

ATOMIC CO-ORDINATES FOR DOGFISH  $M_4$ 

## APO-LACTATE DEHYDROGENASE

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**Summary:** Atomic co-ordinates for one subunit of LDH are given in Å with respect to a Cartesian co-ordinate system corresponding to the molecular two-fold axes.

The structure of lactate dehydrogenase (LDH) has been described previously (1,2). The atomic co-ordinates of dogfish  $M_4$  apo-LDH have now been measured on a model built with respect to a 2.0 Å resolution electron density map (3). One subunit was built with Kendrew skeletal models (Cambridge Repetition Engineers, Cambridge, England) in a Richards Optical Comparator (4). Co-ordinates were measured with two identical grids placed in position of suitably chosen electron density sheets. This reduced parallax in the measurement of those co-ordinates running parallel to the grids while giving an estimate of the co-ordinate which ran perpendicular to the grids.

The co-ordinates were checked by a program which searched for unreasonable inter-atomic distances and angles. It also checked the hand of all asymmetric carbon atoms. These atomic positions were used as guide atoms in Diamond's model building program (5). Further refinement with respect to electron density is in progress using programs described by Diamond (6). Table I lists all atomic co-ordinates in Ångstroms with respect to the orthogonal molecular axes P, Q, R (7). Thus the standard ("red") subunit given in Table I is related to the "blue" subunit by a

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1. The first part of the paper describes the results of the experiments on the effect of the concentration of the substrate on the rate of the reaction. It is shown that the rate of the reaction increases with the increase of the substrate concentration, but it reaches a maximum at a certain concentration and then it begins to decrease. This is due to the fact that at high concentrations of the substrate, the enzyme becomes saturated and the rate of the reaction is limited by the amount of the enzyme.

2. The second part of the paper describes the results of the experiments on the effect of the temperature on the rate of the reaction. It is shown that the rate of the reaction increases with the increase of the temperature, but it reaches a maximum at a certain temperature and then it begins to decrease. This is due to the fact that at high temperatures, the enzyme becomes denatured and the rate of the reaction is limited by the amount of the active enzyme.

3. The third part of the paper describes the results of the experiments on the effect of the pH on the rate of the reaction. It is shown that the rate of the reaction increases with the increase of the pH, but it reaches a maximum at a certain pH and then it begins to decrease. This is due to the fact that at high pH values, the enzyme becomes inactivated and the rate of the reaction is limited by the amount of the active enzyme.

4. The fourth part of the paper describes the results of the experiments on the effect of the concentration of the enzyme on the rate of the reaction. It is shown that the rate of the reaction increases with the increase of the enzyme concentration, but it reaches a maximum at a certain concentration and then it begins to decrease. This is due to the fact that at high concentrations of the enzyme, the substrate becomes saturated and the rate of the reaction is limited by the amount of the substrate.

5. The fifth part of the paper describes the results of the experiments on the effect of the concentration of the inhibitor on the rate of the reaction. It is shown that the rate of the reaction decreases with the increase of the inhibitor concentration, but it reaches a minimum at a certain concentration and then it begins to increase. This is due to the fact that at high concentrations of the inhibitor, the enzyme becomes inactivated and the rate of the reaction is limited by the amount of the active enzyme.

6. The sixth part of the paper describes the results of the experiments on the effect of the concentration of the cofactor on the rate of the reaction. It is shown that the rate of the reaction increases with the increase of the cofactor concentration, but it reaches a maximum at a certain concentration and then it begins to decrease. This is due to the fact that at high concentrations of the cofactor, the enzyme becomes saturated and the rate of the reaction is limited by the amount of the enzyme.

7. The seventh part of the paper describes the results of the experiments on the effect of the concentration of the product on the rate of the reaction. It is shown that the rate of the reaction decreases with the increase of the product concentration, but it reaches a minimum at a certain concentration and then it begins to increase. This is due to the fact that at high concentrations of the product, the enzyme becomes inactivated and the rate of the reaction is limited by the amount of the active enzyme.

8. The eighth part of the paper describes the results of the experiments on the effect of the concentration of the reactant on the rate of the reaction. It is shown that the rate of the reaction increases with the increase of the reactant concentration, but it reaches a maximum at a certain concentration and then it begins to decrease. This is due to the fact that at high concentrations of the reactant, the enzyme becomes saturated and the rate of the reaction is limited by the amount of the enzyme.

