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HISTONE H1 IS A SUBSTRATE FOR LYSYL OXIDASE AND CONTAINS ENDOGENOUS SODIUM BOROTRITIDE-REDUCIBLE RESIDUES

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Summary: Incubation of purified bovine aortic lysyl oxidase with rat liver or calf thymus H1 histone results in the catalytic formation of hydrogen peroxide, indicating the substrate potential of H1 for this connective tissue enzyme. Sodium borotritide-reducible residues consistent with aminoadipic semialdehyde and the lysinonorleucine crosslinkage were generated in H1 by incubation with lysyl oxidase. H1 histone also contains endogenous reducible functions including an unidentitified prominent tritiated peak eluting near tyrosine as well as other lesser peaks, one of which is consistent with lysinonorleucine.

The maturation of elastin and collagen fibers requires the oxidative deamination of peptidyl lysine in these proteins to peptidyl α-aminoadipic-δ-semialdehyde by lysyl oxidase (1). Spontaneous condensations of aldehyde residues then yield a variety of covalent crosslinkages, including the aldol condensation product, Schiff base, and higher-ordered crosslinkage products in these proteins (2). Such lysine-derived crosslinkages are also present in the cell envelope of Escherichia coli (3), in egg shell membrane protein (4), and connectin (5), although there appears to be no other clear example of such products appearing in other proteins.

In assessing the substrate specificity of highly purified lysyl oxidase, we have considered the possibility that the lysine-rich histone proteins might also be acted upon by this enzyme. Histones are the major protein components of chromatin. Histone H2a, H2b, H3 and H4 constitute the core proteins of the nucleosome around which the DNA double helix is wound. Histone H1, the most lysine-rich of the histone molecules, binds to the outside of the nucleosome core particle and also interacts with the inter-nucleosomal linker DNA (6). Modification of the interactions between the cationic histones and the anionic

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nucleic acid may be involved in regulating gene expression (6). Indeed, histones are subject to a variety of charge-altering post-translational modifications including acetylation, phosphorylation, ADP-ribosylation, and N-methylation (6,7). In the present study, we report that histone H1 is a substrate for purified lysyl oxidase *in vitro* and that histone H1 as isolated contains sodium borotritide-reducible residues consistent with the presence of carbonyl functions in this protein.

MATERIALS AND METHODS

Histone H1 was purified from the livers of 200 gram male Sprague Dawley rats by a modification of the method of Johns (8), substituting ethanol for acetone in the final washes of the acid precipitate. Calf thymus histone H1, isolated by the method of Bohm et al. (9), was a product of Boehringer Mannheim Biochemicals. Sodium dodecyl sulfate-15% polyacrylamide gel electrophoresis (10) of each H1 preparation indicated the presence of H1 variants between 20- and 25,000 daltons. Amino acid analyses of each preparation were consistent with published compositions of H1 histone (6). Sodium borotritide (350 mCi/mmole) was a product of ICN Corporation. Insoluble elastin was isolated from bovine *ligamentum nuchae* by repeated autoclaving, as described (11).

Enzyme Purification and Assay Lysyl oxidase was purified from bovine aorta by a modification (12) of the previously described procedure (13) by substituting chromatography through Cibacron Blue-Sepharose for the DEAE cellulose chromatography step. This modification yields an apparently homogeneous preparation of the four lysyl oxidase enzyme variants. Enzyme assays employed the peroxidase-coupled continuous spectrofluorometric method (14).

Amino Acid Analysis Amino acid analyses were performed using a Beckman 119C amino acid analyzer on protein samples hydrolyzed at 105° for 22 hours in 6 N HCl in tubes sealed *in vacuo* after nitrogen purging. Sodium borotritide-reducible protein residues were separately determined from 6 N HCl or 2 N NaOH hydrolysates of reduced protein samples using a Technicon amino acid analyzer equipped with a stream-splitting device, thus permitting the monitoring of the column effluent for radioactivity and for ninhydrin reactivity (15).

