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INVESTIGATION OF THE CONFORMATION OF LACTATE DEHYDROGENASE AND OF ITS CATALYTIC ACTIVITY

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SUMMARY

The conformation and enzymic activity of lactate dehydrogenase has been studied at different pH values. Spectropolarimetric investigations showed a bell-shaped dependence of α-helicity on pH. The correlation between the secondary structure and the rate of pyruvate reduction has been established. The action of the coenzyme (NADH) and of the inhibitor (oxalate) upon the secondary structure of the apoenzyme has been determined. It is shown that the inhibitor alters the apoenzyme structure only in the presence of the coenzyme.

INTRODUCTION

In the previous paper[1] conformational changes were shown to occur in GAPD under the action of various components of the catalytic system (coenzyme, substrate). The dependence of the GAPD molecular conformation and of its catalytic activity on the pH of the medium was also established and the influence of the hydrophobic "nucleus" of the molecule on its optical properties was demonstrated.

This paper presents a study of the behaviour of another enzyme, LDH. LDH is known to catalyze the reduction of pyruvate into lactic acid and the reverse reaction of L-lactate oxidation into pyruvate[2]:

$$\text{LDH} + \text{NADH} + \text{pyruvate} \rightleftharpoons \text{LDH} + \text{NAD}^+ + \text{L-lactate} \qquad (1)$$

The presence of the reduced coenzyme NADH is necessary for the direct reaction and that of NAD^+ is required for the reverse one. This reaction is also known to be inhibited by oxalate[3]. Consequently, this paper deals with investigations of the conformation of LDH and of changes in conformation during interaction with the NADH and NAD^+ coenzymes and with the inhibitor (oxalate).

At the same time, the rate of the reactions (1), catalyzed by LDH, is known to

Abbreviations: GAPD, D-glyceraldehyde-3-phosphate dehydrogenase; LDH, lactate dehydrogenase; ORD, optical rotatory dispersion.

depend on the pH of the medium. This dependency is different for the direct and reverse reactions[4].

The effect of pH on the activity of the enzyme consists in changes in the ionization of various components of the system. Free enzyme, enzyme–substrate complex, or substrate can undergo such changes. Accordingly, several explanations may be put forward for the influence of pH on the catalytic activity: (1) real reversible influence on the reaction rate; (2) influence on the affinity of the enzyme for the substrate; (3) influence on the stability of the enzyme.

When the enzyme is completely saturated with substrate, the major factor at every pH is the ionic form of the enzyme. Since enzymes are proteins whose molecules contain a large number of ionizable groups, some kind of distribution based on different ionized states of the enzyme should exist at every pH value. In all probability, different ionic forms of the enzyme possess different catalytic activity. Variation in the catalytic activity may be due either to the conformational differences between the molecules of the enzyme having different degrees of ionization or to different ionization states of certain groups present in the active centre of the enzyme.

According to the Michaelis–Davidsohn theory[5], the bell-shaped dependence of catalytic activity on pH is caused by the ionization of specific groups of the active centre. KIRKWOOD AND SHUMAKER explained such dependence by charge fluctuations on the enzyme which influence its electrostatic interactions with the substrate[6,7].

Since enzymes are protein molecules containing groups ionized at different pH values, one can expect that their secondary and tertiary structures change when the pH is changed. As shown by the theoretical investigations of model linear polyampholytes[8], a change in the pH of the medium should lead to a change in the α-helix content of the protein molecules, this dependence being governed by the amino acid composition and by the primary protein structure. Proceeding from modern concept of the nature of enzymic action[9], which connect the catalytic activity with conformational lability, one might expect a change of protein conformation to result in a change in its catalytic activity.

In order to elucidate the mechanism by which the pH of the medium influences the catalytic activity of the enzyme, an investigation of its influence upon the rate of the catalytic reactions(1) and upon the LDH conformation was undertaken in this work.

The ORD method was used for studying the LDH conformation as it gives direct information about protein structure.

