SC 11032

Effect of thyroid hormone on the bacteriolytic action of (muramidase lysozyme) *in vitro*

The inhibition *in vivo* of rat-kidney muramidase (*N*-acetylmuramide glucanohydrolase, EC 3.2.1.17, formerly known as lysozyme) by thyroid hormone was reported from this laboratory[1,2]. These results strongly indicated a direct interaction between the hormone and the enzyme. Subsequently L-thyroxine and triiodo-L-thyronine were shown to be inhibitors of muramidase activity *in vitro*, while inorganic iodide, DL-thyronine, and diiodo-L-tyrosine were inactive as inhibitors[3]. It is now possible to report some of the salient features of the interaction *in vitro* of

TABLE I

INHIBITION OF MURAMIDASE ACTIVITY BY THYROID HORMONES

All systems are buffered with 0.066 M phosphate buffer to the pH indicated.

Enzyme	*Hormone*	*pH of pre-incubation and assay systems*	*Average relative lytic activity*
I. 0.09 μM egg-white muramidase	None	7.1[a]	100 (2)[b]
	0.9 μM L-thyroxine	7.1[a]	75 (2)
	1.8 μM L-thyroxine	7.1[a]	53 (2)
	2.7 μM L-thyroxine	7.1[a]	28 (2)
	None	7.45[a]	100 (1)
	0.9 μM L-thyroxine	7.45[a]	70 (1)
	1.8 μM L-thyroxine	7.45[a]	57 (1)
	2.7 μM L-thyroxine	7.45[a]	27 (1)
	None	7.6[c]	100 (2)
	6 μM L-thyroxine	7.6[c]	64 (2)
	None	7.6[d]	100 (2)
	6 μM L-thyroxine	7.6[d]	44 (2)
	None	7.6[a]	100 (2)
	1 μM triiodo-L-thyronine	7.6[a]	70 (2)
	None	7.6[c]	100 (2)
	6 μM triiodo-L-thyronine	7.6[c]	79 (2)
	None	7.6[d]	100 (2)
	6 μM triiodo-L-thyronine	7.6[d]	62 (2)
	None	7.6[c]	100 (2)
	6 μM DL-thyronine	7.6[c]	99 (2)
	None	7.6[d]	100 (2)
	6 μM DL-thyronine	7.6[d]	97 (2)
II.	None	7.6[e]	100 (2)
	6 μM L-thyroxine	7.6[e]	100 (2)
III. 5.5 μg/ml rat-kidney muramidase[f]	None	7.6[g]	100 (5)
	1 μM L-thyroxine	7.6[g]	90 (5)
	2 μM L-thyroxine	7.6[g]	82 (5)
	3 μM L-thyroxine	7.6[g]	59 (5)

[a] Preincubation of enzyme and hormone (or buffer) for 15 min at 37°.
[b] Number of experiments.
[c] Preincubation of enzyme and hormone (or buffer) for 15 min at 25°.
[d] Preincubation of enzyme and hormone (or buffer) for 50 min at 25°.
[e] Thyroxine was added directly to the cell suspension for 5 min before enzyme was added at 25°.
[f] Acetate form of the enzyme; other forms show greater inhibition with thyroxine.
[g] Preincubation of enzyme and hormone (or buffer) for 10 min at 25°.

thyroid hormones and crystalline egg white or homogeneous rat-kidney muramidase[3*].

In all cases, the hormone (1–6 μM) and enzyme (about 0.1 μM) are preincubated either at room temperature or at 37° from 5–50 min before combination with the substrate, *Micrococcus lysodeikticus*[4,5].

The Zeiss PMQII spectrophometer with a model-43 Varicord Photovolt recorder was used to record rate of change of absorbancy at 645 mμ at 25°. Reciprocals of absorbancy are plotted against time in seconds according to the second order reaction, $dx/dt = -kc^2$ which describes this reaction with respect to enzyme concentration and this method is appropriate for muramidases isolated from various sources[3**].

As shown in Table I muramidase is sensitive to the inhibitory action of thyroxine and triiodothyronine under various conditions, but thyronine is not inhibitory. It is obvious that thyroxine is more potent that triiodothyronine in longer preincubation experiments. Preincubation of the hormone alone with the cells (Expt. 2) compared to preincubation of enzyme and hormone before addition to the cells does not lead to inhibition. This means that inhibition is due to an effect upon the enzyme rather than to a rapid modification of the cell-wall substrate by the hormone.

At pH 7.4 the phenolic group of thyroxine is approx. 82% ionized and this group of triiodothyronine is only about 8% ionized[6]; there exists undoubtedly a participation of the phenolic ion in the inhibition process, the iodo substitution being necessary to enhance the ionization.

