Regulation of lactate dehydrogenase activity: Reversible and isoenzyme-specific inhibition of the tetramerization process by peptides

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Summary. The transition of inactive lactic dehydrogenase (LDH) subunits to functional tetramers is controlled by 2 naturally-occurring peptides. One of these peptides inhibits the folding/association/activation process of H-LDH and the other peptide, the reconstitution of M-LDH. NADH and NAD^+ are isoenzyme-specific antagonists of the 2 inhibitory peptides.

The catalytic activity of lactate dehydrogenase (LDH) isoenzymes depends on their tetrameric structure. Low pH or other denaturants lead to dissociation of the isoenzymes into monomers, parallelled by a loss of enzymatic activity. Removal of the denaturing conditions induces reassociation of the monomeric units and reconstitution to the active tetramer[2,3]. This reassociation is strictly correlated with the regain of the catalytic activity[4,5]. Two peptides isolated from human liver isoenzymes specifically inhibit the reactivation of dissociated LDH[6-9]. In addition, some proteolytic enzymes do not attack native tetrameric LDH but rapidly cleave the unfolded subunits[10]. Therefore, the observed peptide inhibition could be due to proteolysis rather than to specific inhibitors. We attempted to exclude this possibility by showing the reversibility of the inhibition. As in our assay system[8,9], dialysis leads to side reactions of the dissociated and unfolded LDH monomers[11]. We demonstrated the reversibility by using specific antagonists of the inhibitors.

Methods. The reassociation/reactivation assay was carried out as previously described[8,9]. Inhibitors were isolated according to Schoenenberger et al.[8] with the modification that, instead of the affinity column, a cation-exchange column was used[9]. The inhibitors exhibited a half-maximal effect on the M-LDH reactivation at a concentration of 20 µg/ml and on the H-LDH reactivation at a concentration of 30 µg/ml (LDH concentrations = 2.5 µg/ml).

Results and discussion. In the absence of inhibitors, NADH or NAD^+ do not influence the reconstitution of dissociated LDH under our experimental conditions. This is in good agreement with results obtained by Rudolph et al.[12]. In the presence of inhibitors, however, the coenzymes act as antagonists. The effect of NADH differs from that of NAD^+.

At identical inhibitor concentration NADH counteracts the inhibited *H-LDH* reconstitution, whereas NAD^+ has only a marginal effect. A significant reversion of the inhibition with NAD^+ is only possible by lowering the inhibitor concentration (fig. 1).

In contrast, the inhibition of *M-LDH* reconstitution is not reversed by NADH under comparable conditions used for the H-LDH system. The observed pattern resembles that of the H-LDH system with NAD^+ as antagonist. In contrast to the H-LDH system, NAD^+ is capable of counteracting the M-LDH inhibitor completely. In the experiments shown in figure 1, the LDH tetramers were dissociated in the presence of the inhibitors. The coenzymes were added at the onset of the reactivation. A partial reactivation in the presence of inhibitors is also possible when the respective coenzyme is added after a time lag (fig. 2). However, the

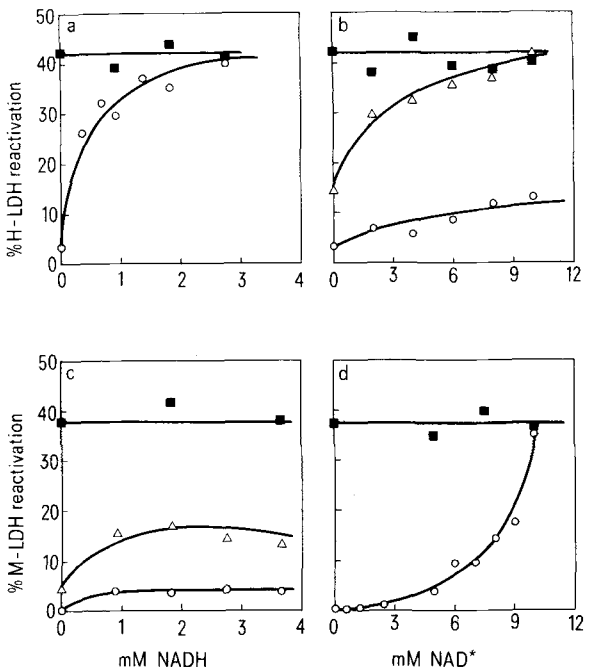

Figure 1. Effect of the coenzymes on the recovery of LDH activities in presence of inhibitors. *a* NADH and *H-LDH*, (○) 100 µg/ml inhibitor, (■) control. *b* NAD^+ and *H-LDH*, (○) 100 µg/ml inhibitor, (△) 50 µg/ml inhibitor, (■) control. *c* NADH and *M-LDH*, (○) 64 µg/ml inhibitor, (△) 32 µg/ml inhibitor, (■) control. *d* NAD^+ and *M-LDH*, (○) 64 µg/ml inhibitor, (■) control.