MATERIALS AND METHODS

Materials. LDH was obtained by JÉCSAI's method[10] from pig skeletal muscle at the Biochemistry Institute of the Hungarian Academy of Sciences (Dr. ELÖDI's laboratory) and was recrystallized three times. The NAD^+ and NADH coenzymes were obtained from the Lawson Corp. (Great Britain). Sodium pyruvate was furnished by the Chemapol Co. (Cezchoslovakia), and DL-lactate was prepared by NEILANDS' method[11] from lactic acid obtained from the Carlo Erba Co. (Italy). Sodium dodecyl sulphate was furnished by the Biochemistry Institute, Budapest, Hungary. Other preparations were 'chemically pure' grade.

Measurements were made in 0.06 M phosphate buffer and in 0.1 M glycine buffer.

Preparation of solutions. Directly before measurements, a small amount of the enzyme suspension in $(NH_4)_2SO_4$ was centrifuged at 4° in a preparative TSUM-1 centrifuge and the precipitate was dissolved in the buffer used. After recentrifugation the enzyme was gel-filtered on columns of Sephadex G-25 saturated with the buffer used. Gel filtration was substituted for titration of the solution; it was conducted on the same columns saturated with buffers having the desired pH.

In the investigation of the LDH–NADH interaction, the coenzyme was added in the ratio of 5–20 moles per mole LDH. In experiments on the NAD^+–LDH interaction, NAD^+ was added in the ratio of 20–1500 moles per mole LDH. When appropriate, 1000 moles oxalate was added per mole LDH, and 400 moles sodium dodecyl sulphate was used per mole LDH.

Enzyme concentration was determined with the SF-4A spectrophotometer by protein extinction in solutions of 0.1 M NaOH at 280 mμ using the value $E^{1\%}_{1\,cm} = 1.29$.

Catalytic activity was determined according to KORNBERG's method[12] by observing the change in absorbance of the reaction mixture at 340 mμ during 1 min after the addition of 1 μg LDH per 3 ml of the reaction mixture.

pH of the solutions was measured with a LPU-01 pH meter to within ± 0.05 units. The measurements were performed both before and after the measurements of ORD.

Measurement of ORD was performed with a Pepol-60 spectropolarimeter (Bellingham and Stanley Co., Great Britain) with oscillating analyser. A xenon lamp was used as light source. The rotation angle was determined to within $\pm 0.002°$. The angle of the prism oscillation was 30°. The measurements were made at room temperature using cells (with fused quartz end-plates) of various lengths: 0.5, 1.0 and 2.2 dm.

On the basis of the ORD curves, the λ_c and K_c constants in Drude's equation, the a_0 and b_0 constants in Moffitt's equation, and the A_{193} and A_{225} constants in the SCHECHTER AND BLOUT equation[13,14] were calculated. In some cases the A_{193} and A_{225} constants were calculated from λ_c and K_c by using the transition formulae[15]:

$$A_{193} = 0.201 \cdot 10^{-8} K_c(\lambda_{225}^2 - \lambda_c^2) \qquad (2)$$
$$A_{225} = -0.148 \cdot 10^{-8} K_c(\lambda_{193}^2 - \lambda_c^2)$$

Circular dichroism was measured with a Jouan-Roussel dichrograph (France) at the Institute of Molecular Biology (Moscow),

RESULTS

Rotatory dispersion curves were obtained for LDH in the pH range between 3.6 and 10.6. The constants λ_c, K_c, a_0, b_0, A_{193} and A_{225} were calculated from these curves. The values obtained are given in Table I.

Table I shows that all the constants of ORD varied when the pH was changed. All the curves are well described by a one-term Drude equation; consequently we chose the λ_c and K_c constants as parameters characterizing the LDH structure. (The value of λ_c is not sensitive to errors in the $[\alpha]$ determinations, *e.g.* to those connected with the concentration determinations, *etc.*)

Fig. 1 presents curves of λ_c and K_c for LDH as functions of pH. Similar curves

TABLE I

EFFECT OF pH ON THE ORD PARAMETERS OF LDH (IN 0.06 M PHOSPHATE BUFFER)

pH	λ_c	$-K_c \times 10^{-5}$	a_0	b_0	A_{193}	A_{225}
3.6	228	142	−280	− 90	38	−309
4.3	244	116	−260	−127	206	−382
5.0	260	93	−183	−160	316	−417
5.3	261	84	−160	−170	295	−383
5.9	272	90	−183	−170	420	−490
6.8	270	93	−178	−190	414	−490
7.0	275	78	−140	−200	392	−443
7.5	272	95	−185	−187	444	−515
7.8	277	86	−160	−220	450	−502
8.5	270	93	−178	−193	414	−490
8.75	271	81	−151	−200	370	−434
9.5	254	84	−167	−150	234	−339
10.0	252	95	−190	−173	344	−441
10.7	231	152	−320	− 96	85	−363

