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Chelating Agents and Regulation of Lactate Dehydrogenase Synthesis

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Changes in lactate dehydrogenase (LDH) activity induced by exposure to chelating agents have been demonstrated in established cell strains, cultured *in vitro*.[1,2] In the cell strain used in the present study (Chang liver), addition of 2,2′-bipyridine or 1,10-phenanthroline to the culture medium, brought about an increased synthesis of LDH. Furthermore, the inducing action of the two compounds was enhanced by the addition of equimolar amounts of ferrous ions.[2]

In order to clarify the mechanism of the action of 2,2′-bipyridine and 1,10-phenanthroline on the regulation of LDH synthesis, it was considered necessary to establish the extent to which their chelating properties are essential for their ability to induce synthesis of LDH. Consequently, a number of structurally related compounds, some of them with no ability to chelate metal ions, were tested to ascertain their effect on LDH synthesis in Chang cells. Tests were also made, where the compounds were added together with equimolar amounts of ferrous ions, in order to investigate if the potentiating action of these ions could be correlated with the formation of a ferrous complex of the compound.

Experimental. Chang cells were grown in suspension cultures, continuously exposed to an atmosphere of 95% air and 5% carbon dioxide. The cultivation and the experiments were carried out in a manner similar to that used for 2,2′-bipyridine and 1,10-phenanthroline in a previous report [2] and the results should thus be directly comparable. 2,2′-Bipyridine, 4,4′-bipyridine, 1,10-phenanthroline, and ferrous ions were added to the cultures in a small volume of saline, the other compounds were dissolved in a small amount of ethanol. The control cultures received a corresponding amount of pure solvent. 2,2′-Bipyridine and 1,10-phenanthroline were obtained from Merck, Darmstadt, Germany, and the other compounds listed in Table 1 from Th. Schuchardt, Munich, Germany.

LDH activity was determined by the method of Stambaugh and Post,[3] and the nitrogen content of the homogenates by a micro-Kjeldahl method.

Results. Of the compounds with chelating abilities listed in Table 1, *i.e.* 2,2′-bipyridine, 1,10-phenanthroline, and its substituted derivatives, 5-nitro-1,10-phenanthroline was the most potent inducer of LDH synthesis, with a high activity in a concentration of 10^{-5} M. This compound was too toxic to be used in the same concentrations as the other compounds ($5\times 10^{-5}-10^{-4}$ M). The action on LDH synthesis was similar to and in the same range as that obtained when the cells were exposed to 2,2′-bipyridine or 1,10-phenanthroline.[2] The effect of 5-nitro-1,10-phenanthroline was enhanced by the addition of ferrous ions. Cells exposed to 4,7-dimethyl-1,10-phenanthroline did not show any significant increase in LDH activity when the compound was used alone. However, upon addition of ferrous ions, this compound was able to stimulate the synthesis of LDH. In contrast to the other chelating compounds used, 2,9-dimethyl-1,10-phenanthroline (Neocuproine) failed to bring about an increased LDH synthesis, but caused a

Table 1. Summary of the effects of 2,2′-bipyridine and related compounds on LDH synthesis. The symbols indicate: + increased activity, 0 no change, 0− slightly decreased activity, −decrease in cell number, compared with control cultures.

Substance	Conc. M	Change in LDH activity	Additional change by Fe^{2+}	Cell number
2,2′-Bipyridine	5×10^{-5} -10^{-4}	+	+	−
1,10-Phenanthroline	5×10^{-5} -10^{-4}	+	+	−
5-Nitro-1,10-phenanthroline	10^{-5}	+	+	−
2,9-Dimethyl-1,10-phenanthroline	5×10^{-5} -10^{-4}	0−	0	−
4,7-Dimethyl-1,10-phenanthroline	5×10^{-5} -10^{-4}	0	+	−
4,4′-Bipyridine	5×10^{-5} -10^{-4}	0	0	0
Phenanthridine (3,4-benzoquinoline)	5×10^{-5}	0	0	0
5,6-Benzoquinoline	5×10^{-5}	0	0	0
7,8-Benzoquinoline	5×10^{-5} -10^{-4}	0	0	0

slightly decreased LDH activity after 48 h, probably due to some toxic action on the cells. The addition of ferrous ions did not change the reaction of the cells to this compound.

All substituted 1,10-phenanthrolines used, except 2,9-dimethyl-1,10-phenanthroline, brought about a pink coloration of the cells, similar to that observed after exposure to 2,2′-bipyridine and 1,10-phenanthroline. This hue became more intense in cells from cultures receiving, in addition, ferrous ions. Similar to 2,2′-bipyridine and 1,10-phenanthroline, all substituted 1,10-phenanthrolines used inhibited growth of the cells in the concentrations used.