The molecularity of the inhibition was determined according to the equation,

$$\log (v/v_i^{-1}) = R \log [I] + \log 1/K_i \qquad (7)$$

where R — number of molecules of inhibitor combining with one molecule of enzyme. A plot of experimental values is shown in Fig. 1 using 0–7.4 μM L-thyroxine and

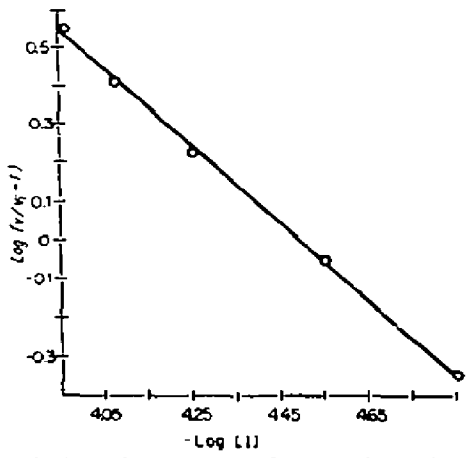

Fig. 1. Molecularity of inhibition of muramidase by L-thyroxine.

0.09 μM egg-white muramidase. The average value for R is 1.04 with a range of 0.75–1.45 in 8 experiments. Similar curves were obtained using triiodo-L-thyronine yielding an average value of 1.16 for R with a range of 1.07–1.25 in 4 experiments.

* G. LITWACK AND S. G. CHAKRABARTI, submitted for publication.
** A. L. N. PRASAD AND G. LITWACK, manuscript in preparation.

Characteristic Michaelis–Menten curves reflect a mode of action similar to irreversible and "pseudo-irreversible"[8] inhibition as shown by Fig. 2A. Similar results are found using triiodo-L-thyronine (Fig. 2B).

Equilibrium-dialysis experiments using L-[^{131}I]thyroxine in a system described for human serum albumin[9] showed no strict adherence to the SCATCHARD equation[10] with muramidase in the system, and in fact all extrapolations yield only a small fraction of one binding site leading to the conclusion that muramidase does not behave like a thyroxine-binding protein. Furthermore, muramidase can be precipitated from solution* by thyroxine suggestive of a dimerization effect[11]. The precipitate is enzymically inactive and exhibits about one-tenth the migration rate of free muramidase towards the cathode in paper electrophoretic experiments.

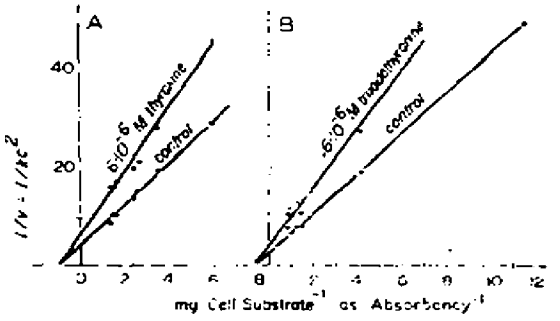

Fig. 2A. Effect of L-thyroxine on muramidase activity showing irreversible to "pseudo-irreversible" inhibition.
Fig. 2B. Effect of triiodo-L-thyronine on muramidase activity showing irreversible to "pseudo-irreversible" inhibition.

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Latency and solubilization of the mitochondrial aspartate transaminase of rat cerebral cortex

Several enzymes present in mitochondrial preparations isolated from mammalian liver[1–4], heart[5,6] and brain[7] are known to exhibit, when assayed under conditions preserving mitochondrial structural integrity, only a portion of the activity that becomes apparent after treatment of these preparations by disruptive procedures. That the use of such procedures fails at times to release the fully active enzyme in soluble form, as judged by non-sedimentation after centrifugation at high speeds, has been shown repeatedly[1,4,5,8,9]. We have recently observed that L-aspartate: 2-oxoglutarate aminotransferase (aspartate transaminase, EC 2.6.1.1) present in mitochondrial fractions of rat cerebral cortex[10,11] exhibits latency and, furthermore, that vigorous treatments are required to render the measurable transaminase activity of such preparations maximal.

Centrifugal fractions of rat cerebral cortex consisting of "heavy" + "light" mitochondria were isolated in 0.25 M sucrose as previously described[12]. Aspartate

TABLE I

EFFECT OF PRETREATMENTS OF HOMOGENATE AND MITOCHONDRIAL FRACTIONS OF RAT CEREBRAL CORTEX ON THE ACTIVITY OF ASPARTATE TRANSAMINASE

Expt.	Pretreatment	Fraction	
		Homogenate (units/g)	Mitochondria (units/g)
1*	None	3570	760
	1 h, 37° + digitonin	4210***	1770***
	20 h, 4°	3400	1080
	43 h, 4°	3170	1360
2*	None	—	454
	Digitonin	—	2520
	1 h, 37°	1730	—
	1 h, 37° + digitonin	2520	
3**	None	—	408
	Digitonin	—	715
	1 h, 37°	—	1500
	1 h, 37° + digitonin	—	1975

* Ti e equivalent: 100 mg/ml.
** Ti e equivalent: 167 mg/ml.
*** A rtate transaminase, specific activity: homogenate, 13.3; mitochondrial fraction, 20.9.
Digiton 0.33 mg/ml.