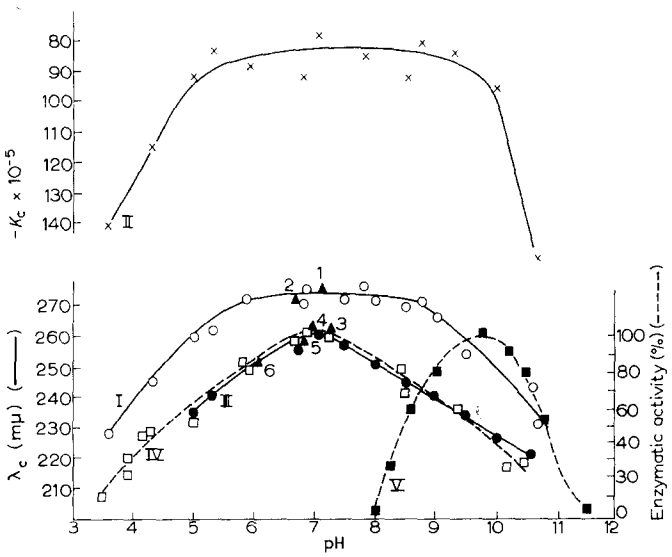

Fig. 1. Curve I (○—○). Dependence of λ_c on pH for LDH in a 0.06 M phosphate buffer. Point 1 was obtained for the change from pH 10 to pH 7; point 2 for the change from pH 4 to pH 6.75. Curve II (×—×). Dependence of K_c on pH for LDH. Curve III (●—●). Dependence of λ_c on pH for LDH in the presence of NADH. Curve IV (□— — —□). Dependence of enzymic activity of LDH on pH for the reduction of sodium pyruvate into lactate. 100% activity is taken as giving $\Delta A_{340\ m\mu}$/min = 0.550 for the system: $3.3 \cdot 10^{-4}$ M sodium pyruvate; $1.77 \cdot 10^{-7}$ M NADH; 0.06 M phosphate buffer; $2.2 \cdot 10^{-9}$ M LDH. Point 3 was obtained when the pH of the solution of LDH changed from pH 4.0 to 7.3; point 4, from pH 8.5 to 7.1; point 5, from pH 9.5 to 7.0; point 6, from pH 10.5 to 6.3. Curve V (■— — —■). Dependence of enzymic activity on pH for the oxidation of L-lactate. 100% activity is taken as giving $\Delta A_{340\ m\mu}$/min = 1.050 for the system: $5.4 \cdot 10^{-3}$ M NAD^+; $2.5 \cdot 10^{-2}$ M DL-lactate; $2.2 \cdot 10^{-9}$ M LDH; 0.1 M glycine buffer.

were obtained for a_0 and b_0. Fig. 1 shows that the dependence of λ_c and K_c on pH was bell-shaped and maximum values of λ_c (and of b_0) were in the range of neutral pH. When the pH was changed to acidic or alkaline values, the values of λ_c and b_0 decreased while those of K_c and a_0 increased. The change in the ORD constants in the pH range 4 to 10.6 was reversible. Fig. 1 gives the values of λ_c obtained when the LDH solution passed from pH 4 or 10.6 to pH 7.0.

