PEPTIDE INHIBITORS OF LACTIC DEHYDROGENASE (LDH) I: SPECIFIC INHIBITION OF LDH-M_4 AND LDH-H_4 BY INHIBITOR PEPTIDES I AND II.

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We have recently isolated and characterized two peptides (I and II) from human urine which inhibit both human and rabbit muscle lactic dehydrogenase (Schoenenberger and Wacker, 1965). I has a molecular weight of 1500 and that of II is about 2000 (Wacker and Schoenenberger, unpublished).

When the effect of these peptides was measured using crystalline rabbit muscle LDH (Beisenherz et al., 1953) complete inhibition could not be obtained with either peptide alone. While Peptide I maximally inhibited rabbit muscle LDH activity 85%, II inhibited only 15%. A mixture of the two peptides, however, abolished LDH activity completely. The LDH of rabbit muscle is known to consist of approximately

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85% M subunits and 15% H subunits (Kaplan, 1964). This suggested to us that each of these peptides specifically affected the activity of only one of these subunits. Accordingly the inhibition caused by Peptides I and II was measured using the homogenous isozymes of LDH-M_4 of rabbit muscle and LDH-H_4 of pig heart (Table 1). Peptide I inhibits LDH-M_4 but it is inactive toward LDH-H_4. Conversely II inactivates LDH-H_4 but does not affect LDH-M_4.

Table 1. Inhibition of LDH isozymes by Inhibitor Peptides I and II.

Isozyme	Inhibitor Peptide I Vi/Vc	Inhibitor Peptide II Vi/Vc
LDH-M_4	0.65	0.98
LDH-H_4	1.00	0.66

Assays were carried out at 25° in a Beckman DU spectrophotometer equipped with a Gilford automatic cell changer and recording attachment. The final volume of the assay mixture was 3 ml. and the controls contained 5.2×10^{-2} M sodium pyrophosphate, pH 8.8, 2.3×10^{-3} M lactic acid and 5.2×10^{-3} M DPN, (Wacker et al., 1956), inhibited assay mixtures also contained approximately 10^{-9} M Inhibitor Peptide I or II. Reactions were started by addition of the LDH-M_4 or LDH-H_4 to yield a final concentration of 3.6×10^{-10} M. LDH-M_4 and LDH-H_4 were obtained from Boehringer, Mannheim, W. Germany.

Table 2. Inhibition of LDH-M_4 by Inhibitor Peptide I.

Reaction	Vi/Vc
Lactate to pyruvate	0.65
Pyruvate to lactate	0.65

Conditions for measurements of the reaction from lactate to pyruvate were the same as in Table 1. The reaction from pyruvate to lactate was measured by the method of Henry et al. (1960). Conditions were otherwise the same as in Table 1.

The effect of Peptide I on the kinetics of LDH-M_4 has been studied in some detail. It inhibits the LDH-M_4

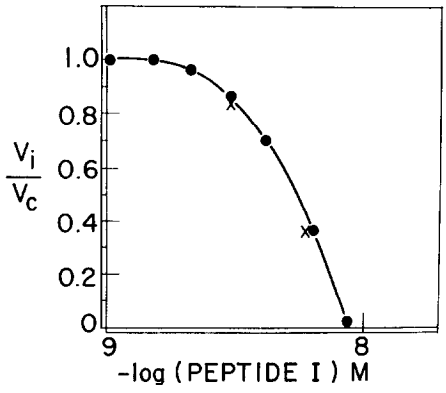

Figure 1. Inhibition of LDH-M_4 by Inhibitor Peptide I. Enzyme and inhibitor were preincubated for 50 minutes in 0.1 M K_2HPO_4, pH 7.0. 0.1 ml of the preincubated mixture was added to 2.9 ml of a reaction mixture containing 5.2×10^{-3} M DPN, 2.3×10^{-3} M lactic acid and 5.2×10^{-2} M sodium pyrophosphate, pH 8.8, and the reaction measured as described in Table 1. The fractional activity, Vi/Vc is plotted against the logarithm of the peptide concentration in the final reaction mixture. The X's indicate the effect of dilution. See text.