Reduction with Sodium Borotritide Histone H1 (1 mg) was dissolved in 0.5 ml of 0.05 M sodium borate, pH 8.2, and reduced with 1.5 mCi of sodium borotritide (specific activity 157 mCi/mmole) at 25° for 1 hr. Reduction was stopped and excess borotritide decomposed by addition of 50% acetic acid to pH 3. Samples were dried under a stream of nitrogen and rehydrated repeatedly. Reduced histones were desalted by passage through a 1 X 20 cm column of Bio Gel P-2 eluting with 1 % acetic acid and then lyophilized in preparation for acid or base hydrolysis. Insoluble elastin was reduced under identical conditions but was freed of salts and excess borotritide by washing on a sintered glass funnel with water and then dried with ethanol and ether.

RESULTS

Incubation of Histone with Lysyl Oxidase Preparations of H1 histone from rat liver or calf thymus were incubated in the peroxidase-coupled assay to test their potential as substrates for lysyl oxidase. Each of the histone

samples were readily oxidized by lysyl oxidase, indicated by the catalytic release of hydrogen peroxide (Figure 1). The oxidation of the 1,5-diaminopentane and of the histone substrates was largely inhibited by 0.2 mM β-aminopropionitrile, an irreversible and selective inhibitor of lysyl oxidase (12).

Demonstration of Oxidation Products in Histones Samples of rat liver or calf thymus H1 were incubated in the presence or absence of lysyl oxidase and were then reduced with sodium borotritide, base hydrolyzed, and analyzed by split-stream amino acid analysis. Elution positions of tritiated peaks deriving from histone H1 were compared to those of the reduced derivatives of the aldehyde and crosslinkage products of base-hydrolized ligament elastin, chromatographed under the same conditions. As shown (Figure 2), there are tritiated peaks in each of the H1 samples which had been incubated in the absence of lysyl oxidase. These endogenous, borotritide-reducible residues include a prominent peak eluting near tyrosine and lesser peaks eluting near the positions of glutamate, ammonia and lysine, respectively. Incubation of the histones with lysyl oxidase generates a prominent new peak eluting at the

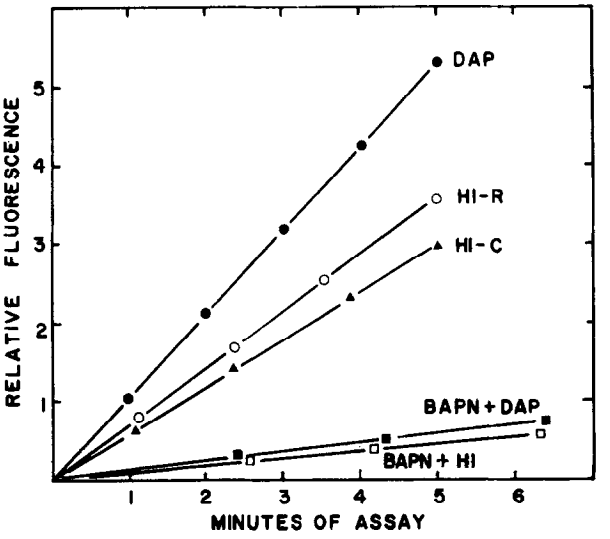

Figure 1. Oxidation of Histone H1 by Lysyl Oxidase. Rat liver (H1-R) or calf thymus (H1-C) H1 histone was present at 0.5 mg in 2 ml of the peroxidase-coupled assay for lysyl oxidase-dependent hydrogen peroxide formation (14). Assays were initiated by the addition of enzyme. DAP, 0.01 M 1,5-diaminopentane; BAPN, 0.2 mM β-aminopropionitrile present in the assay.