To obtain additional information on the structure of LDH and on the changes occurring when the pH of the medium was varied, we plotted A_{225} as a function of A_{193} from the SCHECHTER–BLOUT equation[13] for several pH values. Fig. 2 gives straight lines I and II plotted for $\varepsilon > 30$ (aqueous solutions) and $\varepsilon < 30$ (organic solvents) in the plot of SCHECHTER AND BLOUT[13,14], and points of A_{225} as functions

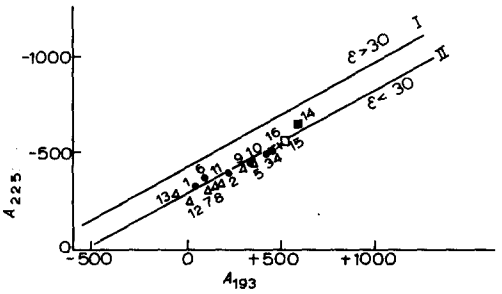

Fig. 2. Plot of the SCHECHTER AND BLOUT constants A_{225} *versus* A_{193}. Line I is the empirical formula of SCHECHTER AND BLOUT for aqueous solutions with $A_{225} = -0.55 \cdot A_{193} - 430$ ($\varepsilon > 30$). Line II is for organic solvents, with $A_{225} = -0.55 \cdot A_{193} - 280$ ($\varepsilon < 30$). Points 1–6 (●): LDH in a 0.06 M phosphate buffer. 1, pH 3.6; 2, pH 4.3; 3, pH 5.9; 4, pH 7.5; 5, pH 10.0; 6, pH 10.7. Points 7–13 (△): LDH in the presence of NADH. 7, pH 5.0; 8, pH 5.3; 9, pH 6.8; 10, pH 7.5; 11, pH 8.5; 12, pH 10.0; 13, pH 10.6. Point 14 (■): LDH *plus* sodium dodecyl sulphate (in the weight ratio 1:1), pH 7.0. Point 15 (□): LDH *plus* oxalate, pH 7.0. Point 16 (×): LDH *plus* NADH *plus* oxalate, pH 7.0.

of A_{193} for LDH at various values of pH. As Fig. 2 shows, these points for LDH fall on line II ($\varepsilon < 30$) as do those for GADP (see ref. 1).

When the pH was varied, the points moved along line II without abandoning it. When LDH was treated with sodium dodecyl sulphate in the ratio 1:1 by weight (400 moles sodium dodecyl sulphate per 1 mole LDH), the points shifted somewhat upwards. As in the case of GAPD, the treatment of LDH with sodium dodecyl sulphate produced changes in the K_c and a_0 constants while the values of λ_c and b_0 remained unchanged.

We investigated the influence of NADH and NAD^+ upon the structure of LDH at various values of pH. NADH and NAD^+ are optically active compounds. It was established that in the pH range from 10.6 to 5.3 NADH possessed a stable structure characterized by a definite rotatory dispersion (Fig. 3). In the acid region (below pH 5.3), the curves of the NADH dispersion began to change with time. At pH 3.6 the ORD for NADH underwent a drastic change (Fig. 3). We would suggest that NADH is stable only in the pH range above 5.3 and that in the acid region it begins to decompose. Hence, the influence of NADH upon LDH was determined in the pH range 5.3 to 10.6. Curves of rotatory dispersion for LDH *plus* NADH were obtained

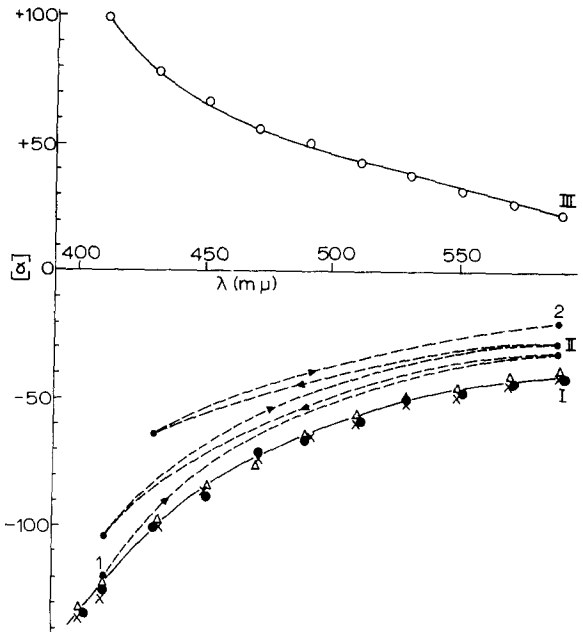

Fig. 3. Dependence of $[\alpha]$ on λ for NADH in 0.06 M phosphate buffer at various values of pH. Curve I (—): ●, pH 5.3; ×, pH 7; △, pH 10.6. Curve II (— — —): Change with time in the dependence of $[\alpha]$ on λ at pH 4.3. Beginning of measurements, point 1. End of measurements, point 2. Curve III (○—○): pH 3.5.