The non-chelating compounds, *i.e.* 4,4′-bipyridine, phenanthridine, 5,6-benzoquinoline, and 7,8-benzoquinoline did not affect the synthesis of LDH and no inhibition of cell growth was observed with the concentrations used.

Discussion. The observation, that the non-chelating analogues of 2,2′-bipyridine and 1,10-phenanthroline were not able to induce LDH synthesis, while of the chelating compounds used, only 2,9-dimethyl-1,10-phenanthroline was completely unable to induce LDH synthesis, strongly suggests that the action on LDH synthesis is associated with the ability of these compounds to form complexes with metal ions. It is further suggested that the identity of the metal involved is iron, a hypothesis supported by the fact that the chelating compounds which were active as inducers of LDH synthesis, have high avidity for ferrous ions, whereas 2,9-dimethyl-1,10-phenanthroline, which does not form the typical colored complexes with ferrous ions,[4] was unable to induce LDH synthesis.

It has been proposed that chelating agents exert their LDH-inducing activity by interfering with metals mediating the repressive action of oxygen on LDH synthesis.[1] It appears plausible that the LDH-inducing compounds form complexes with protein-bound ferrous ions, thereby causing

changes in their chemical reactivity. This view is consistent with the observation that cells exposed to 2,2′-bipyridine, 1,10-phenanthroline, and their LDH-inducing derivatives obtain a pink coloration, assumedly due to complex-bound ferrous ions. The formation of complexes with intracellular ferrous ions could possibly bring about conditions to some extent resembling hypoxia, a condition known to stimulate LDH synthesis in Chang cells.[5,6]

However, if the formation of complexes with intracellular ferrous ions would be the only mechanism causing the increased LDH synthesis, it could be expected that the addition of ferrous ions, similar to cobaltous ions,[2] would abolish the action of the chelating agents on LDH synthesis analogously to the abolishment of the fungistatic action of 2,2′-bipyridine and 1,10-phenanthroline by the addition of various metal ions.[7] Since this is not the case, it appears probable that the chelating agents may also act by some other mechanism. It is known that chelates of metal ions often show catalytic properties in reactions involving oxygen. Ferrous complexes of 1,10-phenanthroline and some of its substituted derivatives have been reported to possess a certain catalase activity.[8] Thus it also appears possible that the complexes of 2,2′-bipyridine, 1,10-phenanthroline and some of their substituted derivatives interfere with some reaction of importance to the synthesis of LDH.

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Heats of Combustion Diethyl Ether and 1,1-Diethoxyethane

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Because the heats of combustion, $\Delta H_c°(g)$, of diethyl ether and 1,1-diethoxyethane were required for a recent derivation of a bond-bond interaction scheme for aliphatic alcohols, ethers, and acetals,[1] this paper reports the measurement of these quantities.

1,1-Diethoxyethane was prepared from acetaldehyde and ethanol by allowing the mixture to stand overnight over anhydrous calcium chloride in a stoppered flask. The water layer was discarded and the excess alcohol distilled off. After several redistillations from sodium to remove the remaining alcohol and water, a 99.95 % pure sample was obtained. B.p. 102−104°C, n_D^{20} 1.3811, d_4^{20} 0.8218.

Diethyl ether was commercial product of E. Merck AG (guaranteed reagent), which was purified carefully and distilled several times from sodium. B.p. 36.0°C, n_D^{20} 1.3523, d_4^{20} 0.7077. Purity≥99.99 % by gas chromatographic analysis.

Samples of these compounds were sealed in thin soda glass ampoules and burned in oxygen in an adiabatic bomb calorimeter No. 1221 manufactured by Parr Instruments Co., Illinois, USA, as described earlier.[2–4] The energy equivalent of the standard calorimeter system, $e°$(calor), was 1359.13±0.33 cal/°F. The experimental results are presented in Table 1.

The heat of vaporization of 1,1-diethoxyethane, 7.82 kcal·mole^{-1} (*Intern. Crit. Tables*), at the boiling point was corrected to refer to 25°C (9.04 kcal·mole^{-1}) employing the equation proposed by Watson.[5] The heat of vaporization of diethyl ether, 6.36 kcal·mole^{-1}, was that reported by Pilcher *et al.*[6]

The value, −657.96±0.44 kcal·mole^{-1}, obtained for the heat of combustion of gaseous diethyl ether is in close agreement with the value, −657.52±0.19 kcal·mole^{-1}, reported by Pilcher *et al.*[6] and with the value, −658.1 kcal·mole^{-1}, reported by Stohmann as corrected by Pilcher *et al.*[6] No reliable data have been reported for 1,1-diethoxyethane.