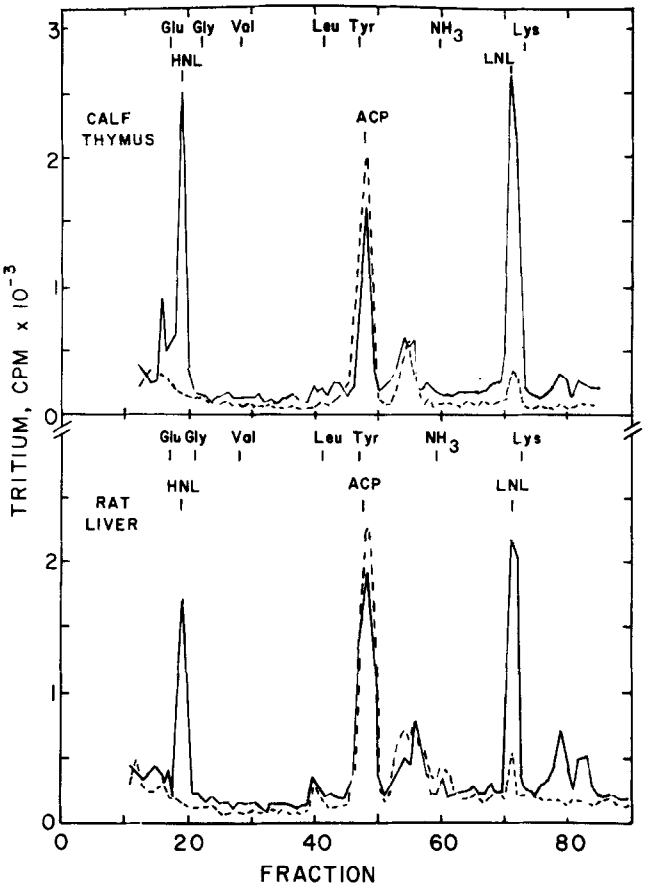

Figure 2. Amino Acid Analyses of Sodium Borotritide-Reducible Residues in Calf Thymus (top) or Rat Liver (bottom) H1. (——), lysyl oxidase-induced profile; (- - -), endogenous profile. HNL, hydroxynorleucine; ACP, aldol condensation product; LNL, lysinonorleucine. Positions of amino acid standards are shown.

position of hydroxynorleucine and markedly increases the magnitude of the endogenous peak eluting at lysinonorleucine. Hydroxynorleucine is the reduced derivative of aminoadipic semialdehyde, the initial product of lysyl oxidase action, while lysinonorleucine is the reduced derivative of the Schiff base crosslinkage between aminoadipic semialdehyde and an unmodified lysine residue (2). Thus, it appears that aminoadipic semialdehyde and the Schiff-base crosslinkage are generated in both samples of H1 by incubation with lysyl oxidase. There also are other lysyl oxidase-dependent peaks eluting after lysine. A separate analysis of a reaction mixture containing only lysyl oxidase established that none of the peaks shown in Figure 2 can be accounted for as deriving from the enzyme, itself. Except for the marked increase in the

peak at lysinonorleucine, none of the endogenous peaks are markedly affected by the incubation with lysyl oxidase, including the prominent endogenous peak near tyrosine. It is noteworthy that the lysyl oxidase-induced and endogenous profiles of tritiated peaks in calf thymus H1 are essentially the same as those in the rat liver preparation, suggesting that these results may be applicable to H1 histone of various sources.

The distribution of tritiated peaks as well as the total tritium content within each of the endogenous peaks are essentially the same after hydrolysis of the borotritide-reduced samples of H1 by 6 N HCl or by 2 N NaOH. The acid stability of the endogenous peak at tyrosine argues against its identity as the reduced aldol condensation product, since this crosslinkage is largely destroyed by acid hydrolysis (15). However, the acid- and base-stability of the lesser peak at the position of lysinonorleucine is consistent with its identification as this reduced Schiff base crosslinkage (15). Further, incubation of rat liver H1 in 0.1 N NaOH for one hour at 37° prior to reduction with borotritide did not significantly alter the resulting profile of endogenous peaks from those seen in Figure 2. Thus, the endogenous peaks likely do not originate from a reducible ester linkage between a histone glutamyl residue and an ADP-ribose group, consistent with the lability of this derivative of H1 to hydrolysis by 0.1 N NaOH at 37° for 3 minutes (16). Other known modifications, including N-methylation, N-acetylation, or phosphorylation of H1 (6) do not appear to be likely sources of incorporation of acid- or base-stable tritum from sodium borotritide reduction.