and from them the λ_c, K_c, a_0, b_0, A_{193} and A_{225} constants were calculated for LDH. The values obtained are given in Table II. Fig. 1 gives the λ_c curve as a function of pH for LDH in the presence of NADH and shows that NADH changed the values of the λ_c constants for LDH, lowering them differently for different values of pH. The curve of λ_c as a function of pH for LDH in the presence of NADH has the same bell-shaped character but NADH made the "bell" of the apoenzyme narrower. At all pH values

TABLE II

EFFECT OF NADH ON THE ORD PARAMETERS OF LDH (IN 0.06 M PHOSPHATE BUFFER AT THE DESIRED pH)

pH	λ_c	$-K_c \times 10^{-5}$	a_0	b_0	A_{193}	A_{225}
5.0	236	117	−250	− 43	119	−320
5.3	241	103	−213	− 60	155	−318
5.9	253	90	−210	−106	241	−356
6.8	256	105	−160	−153	314	−440
7.0	261	88	−151	−190	308	−403
7.5	258	106	−180	−133	340	−460
8.0	251	80	−170	−100	198	−305
8.5	245	91	−186	− 90	175	−314
9.0	241	86	−180	− 80	129	−265
9.5	235	91	−175	− 60	85	−243
10.0	228	110	−213	− 30	30	−240
10.6	221	176	−362	− 20	−62	−302

NADH produced a decrease in the λ_c constant. It should be noted that at the same time the K_c (and a_0) constants did not undergo any considerable change. When NADH was added, the points in the SCHECHTER–BLOUT plot (Fig. 2) moved along the line II with a certain tendency to shift downwards from line II (in a direction away from line I).

Changes in the ORD constants for LDH under the action of NADH were reversible. If NADH was removed by dialysis on a column of Sephadex G-25, the initial values of the constants were restored.

The curves of rotatory dispersion and circular dichroism were obtained for LDH *plus* NADH in the wavelength region 300 to 590 mμ. Neither anomalous dispersion, nor circular dichroism were observed in the absorption band of the bound NADH.

In studying the influence of NAD^+ upon the structure of LDH we did not observe any important effects. It is noteworthy that the affinity of NAD^+ for LDH is much less pronounced than that of NADH ($K_s = 10^{-4}$ M for NAD^+, while for NADH $K_s = 10^{-5}$ M). Usually the ratio of 15 000 moles NAD^+ per mole LDH is used in enzymic reactions. In our experiments we used the ratio of 20–1500 moles NAD^+ per mole LDH. No important changes in λ_c were observed when small amounts of NAD^+ were used, while large NAD^+ concentrations produced a large error in the determination of the rotation of the LDH apoenzyme.

In order to establish the mechanism of the action exerted by pH on the catalytic activity of the enzyme, we obtained the curves of the rates of the catalytic reactions (the direct and the reverse ones) as functions of pH. The curves are shown in Fig. 1. They are bell-shaped, but the positions of the rate maxima are different: the sodium pyruvate reduction had a maximum rate of reaction around neutral pH while the maximum rate of the L-lactate oxidation was in the alkaline range. The change in reaction rates in the pH range 4.0 to 10.5 was reversible. When the LDH solution passed from pH 4 or pH 10.5 to neutral pH (approx. 7), the enzymic activity was completely restored (Fig. 1). As has been shown in several studies, oxalate is not competitive as an inhibitor for pyruvate but is competitive and an inhibitor for lactate[3].

NOVOA AND SCHWERT[16] have shown that the addition of substrates and inhibitors to the enzyme does not take place before the coenzyme is introduced. We studied the influence of oxalate on LDH in the presence of the NADH coenzyme and in its absence. Results are given in Table IV, which shows that in the absence of NADH the oxalate did not produce changes in the ORD constants for LDH. In its presence the addition of oxalate to the enzyme–coenzyme complex led to a marked increase of λ_c and b_0. It is interesting to note that at the same time K_c and a_0 underwent hardly any change.

