by the haphazard alignment of precursors. It may be assumed that the agent concerned with the selection and the arrangement of the nucleotides in the living cell has so far escaped identification. Whether it operates in a manner similar to that suggested before[2], remains to be seen.

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Sodium sulfide inhibition of liver lactic dehydrogenase*

Inhibition of carbonic anhydrase[1], trypsin and chymotrypsin[2], carboxypeptidase[3,4], desoxyribonuclease[5], and glutamic dehydrogenase[6] by Na_2S has been reported in recent years. The first interpretation of sulfide inhibition might be that metal ions were removed as insoluble sulfides or that reductive cleavage of important disulfide bonds might be involved. The latter process usually leads to irreversible inactivation. However, in the case of the experiments to be reported here, another view of the inhibition is necessary.

It seems likely that the principal ionic species of sulfide at pH 8.5 will be SH^-. Under these conditions Na_2S showed a reversible, sensitive inhibition of highly purified rat liver lactic dehydrogenase (LDH)[7]. At concentrations of Na_2S as low as 10^{-3} to $10^{-5}\,M$ marked inhibition, depending on exact conditions, was observed. LDH (2 ml) and 0.01 M sulfide (1 ml) were usually mixed with 2 ml M glycine buffer, pH 8.5, 0°, and allowed to stand for a few minutes, following which aliquots were diluted about 50 times with 0.4 M glycine. For analysis 0.6 ml were added to a Beckman cuvette along with Veronal buffer, sodium D,L-lactate and DPN^+. The final cuvette volume was 3.6 ml. The reaction was initiated by adding DPN^+. In a typical experiment the final cuvette concentrations were:

[LDH], $2\cdot10^{-10}\,M$; [Na_2S], $1.0\cdot10^{-5}\,M$; [Glycine], 0.06 M; [Veronal], 0.03 M; [Sodium D,L-lactate], 0.11 M; [DPN^+], $1.5\cdot10^{-4}\,M$; pH 8.6.

As can be seen in Table I, reversal of sulfide inhibition of LDH could be accomplished by a variety of procedures, the common denominator of which turned out to be the removal or displacement of SH^-. Thus such apparently diverse operations as (1) dilution; (2) dialysis; (3) LDH precipitation and re-solution; (4) increase in ionic strength; (5) addition of such chelating reagents as Versene, *o*-phenanthroline, α,α'-dipyridyl, and pyrophosphate; (6) addition of Zn^{++}, Fe^{++}, or Mn^{++}; (7) oxidation of SH^- by ferricyanide or *o*-iodosobenzoate all served to reverse sulfide inhibition.

TABLE I

SULFIDE INHIBITION OF LDH AND ITS REVERSAL

System	[Na_2S] *in cuvette* $M \times 10^{-5}$	[*Reagent*] *in cuvette* *M*	*Inhibition* %
Na_2S	1.18	—	92
Na_2S	0.58	—	78
Na_2S	0.14	—	10
Na_2S + NaCl	1.66	$6.6\cdot10^{-2}$	68
Na_2S + versene	1.66	$6.6\cdot10^{-5}$	10
Na_2S + Zn^{++}	1.18	$2.0\cdot10^{-4}$	0
Na_2S + *o*-phenanthroline	1.18	$1.3\cdot10^{-3}$	25
Na_2S + $K_3Fe(CN)_6$	3.80	$8.8\cdot10^{-4}$	0

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Purified liver LDH does not contain a heavy metal cation, as shown by spectrographic, X-ray fluorescence* or chemical analysis, although it is possible that the pure enzyme may contain Mg^{++}.

The most potent inhibition of sulfide was encountered when LDH was preincubated with DPN^+ and sulfide, the reaction in the cuvette being initiated by the addition of lactate. This observation suggested that interaction between DPN^+ and SH^- lay at the heart of the inhibition. Spectrophotometric measurements made with the Cary Recording Spectrophotometer, Model 11, showed an absorption maximum at 318–320 mμ when *both* DPN^+ and LDH were added to Na_2S-containing systems (Fig. 1). In the absence of LDH, a mixture of DPN^+ and Na_2S showed no spectral change. Thus it seems that the role of LDH may involve favoring the resonance hybrid form of DPN^+ which has an electron pair deficiency at position 4 in the pyridine ring. Na_2S additions do not affect the absorption spectrum of DPNH, either free or in the presence of LDH.

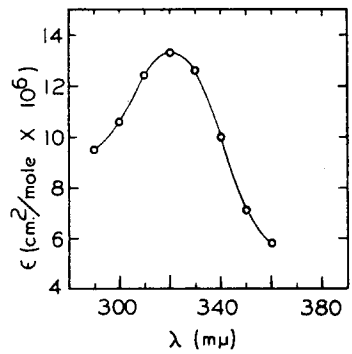
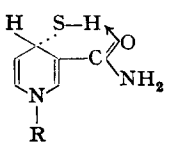


Fig. 1. Difference spectrum: Curve I *minus* Curve II, Cary Recording Spectrophotometer. Curve I: LDH, DPN^+, Na_2S. Reference solution, LDH, Na_2S. Curve II: DPN^+, Na_2S. Reference solution, Na_2S.

$[LDH] = 3.66 \cdot 10^{-6} M$. $[DPN^+] = 8.3 \cdot 10^{-5} M$. $[Na_2S] = 1.54 \cdot 10^{-3} M$. Phosphate buffer, $\Gamma/2 = 0.04$, pH 7.8. ε calculated for ratio: 2 DPN^+/1 LDH.

Addition of Versene to a system containing LDH, DPN^+ and Na_2S led to a considerable decrease of the 320 mμ absorption. Such an addition comdletely reversed sulfide inhibition.

The $DPN^+ \cdot LDH \cdot SH^-$ complex may possibly be written as shown below, and is quite similar to the CN^-, HSO_3^-, $NHOH^-$ and other anion complexes thoroughly described by MEYERHOF, OHLMEYER AND MOHLE[8] and by KAPLAN and co-workers[9]. It is interesting to note that liver LDH is strongly inhibited by HSO_3^-, but not by CN^- or $NHOH^-$.



It is apparent that the appearance of an absorption band at 320 mμ will permit the determination of the number of moles of DPN^+ which can be bound per mole of LDH (126,000 *g*). Experiments indicate that two moles of DPN^+ are bound, thus indicating the presence of two catalytically active reaction sites. Our experiments and those of others[10,11,12,13] are consistent with a reaction mechanism for LDH which would involve a spatial orientation of the lactate and DPN^+ molecules, hydride ion transfer[14] from lactate to the appropriate resonance hybrid of DPN^+, proton removal from the hydroxy group of lactate, and stabilization by formation of a carbonyl group to yield pyruvate.

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