Injection of L-triiodothyronine resulted in a significant increase in the specific activities of all the fetal lysosomal enzymes studied in both forebrain and cerebullum (figure). Comparing total activities in forebrain of control and $T_3$-treated groups showed small and statistically insignificant differences; only total activity of $\alpha$-L-fucosidase was significantly increased. The differences in total activities of all enzymes studied in cerebellum between the control and $T_3$-treated rats were not significant. Administration of cortisone decreased both specific and total activities of $\beta$-galactosidase, $\beta$-glucuronidase, N-acetyl-$\beta$-glucosaminidase, and $\alpha$-L-fucosidase in forebrain (figure). In cerebellum, cortisone administration caused no significant changes in specific or total activity (differences between controls and cortisone-treated group were less than 5%).

Experiments have thus shown that administration of triiodothyronine or cortisone to pregnant rats can influence the activity of several fetal brain acid hydrolases. It is noteworthy that cortisone influenced glycosidases in the forebrain, but no effect was seen in cerebellum. Mechanisms involved in these effects are open for further studies.

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## Evidence for the nonhydrophobic interaction of aromatic, nitrogen-containing compounds with the coenzyme binding site of a NAD-linked D-lactate dehydrogenase

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*Summary.* Quantitative structure activity relationships suggest that the binding of quinoline and phenanthroline analogs to D-lactate dehydrogenase from the barnacle, *Balanus nubilus* Darwin, does not involve primarily hydrophobic effects. This phenomenon appears to exist also for other lactate dehydrogenases.

A variety of polyaromatic compounds have been demonstrated to bind at the coenzyme binding sites of diphosphopyridine nucleotide-linked dehydrogenases. For example, salicylate[1], cibacron blue[2] and simple aromatic hydrocarbons such as benzene and naphthalene[3] are effective competitive inhibitors with respect to the coenzyme in a variety of dehydrogenases. Aromatic metal chelators (o-phenanthroline, 8-hydroxyquinoline) and their nonchelating analogs have been shown to catalytically inhibit yeast alcohol dehydrogenase[4], pig heart s-malate dehydrogenase[5], human liver aldehyde dehydrogenase[6] and beef liver glutamate dehydrogenase[7]. Anderson and Anderson[8] observed that the effectiveness of nicotinamide chlorides as inhibitors of yeast alcohol dehydrogenase was related to the length of hydrocarbon side chains attached to the nicotinamide ring. A linear relationship was observed between chain length and the inhibitory capacity of the compound, suggesting hydrophobic interactions were involved at the coenzyme binding site. Since aromatic chelators and their nonchelating analogs are hydrophobic in nature, a number of workers have invoked similar hydrophobic explanations for the mode of inhibition of these compounds on diphosphopyridine nucleotide-linked dehydrogenases[4-7]. Studies reported here using quantitative structure activity relationships suggest that the mode of binding of quinoline and phenanthroline analogs to a D-lactate dehydrogenase is not hydrophobic.

In kinetic studies it has been observed that the D-lactate dehydrogenase (E.C. 1.1.1.28) from the muscle of the giant barnacle, *Balanus nubilus* Darwin, is instantaneously inhibited by a wide range of aromatic metal chelators and their nonchelating analogs[9]. In all cases, the pattern of inhibition by quinoline and phenanthroline congeners is competitive with respect to the coenzyme, NADH, suggesting that these compounds bind at the coenzyme site on the enzyme molecule. In order to obtain a clearer picture as to whether hydrophobic (i.e. water desolvation) interactions are involved in the binding of these aromatic compounds to *B. nubilus* D-lactate dehydrogenase, we have analyzed our results using quantitative structure activity relationships (QSAR) as developed by Hansch and coworkers[10-12]. This type of analysis involves the correlation of the inhibitory capacities of compounds with certain of their physicochemical characteristics. We have directed our attention at 2 particular parameters, the logarithm of the octanol-water partition coefficient (log P) and the Lorentz-$_{\text{Lorenz}}$ molar refractivity (MR). The partition coefficient appears to correlate substituent interactions in the hydrophobic regions of enzymes. It presumably models the hydrophobic effect as determined by desolvation of substituent and enzyme. The molar refractivity is not well understood at the physical level but is thought to reflect the overall electronic configuration of the molecule[13]. This is the result of both the constitutive nature and number of atoms involved (leading to an additive parameter related to molecular volume or 'bulkiness' for organic molecules) and intra- and intermolecular electronic distortions (a polarizability parameter). Log P values have been determined experimentally and MR values were determined by calculation[10-12] for each of the inhibitory compounds. Linear regressions were calculated correlating log 1/apparent $K_i$ with either log P or MR. The results of the QSAR's are listed in table 1. The correlation coefficient of equation 1 (table 1) for *B. nubilus* D-lactate dehydrogenase is very low ($r = 0.498$), whereas a relatively high correlation is observed (equation 2, table 1) for this enzyme when one looks at molar refractivity

($r = 0.891$). These results suggest that the interaction of the ligands with the enzyme do not involve primarily hydrophobic effects at least in the operational sense of desolvation. This point is further strengthened by the noncolinearity of the squared correlation matrix for the log P and MR variables in this study. Similar analysis of inhibition by aromatic compounds on potato tuber (table 2) and rabbit muscle[11] lactate dehydrogenases revealed a high correlation with molar refractivity, suggesting that the relationship may be of a general nature for at least lactate dehydrogenases. Pauling and Pressman in 1945 first reported a clear correlation between biological effectiveness and molar refractivity for hapten-antibody interactions[16]. Additionally, Hansch and Calef[17] have reported a high correlation between molar refractivity of substituted aryl esters and the measured apparent $K_m$'s resulting from the hydrolysis of these compounds by papain.