DISCUSSION

As Fig. 1 and Table I show, LDH in the neutral pH range (6 to 8.75) possesses a stable structure characterized by rather high λ_c and b_0 values, corresponding approximately to an α-helix content of 40%. LDH as well as GAPD can be included in the first group of globular proteins according to JIRGENSONS' classification[17]. The fact that the points in the SCHECHTER–BLOUT plot shift from line I and fall on line II is

evidently due (as for GAPD) to the influence of a strong hydrophobic nucleus of the molecule upon the ORD constants. The existence of such a nucleus is also indicated by data concerning deuterium-exchange kinetics[18], the spectrophotometric titration of tyrosine amino acid residues[18] and the iodination of LDH[18].

When the pH was varied in the range between 3.6 and 10.7, reversible changes in the secondary structure of LDH were observed, expressed in a change of α-helicity. Its dependence on pH was bell-shaped and this corroborates theoretical calculations[8].

When the pH was varied, the points in the SCHECHTER–BLOUT plot moved along line II without abandoning it. It is reasonable to assume that partial changes in the secondary structure of LDH, occurring when the degree of ionization is varied, do not cause a major reconstruction of all the tertiary structure. This is confirmed by the reversible restoration of the secondary structure and of the catalytic activity of LDH.

In all probability, LDH possesses a much more rigid and stable tertiary structure than GAPD since the changes in pH from 3.6 to 10.7 and the action in sodium dodecyl sulphate on LDH did not produce a complete unfolding of the molecule nor a disappearance of the hydrophobic nucleus. Under the action of sodium dodecyl sulphate the points shifted somewhat upwards towards line I but did not reach it. Investigation of the hydrodynamic properties of LDH has shown that under the influence of sodium dodecyl sulphate at 0.008–0.01 M, no significant changes in the subunit structure take place (the viscosity varies from 0.03 dl/g to 0.04 dl/g (ref. 19)). For GAPD under the influence of the same amounts of sodium dodecyl sulphate the viscosity changes from 0.03 dl/g to 0.09 dl/g (ref. 20). This signifies that more important changes in the subunit structure take place. The fact that the NADH coenzyme influences the secondary structure of LDH (and consequently interacts with the apoenzyme) when sodium dodecyl sulphate is present in a 1:1 ratio, serves as additional evidence for the incomplete destruction of the LDH molecule under the action of sodium dodecyl sulphate (Table III). A comparison of all the results indicates that the structure of GAPD is more labile while that of LDH is more stable with respect to the action of pH and sodium dodecyl sulphate.

Table II and Fig. 1 show that NADH changed the LDH structure causing partial uncoiling of the helices, which was revealed by a decrease in the λ_c and b_0

TABLE III

EFFECT OF SODIUM DODECYL SULPHATE UPON THE ORD PARAMETERS OF LDH (IN 0.06 M PHOSPHATE BUFFER, pH 7.0)

Preparations and conditions of measurements	λ_c	$-K_c \times 10^{-5}$	a_0	b_0	A_{192}	A_{225}
LDH	275	78	−175	−230	392	−443
LDH *plus* sodium dodecylsulphate (1:1, w/w) (400 moles sodium dodecyl sulphate per mole LDH)	279	108	−210	−227	591	−649
LDH *plus* sodium dodecyl sulphate *plus* NADH (400 moles sodium dodecyl sulphate and 8 moles NADH per mole LDH)	266	108	−214	−173	436	−536

constants. These structural changes in LDH were different at different values of pH. It should be noted that when λ_c and b_0 decreased, no significant changes in K_c and a_0 were observed. This stability of the K_c and a_0 constants with the simultaneous changes in λ_c and b_0 may have been due to the compensation of changes in the secondary structure of the molecule by changes in its tertiary structure.