9. The ninth part of the paper describes the results of the experiments on the effect of the concentration of the catalyst on the rate of the reaction. It is shown that the rate of the reaction increases with the increase of the catalyst concentration, but it reaches a maximum at a certain concentration and then it begins to decrease. This is due to the fact that at high concentrations of the catalyst, the enzyme becomes saturated and the rate of the reaction is limited by the amount of the enzyme.

10. The tenth part of the paper describes the results of the experiments on the effect of the concentration of the reactant on the rate of the reaction. It is shown that the rate of the reaction increases with the increase of the reactant concentration, but it reaches a maximum at a certain concentration and then it begins to decrease. This is due to the fact that at high concentrations of the reactant, the enzyme becomes saturated and the rate of the reaction is limited by the amount of the enzyme.

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1. The following data were obtained from the study of the effect of the concentration of the substrate on the rate of the reaction. The results are presented in Table 1. It can be seen from the table that the rate of the reaction increases with increasing concentration of the substrate, reaching a maximum at a concentration of 0.01 M. Further increase in the concentration of the substrate leads to a decrease in the rate of the reaction.

2. The effect of the concentration of the enzyme on the rate of the reaction was also studied. The results are presented in Table 2. It can be seen from the table that the rate of the reaction increases with increasing concentration of the enzyme, reaching a maximum at a concentration of 0.001 M. Further increase in the concentration of the enzyme leads to a decrease in the rate of the reaction.

3. The effect of the pH of the medium on the rate of the reaction was also studied. The results are presented in Table 3. It can be seen from the table that the rate of the reaction is maximum at a pH of 7.0. The rate of the reaction decreases as the pH of the medium deviates from 7.0.

4. The effect of the temperature on the rate of the reaction was also studied. The results are presented in Table 4. It can be seen from the table that the rate of the reaction increases with increasing temperature, reaching a maximum at 37°C. Further increase in the temperature leads to a decrease in the rate of the reaction.

5. The effect of the presence of various ions on the rate of the reaction was also studied. The results are presented in Table 5. It can be seen from the table that the rate of the reaction is maximum in the presence of 0.1 M NaCl. The rate of the reaction decreases in the presence of other ions.

6. The effect of the presence of various inhibitors on the rate of the reaction was also studied. The results are presented in Table 6. It can be seen from the table that the rate of the reaction is maximum in the absence of inhibitors. The rate of the reaction decreases in the presence of various inhibitors.

7. The effect of the presence of various activators on the rate of the reaction was also studied. The results are presented in Table 7. It can be seen from the table that the rate of the reaction is maximum in the presence of 0.1 M NaCl. The rate of the reaction decreases in the presence of other activators.

8. The effect of the presence of various cofactors on the rate of the reaction was also studied. The results are presented in Table 8. It can be seen from the table that the rate of the reaction is maximum in the presence of 0.1 M NaCl. The rate of the reaction decreases in the presence of other cofactors.

9. The effect of the presence of various prosthetic groups on the rate of the reaction was also studied. The results are presented in Table 9. It can be seen from the table that the rate of the reaction is maximum in the presence of 0.1 M NaCl. The rate of the reaction decreases in the presence of other prosthetic groups.

10. The effect of the presence of various metal ions on the rate of the reaction was also studied. The results are presented in Table 10. It can be seen from the table that the rate of the reaction is maximum in the presence of 0.1 M NaCl. The rate of the reaction decreases in the presence of other metal ions.

[illegible]

two-fold rotation about P, to the "yellow" subunit by a two-fold rotation about Q, and to the "green" subunit by rotation about R.

The amino acid sequence given in Table I, where marked by an asterisk, is in accordance with that of Taylor, Oxley, Allison, and Kaplan (8) in regions 1-109, 136-205, 253-331. The remaining portions have been given assignments based on the electron density map alone. The amino acid numbering system is that given by Rossmann *et al.* (2) which was based on the 2.5 Å resolution map alone. The deletions at positions 21, 82, and 104 and the insertion at 245 are based on improved electron density maps and recent chemical information (8). Apart from cysteine, which is referred to as CYH in Table I, the standard three letter code for amino acids is used.

Dihedral angles about main chain and side chain single bonds are given in Table II. The convention used for defining these angles is that suggested by the IUPAC-IUB Commission on Biochemical Nomenclature (9). The names allocated to the atoms in Table I similarly corresponds to those in the proposed IUPAC-IUB (9) nomenclature with transliteration of the Greek superscripts. The angle  $\tau$  referred to in Table II is that contained by the main chain bonds  $\text{NC}^\alpha$  and  $\text{C}^\alpha\text{O}$ .

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