Although the exact nature of the ligand and protein interaction is not revealed by the high MR correlation, the results suggest that it may involve a more polar interaction. This interpretation is consistent with studies showing the binding of quinolines and phenanthrolines at the pyridine part of the coenzyme binding site. Anderson and Reynolds[18] have demonstrated via multiple inhibition studies that quinoline, o-phenanthroline and benzoquinoline bind at the nicotinamide binding site of yeast alcohol dehydrogenase. Recent X-ray studies show that o-phenanthroline binds at the nicotinamide, rather than the adenine, region of the coenzyme binding site of horse liver alcohol dehydrogenase[19]. Since X-ray studies have demonstrated that the binding of the adenine portion of NADH to lactate dehydrogenase is facilitated almost exclusively by hydrophobic interactions[20], we suggest that the inhibitors used in the present study bind at the pyridine site of *B. nubilus* D-lactate dehydrogenase. Holbrook et al.[20] have shown that the residues interacting with the pyridine portion of NAD are valine (2), histidine, glutamate and lysine. Additionally, the adjacent substrate site is composed of arginine (2), histidine and serine residues. This would be expected to lead to a polar interaction with ligands, which is consistent with the high correlation of inhibitory capacity with molar refractivity and a lack of correlation with hydrophobicity, observed in the present study.

Table 1. Catalytic inhibition and QSAR analysis of the inhibition of *B. nubilus* D-lactate dehydrogenase by aromatic metal chelators and their nonchelating analogs

| Compound | Chelator | Log $1/K_i$ | Log P | MR |
|---|---|---|---|---|
| 2,9-Dimethyl-o-phenanthroline | + | 4.39 | 2.90 | 64.23 |
| 7,8-Benzoquinoline | − | 3.82 | 3.42 | 54.88 |
| m-Phenanthroline | − | 3.68 | 2.51 | 54.99 |
| o-Phenanthroline | + | 3.60 | 1.78 | 54.99 |
| p-Phenanthroline | − | 3.54 | 2.05 | 54.99 |
| 3,4-Benzoquinoline | − | 3.48 | 3.42 | 54.88 |
| 5-Hydroxyquinoline | − | 3.44 | 1.42 | 41.29 |
| 8-Methoxyquinoline | − | 3.07 | 1.84 | 45.91 |
| 8-Hydroxyquinoline | + | 3.04 | 2.02 | 41.29 |
| Quinoline | − | 2.75 | 2.03 | 39.47 |

* Equation 1: $\log 1/K_i = 2.71 + 0.329 \log P$
$n = 10$ $r = 0.498$ $s = 0.422$
Equation 2: $\log 1/K_i = 0.940 + 0.050$ MR
$n = 10$ $r = 0.891$ $s = 0.221$

* Symbols as follows: n, number of inhibitors; r, correlation coefficient; s, SD from the regression.

Assays were performed using at least 2 NADH concentrations ($1\text{–}6 \times 10^{-5}$ M), 6.7 mM sodium pyruvate and at least 6 inhibitor concentrations. Apparent inhibition constants ($K_i$'s) were calculated from graphical plots of 1/velocity vs. inhibitor concentration. The enzyme was purified by the method of Ellington and Long[14]. Equations represent linear regression equations of log $1/K_i$ vs. either log P or MR.

Table 2. QSAR analysis of the inhibition of potato tuber L-lactate dehydrogenase

| Compound | Log $1/K_i$ | Log P | MR |
|---|---|---|---|
| Hydrocinnamate | 1.17 | 1.84 | 41.88 |
| Benzoate | 1.30 | 1.87 | 32.29 |
| 3,4-Dihydroxybenzoate | 1.56 | 1.21 | 35.93 |
| 2,4-Dihydroxybenzoate | 1.92 | 2.06 | 35.93 |
| 3,5-Dihydroxybenzoate | 2.19 | 1.13 | 35.93 |
| m-Nitrocinnamate | 3.35 | 1.85 | 49.60 |
| Coumarin | 3.52 | 1.39 | 41.21 |
| 1,5-Dihydroxynaphthalene | 3.82 | 2.31 | 46.26 |
| p-Coumarate | 4.00 | 1.61 | 45.09 |
| p-Nitrocinnamate | 4.19 | 1.85 | 49.60 |
| 4-Hydroxyquinoline-2-COOH | 4.30 | 1.71 | 47.19 |
| 7,8-Dihydroxy-4-methylcoumarin | 4.44 | 0.87 | 49.47 |
| 3,4-Dihydroxycinnamate | 4.47 | 1.05 | 46.91 |

* Equation 1: $\log 1/K_i = 4.020 + 0.580 \log P$
$n = 13$ $r = 0.197$ $s = 1.303$
Equation 2: $\log 1/K_i = -4.452 + 0.176$ MR
$n = 13$ $r = 0.843$ $s = 0.714$

* Symbols are as follows: n, number of inhibitors; r, correlation coefficient; s, SD from the regression.

The experimental values are those of Rothe[15]. Equations represent linear regression equations of log $1/K_i$ vs. either log P or MR.

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