The coenzyme is known to make the molecule "more dense" (from results of deuterium-exchange kinetics and the spectrophotometric titration of tyrosine residues[21]) and to increase its rigidity. Experiments with sodium dodecyl sulphate indicated that the unfolding of the molecules caused by detergents was accompanied by increases in the K_c and a_0 constants while the λ_c and b_0 constants retained their values, and points in the SCHECHTER–BLOUT plot shifted somewhat upwards from line II. One might suggest that the increase in density of the molecule results in a decrease in the K_c and a_0 constants and in a shift downwards of the points from the SCHECHTER–BLOUT line II. A small shift of this kind was observed for LDH *plus* NADH complexes (Fig. 2), while a decrease in the K_c and a_0 constants probably compensated for the increase caused by the uncoiling of the helices.

A change in the LDH structure under the action of the NADH coenzyme directly confirms KOSHLAND's induced structural fit concept in the enzyme–coenzyme system. In this reaction NADH plays the part of the "conformational cofactor", just as NAD^+ does for GAPD.

As we have already mentioned, no significant changes were observed in the structure of LDH under the action of NAD^+. It is interesting to note that the greatest conformational changes in the apoenzyme were observed under the influence of the coenzymes having the highest affinity for the apoenzyme. Thus NAD^+, being the "conformational cofactor" for GAPD ($K_s = 10^{-7}$ M), had practically no influence on the structure of LDH ($K_s = 10^{-4}$ M). On the other hand, NADH, the "conformational cofactor" for LDH ($K_s = 10^{-5}$ M) causes hardly any change in the secondary structure of GAPD ($K_s = 10^{-6}$ M).

The fact that NADH plays the part of the "conformational cofactor" for LDH is confirmed by investigations of the LDH–NADH–oxalate complex. As is evident from the results of studies on the kinetics of the reactions catalyzed by LDH, a certain order in the binding of reacting molecules to the enzyme is observed. Using the method of equilibrium centrifugation, NOVOA AND SCHWERT[16] showed that the binding of the oxalate and oxamate inhibitors is possible only after the coenzymes are bound to LDH. The evidence obtained by us—a change in the structure of LDH under the influence of NADH—suggests that these conformational changes provide the possibility for the binding of oxalate to LDH. In their turn, new conformational changes occur as a result of the binding of the inhibitor to the LDH–NADH complex (Table IV).

Comparison of the curves of λ_c for LDH (remembering that λ_c characterizes molecular structure) and of enzymic activity as functions of pH (Fig. 1) enables us to draw the following conclusion: that for the reduction of pyruvate to lactate a correlation exists between the secondary structure of LDH and its enzymic activity. The maximum LDH activity was observed at those pH levels at which LDH was characterised by the maximum λ_c values. Nevertheless, a complete coincidence of the curves was not observed: λ_c remained constant in the pH range 6 to 8.75 while the enzymic activity changed in this range. Complete correlation between these curves was observed only when the NADH coenzyme was added. A comparison between the

TABLE IV

EFFECT OF OXALATE ON THE ORD PARAMETERS OF LDH (IN 0.1 M TRIS BUFFER, pH 7.0)

Preparation and conditions of measurements	λ_c	$-K_c \times 10^{-5}$	a_0	b_0	A_{193}	A_{225}
LDH	277	95	−175	−230	498	−555
LDH *plus* oxalate (1000 moles oxalate per mole LDH)	276	95	−177	−220	488	−548
LDH *plus* NADH (20 moles NADH per mole LDH)	259	103	−180	−160	340	−454
LDH *plus* NADH *plus* oxalate (20 moles NADH and 1000 moles oxalate per mole LDH)	275	95	−185	−225	476	−540

λ_c (pH) curves for LDH *plus* NADH with those of the dependence of catalytic activity on pH shows complete correlation. This enables us to draw a conclusion concerning the direct part played by the secondary structure of the enzyme in the dependence of its catalytic activity on pH.

Evidently, in several cases, not only the ionization of specific groups of the active centre but also the structure of the molecule as a whole play definite roles in the dependence of the catalytic activity on pH.

A comparison of the curves of the secondary structure of LDH and of its catalytic activity as functions of pH for the oxidation of L-lactate does not reveal any correlation between these two properties. Thus we may also conclude that a strict and definite structure of the LDH molecule is necessary for the maximum reaction rate in this case. A structural change in any direction (increase or decrease in the α-helicity) produces a decrease in the catalytic activity.

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