Me), 4.29(1Hx2,t, 7.5Hz,α-CH)and a low field singlet(1Hx2)at δ8.95, typical of protons at C-2 of idazole rings[5]. Additional evidence for the symmetrical dimeric structure 2 is ovided by the ^{13}C nmr (D_2O) spectrum showing only 7 signals attributable to the H_2-CH(NH_2)COOH groupings(δ25.5,54.5 and 173.3) and to the N-methylated imidazole ngs(δ34.0,130.0,134.8, and 141.1).

The position of the N-Me group and the stereochemistry of the chiral centre llows from desulphuration of 2 with Raney Nickel in aq EtOH (30min under reflux) fording 1-methyl-L-histidine, $[\alpha]_D^{20}$=+6.8(c=0.85,in HCl), identified by comparison th an authentic sample.

In contrast with the wide distribution of 2-thiolhistidine metabolites,e.g. gothioneine, in a variety of organisms[6], 5-thiolhistidine derivatives have never en found in nature as free aminoacids. It is noteworthy, however, that the 5-iolhistidine moiety is found incorporated into the structure of adenochrome, a ique iron(III)-binding peptide from *Octopus vulgaris*.[7]

Studies on the biosynthesis and the precise role of 1 and 2 in the development sea urchin eggs are now in progress.

References and Notes

Supported by a grant from "Progetto Finalizzato per la Chimica Fine e Secondaria" CNR (Rome)

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The compound gave satisfactory elemental analyses.

In D_2O solution the C-5 proton of 2-thiolhistidine resonates at 7.02

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(Received in UK 13 May 1982)