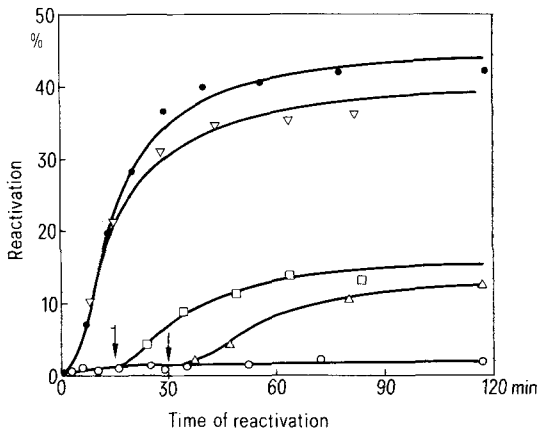

Figure 2. Exclusion of interfering proteolysis by counteracting the inhibition with antagonistic coenzyme. (●), Reactivation of H-LDH without inhibitor and without NADH or NAD^+. All open symbols represent reactivation experiments with 100 µg/ml H-LDH inhibitor in the reactivation mixture. (○) No NADH was added to the reactivation mixture. 0 min (▽); 15 min (□); and 30 min (△) after the start of the reactivation NADH in a minimal volume to avoid dilution of the medium was added yielding a final NADH concentration of 1.5 mM.

yield of recovered enzyme is drastically reduced. This may reflect the importance of competing side reactions of the dissociated LDH monomer in the in vitro system[11].

Conclusion. The purpose of this investigation was to discriminate between specific peptide inhibitors of the reactivation process and a hypothetical proteolytic enzyme effect which could lead to a similar kinetic behavior[10]. Since coenzymes often have the ability to protect their apoenzymes against proteolytic degradation, we had to design an experiment which excludes the trivial possibility that the reduced inhibition in the presence of coenzymes is due to a coenzyme-induced folding of the monomers which could lead to resistance against proteolytic enzymes. The fact that the reactivation is induced even after prolonged exposure to the inhibiting principle (fig. 2) unambigously demonstrates that the blocked reconstitution is truly reversible and therefore cannot be due to proteolysis.

Further consideration deserves the isoenzyme specific effect of the 2 coenzymes. The occurrence of 2 discrete isoenzyme specific inhibitors[9] which are susceptible to different antagonists suggests that the biological significance of the existence of 2 LDH-types might be due to different regulatory properties rather than differing metabolic functions.

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Trace metals and melanogenesis[1]

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Summary. Emission spectroscopic analysis of amphibian and mammalian skin ash for the study of trace metals shows that nickel, lead and tin may play some role in the pigmentation-depigmentation process.

In a previous communication Chakraborty et al.[3] showed that the activity of the melanogenic enzyme, tyrosinase (a monooxygenase) is inversely related to the one of tryptophan pyrrolase (a dioxygenase) during experimental depigmentation, and pigment regeneration by psoralen, in *Bufo melanostictus.* The role of copper is well documented in the tyrosinase action[4]. It is known that nickel in plant systems can increase the monooxygenase activity of catalase[5] while it inhibits the dioxygenase, peroxidase[6]. Lack of any report regarding the involvement of metals other than copper in depigmentation and melanogenesis prompted us to find out whether during experimental depigmentation or regeneration of pigment with pigmentogenic drugs, the normal concentration of trace metals is affected in the amphibian and mammalian skins.

In the present communication, the levels of lead, arsenic, nickel, cobalt, copper and tin in the experimental depigmentation and regeneration of pigment with psoralen (plus sunlight) in *B. melanostictus* have been investigated. Comparative studies of different trace metals in pigmented rat skin with those of albino rat skin have also been done.

Materials and methods. 90 Indian male toads *(B. melanostictus)* of 40–50 g b.wt were used for the experiment; of these, 60 were fed hydroquinone (100 µg/toad/day) for 7 days. On the 8th day 30 of the hydroquinone-treated toads and 30 normal toads were sacrificed for analysis of trace metals in the ventral skin tissues. The remaining 30 toads were fed psoralen (1 mg/toad/day) and kept under ordinary sunlight for 7 days. These toads were sacrificed on the 15th day. Ventral skins were dissected out.

6 pigmented Bandicoot rats of 200–250 g b.wt and 6 albino rats (Wistar strain) of the same b.wt, maintained for a few days on the same diet and in the same atmosphere, were sacrificed for analyses of trace metals in the skin tissues.

For dissection of the skin we used a stainless steel blade. The skins were cut into pieces, washed with deionized water and dried in an air oven. Ashing of the tissues was done by the oxidizing flame of a Bunsen burner keeping the tissues in a silica crucible. The ashes were analyzed for trace metals using a Jarrel Ash Emmission Spectrograph (JAES).

Results and discussions. Table 1 shows that during induced depigmentation, levels of lead and tin in the skin of *B. melanostictus* increase abnormally and during regeneration of pigmentation with oral administration of psoralen (plus sunlight) about normal levels have been obtained in

Table 1. Emission spectroscopic data for hydroquinone and psoralen treated toad skins

	Mean result (ppm) ± SD				
	Pb	Sn	Ni	Co	Cu
Normal	160 ± 9	250 ± 13	80 ± 3	18 ± 3	25 ± 2
Hydroquinone-fed toads (depigmentation)	250 ± 12*	430 ± 12*	80 ± 7 (NS)	15 ± 3 (NS)	22 ± 2 (NS)
Psoralen plus sunlight (after recovery)	180 ± 11*	150 ± 7*	70 ± 8 (NS)	12 ± 3 (NS)	25 ± 3 (NS)

Number of trials (n) = 6. Each trial contains ventral skin ash from 5 toads. *$p < 0.001$; NS = not significant.