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catalyzed oxidation of lactate and reduction of pyruvate to exactly the same extent (Table 2) and as a function of the concentration of Peptide I (Fig. 1). Activity is completely abolished at a concentration of 9×10^{-9} M Peptide I. Dilution reversed the inhibition completely; the partial activity was measured in the presence of Peptide I at a concentration of 6×10^{-9} M (the lower X, Fig. 1) after 3 minutes an aliquot was removed and added to a reaction mixture containing DPN, lactate and enzyme in amounts such that the final concentration of these reactants were unchanged while the peptide concentration was reduced to 3×10^{-9} M. The fractional activity which is observed is that which would be predicted for the concentration of inhibitor after dilution

(the upper X, Fig. 1). The inhibition can also be reversed by dialysis of the preincubation mixture against 0.1 M K HPO_4, pH 7.0 for 24 hours.

In these experiments the concentration of enzyme in the reaction mixture was 1.5×10^{-10} M, close to that of Peptide I. With such an effective inhibitor the inhibition would be expected to depend on enzyme concentration (Straus and Goldstein, 1943; Goldstein, 1944; and Hoch et al., 1960) since the concentration of inhibitor and enzyme differ only by an order of magnitude. This expectation is borne out since an increase in the concentration of LDH-M_4 reduces the inhibition (Fig. 2).

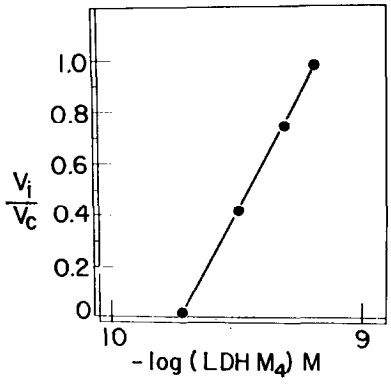

Figure 2. Effect of Enzyme Concentration in the Inhibition of LDH-M_4 by Inhibitor Peptide I.
Final concentration of Inhibitor Peptide I = 6×10^{-9} M. Enzyme and inhibitor were preincubated for 1 hr. in 0.1 M K_2 HPO_4, pH 7.0. 0.1 ml of the preincubated mixture was added to 2.9 ml of the same reaction mixture as in Fig. 1 and the activity measured.

The inhibition of LDH-M_4 by Peptide I appears to be competitive both with DPN and lactate as indicated by preliminary studies. The detailed kinetics of both inhibitors are being assessed currently.

The inhibitory properties of the two peptides would be expected to be related to their composition and structure (Schoenenberger and Wacker, 1965). Both peptides I and II contain lysine and their activity is abolished by trypsin digestion. Peptide II contains tyrosine and its function is therefore also destroyed by chymotrypsin (Table 3). In addition both carboxypeptidase A and Nagarse inactivate the peptides.

Table 3. Effect of Proteolytic Digestion on the Activity of Inhibitor Peptides I and II.

Proteolytic Enzyme	Inhibitor Peptide	Time of Digestion Vi/Vc 0	Vi/Vc 60 min.
Trypsin	I	0.64	1.0
	II	0.66	1.0
Nagarse	I	0.62	1.0
	II	0.66	1.0
Carboxypeptidase A	I	0.63	1.0
	II	0.60	1.0
Chymotrypsin	I	0.62	0.61
	II	0.64	1.0

1 mgm of each proteolytic enzyme was added to 10 ml of an 8 x 10^{-9} M solution of inhibitor. Aliquots were removed at 0 and 60 min. boiled to destroy the proteolytic enzyme and then assayed for inhibitory activity as described in Table 1.

These studies demonstrate for the first time the unsuspected existence of naturally occurring peptide inhibitors of a pyridine-nucleotide dependent dehydrogenase such as lactic dehydrogenase. The remarkable specificity of these peptides, each of which inhibits only one isozyme of LDH is striking. Control of metabolic processes by several inhibitory mechanisms, including repression and induction of enzyme synthesis (Jacob and Monod, 1961), feedback inhibition of

synthetic pathways (Umbarger, 1956; Yates and Pardee, 1956; Gerhart and Schachman, 1965) and substrate inhibition (Kaplan and Goodfriend, 1964) are now well established. The present data suggest that inhibition of enzymes by naturally occurring peptides may represent yet another type of control mechanism. The specificity of the inhibition, the low concentration of peptide required to effect the inhibition, and the apparent competition with both substrate and coenzyme all appear to support such an hypothesis. More importantly, preliminary studies indicate that similar peptide inhibitors are present not only in urine but also in both human cardiac and skeletal muscle (Wacker and Schoenenberger, unpublished) sites where their inhibitory activity could directly mediate the energy requirements of the organism.

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