DISCUSSION

The present results reveal that histone H1 is readily oxidized by lysyl oxidase in <u>in vitro</u> assays, evidenced both by the enzyme-dependent and BAPN-inhibitable release of hydrogen peroxide and by the increase of sodium borotritide-reducible functions in the incubated histone samples. The susceptibility of histone to this enzyme may relate both to its abundance of lysine residues as well as its net cationic charge. Thus, the activity of lysyl oxidase toward an insoluble elastin substrate is dramatically increased

if elastin is rendered cationic by bound cationic amphiphilic ligands (17). Although a systematic review of potential protein substrates of lysyl oxidase is not available, we have noted that native or denatured forms of bovine serum albumin or of catalase are not oxidized by this enzyme. It also is of particular interest that elastin (18) and histone H1 (6) each contain -lys-ala-ala-lys- and -lys-ala-ala-ala-lys- sequences. The lysyl oxidase-catalyzed deamination of one lysine in the former and both lysines in the latter sequence can yield, respectively, the lysinonorleucine and aldol condensation product crosslinkages in elastin (18). Thus, these sequence similarities may also underlie the generation of aldehyde and crosslinkage products in histone by lysyl oxidase.

The total endogenous borotritide-reducible functions were estimated to constitute 0.5 mole per mole of H1, assuming that each mole of reducible residue incorporates one gram atom of tritium. The properties of the lesser endogenous peak eluting near lysine is consistent with those of lysinonorleucine, raising the possibility that H1 may be susceptible to oxidative deamination in vivo. The other more prominent endogenous peaks are subjects of continued investigation. Thus, it appears that derivatives of histone residues with carbonyl reactivity, indicated by reducibility by sodium borotritide, may represent as yet unidentified products of post-translational modification(s) of H1.

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REFERENCES

1. Siegel, R.C. (1979) Int. Rev. Conn. Tiss. Res. 8, 73-118.
2. Gallop, P.M. and Paz, M.A. (1975) Physiol. Rev. 55, 418-487.
3. Merelman, D. and Siegel, R.C. (1979) J. Biol. Chem. 254, 571-574.
4. Harris, E., Leach, R., and Blount, J. (1980) Science 208, 55-56.
5. Fujii, K. Kimura, S., and Maruyama, K. (1978) Biochem. Biophys. Res. Commun. 81, 1248-1253.
6. Isenberg, I. (1979) Annu. Rev. Biochem. 48, 159-191.
7. Kornberg, R.D. (1977) Annu. Rev. Biochem 44, 725-774.
8. Johns., E. (1964) Biochem. J. 92, 55-59.
9. Bohm, E., Strickland, M., Thwaits, B., van der Westhuzen, D., and van Holt, C. (1973) FEBS Lett. 34, 217-221.

10. Laemmli, U. (1970) Nature 227, 680-685.
11. Partridge, S.M., Davis, H.F., and Adair, G.S. (1955) Biochem. J. 61, 11-14.
12. Tang,S.-S., Trackman, P.C., and Kagan, H.M. (1983) J. Biol. Chem. 258, 4331-4338.
13. Kagan, H.M., Sullivan, K.A., Olsson, T.A., and Cronlund, A.L. (1979) Biochem. J. 177, 203-214.
14. Trackman,P.C., Zoski, C.G., and Kagan, H.M. (1981) Anal. Biochem. 113, 336-342.
15. Lent,R.W., Smith, B., Salcedo, L.L., Faris, B. and Franzblau, C. (1969) Biochemistry 8, 2837-2845.
16. Riquelme, P.T., Burzio, L.O., and Koide, S.S. (1979) J. Biol. Chem. 254, 3018-3028.
17. Kagan, H.M., Tseng, L., and Simpson, D.E. (1981) J. Biol. Chem. 256, 5417-5421.
18. Foster,J.A., Rubin, L., Kagan, H.M., Franzblau, C., Bruenger, E., and Sandberg, L.B. (1974) J. Biol. Chem. 249, 6191-6196.