Effect of Magnetic Field on Ascorbic Acid Oxidase Activity, I

V. S. Ghole

Division of Biochemistry, Department of Chemistry
University of Poona, Poona 411007, India

P. S. Damle

Division of Biophysics, Department of Physics
University of Poona, Poona 411007, India

W. H.-P. Thiemann

Physikalische Chemie, Fachbereich 2 - Chemie, Universität Bremen, Postfach 330440, D-2800 Bremen 33, Bundesrepublik Deutschland

Z. Naturforsch. **41c,** 355–358 (1986);
received October 12, 1984/November 25, 1985

Magnetochemistry, Enzymatic Induction, Ascorbic Acid Oxidase, Mixed Type Inhibition, Homogeneous Magnetic Field

A homogeneous magnetic field of 1.1 T strength exhibits a significant influence on the activity of the enzyme ascorbic acid oxidase *in vitro*. A Lineweaver-Burk plot of the reaction shows the typical pattern of a mixed-type inhibition, *i.e.* a larger rate of reaction at low substrate concentrations and a smaller rate of reaction at high substrate concentration than that of the control without magnetic field applied.

Introduction

Magnetic fields have been reported to affect living systems in various ways [1]. Many experiments have been described in particular to demonstrate the effects of magnetism on *in vivo* systems. But often it is very difficult to get a straight-forward insight into the mechanism of the response of a living system to the magnetic field as the system is complex and feedback effects may obscure simple models. In order to arrive at a better understanding of the interaction of magnetic fields with living systems it appears logical to study first *in vitro* systems with only a minimum of controllable variables. Enzymes play an important role as biological key compounds and as chemically well defined macromolecules. In this study we have investigated the interaction of a static homogeneous magnetic field of 1.1 T fields strength with the activity of the enzyme ascorbic acid oxidase (E.C. No. 1.10.3.3). Few works had been published on enzymes such as catalase and trypsin in strong magnetic fields, where an increase of activity had been reported on catalase, while the results with trypsin are somewhat contradictory [2–5].

Reprint requests to Prof. Dr. W. Thiemann.

Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen
0341–0382/86/0300–0355 $ 01.30/0

Materials and Methods

The enzyme ascorbic acid oxidase was extracted from cucumber by usual procedures and purified by 50% *v/v* acetone precipitation. Traces of acetone were removed under vacuum at low temperature. The activity of the enzyme was defined by measuring the residual non-reacted ascorbic acid substrate in the reaction mixture by the 2,6-dichlorophenolindophenol method [6]. The enzyme unit is defined as 1 unit oxidizing 1 micromol ascorbic acid per minute. To avoid temperature effects and other interfering variations a double horse shoe magnet producing 1.1 T at the center of the poles was used. All reactions were carried out within the small gap of 8 mm width for which a special type of glass cell was constructed. Each individual experiment described below was performed in triplicate: while the one cell was exposed to the magnetic field, the other one was placed at a distance of 5 meters from the magnet, and the incubation was started by adding the enzyme at the same time and stopped after the same time elapsed in both cells in parallel. (The identical influence of Earth's magnetic field in both cells was ignored.) Reactions were carried out at 30 °C which temperature was kept constant during the experiment; the solutions were stirred with a small magnetic bar to compensate for any migration effect of colloidal particles caused by a residual inhomogenicity of the field applied.

Experiments

Some introductory single experiments are reported below, before the variation of the rate of reaction with the substrate concentration was determined in order to measure the kinetic parameters and to deduce the mechanistic model of magnetic interaction.

Experiment No. 1

5.7 μmol of ascorbic acid and 1 unit of enzyme was mixed in 1 ml phosphate buffer and incubated for exactly 5 minutes inside and outside the magnetic field as described above.

Result

Enzyme activity outside the magnetic field: 0.27 units; enzyme activity inside the magnetic field: 0.21 units; hence the enzyme activity was decreased by magnetic field by 22%.

Experiment No. 2

5.7 μmol ascorbic acid and 1 unit of enzyme was mixed in 1 ml and incubated for 30 minutes inside and outside the magnetic field; thereafter an amount of another 5.7 μmol ascorbic acid was added to the mixture, which was reincubated for another 5 minutes.

Result

Enzyme activity outside magnetic field: 0.136 units; enzyme activity inside magnetic field: 0.088 units; hence the enzyme activity was decreased by the magnetic field by 35%.

Experiment No. 3

1 unit enzyme *alone* was exposed to the magnetic field for 30 minutes. (Control the same without the field!) The cell was removed thereafter from the magnet and 5.7 μmol ascorbic acid added, both sample and control were incubated for another 5 minutes outside the magnetic field.

Result

Activity of the enzyme sample which had not experienced the magnetic field: 0.28 units; activity of the enzyme which had experienced an exposure to the magnetic field prior to reaction: 0.27 units; hence the activity of the enzyme was decreased only by 5.5% by the magnetic field, which lies within the margin of variance.

Experiment No. 4

The exposed compounds were reversed in their sequence: this time 5.6 μmol ascorbic acid substrate *alone* was exposed to the magnetic field for 30 minutes. (Control outside the field!) Thereafter 1 unit enzyme was added and incubation carried out in both cells outside the field for another 5 minutes.

Result

Activity of the enzyme with the substrate not exposed to the magnetic field: 0.28 units; activity of the enzyme with the substrate exposed to the magnetic field prior to reaction: 0.27 units; hence the activity of the enzyme reacting with the exposed substrate was decreased by 5.5% by the field, which lies within the margin of statistical variance.

Experiment No. 5

In this experiment the kinetic parameters V_{max} and K_s were determined through the classical Michaelis-Menten plot of the variation of enzymic rate with concentration of the substrate: 19 individual experiments with varying of the substrate concentrations in equal steps ranging from 0.11 mM to 5.1 mM were performed in the magnetic field as well as simultaneously outside the field. The readings were taken after each 5 minutes reaction time. The final results of this experimental series are given in Table I, which are derived from the direct (Fig. 1) and reciprocal plot (Fig. 2) of reaction rates versus substrate concentrations.

Results and Discussion

The experiments no. 1, 2, 3, and 4 show clearly that the enzyme activity is strongly influenced by a homogeneous magnetic field. Conclusions are drawn from this observation as follows: A magnetic field of 1.1 T inhibits the enzyme activity to a degree of 22% at short reaction times and to a degree of 35% at longer reaction times: the inhibition is not directly proportional to the reaction time. The magnetic field obviously does not act upon either the enzyme or the substrate alone prior to the reaction when both reaction partners are present at the same time: the effect must be primarily a kinetic one, not a thermodynamic one changing the conformation of enzyme or substrate in a permanent way (*cf.* experiment no. 3 and 4). The Fig. 1 evaluating experiment no. 5 demonstrates the type of inhibitory effect of magnetic interaction: The control experiment supposedly obeys the classical Michaelis-Menten behavior as given by equation (1):

$$E + S \underset{}{\overset{K_s}{\rightleftharpoons}} ES \overset{K_p}{\rightleftharpoons} E + P. \qquad (1)$$

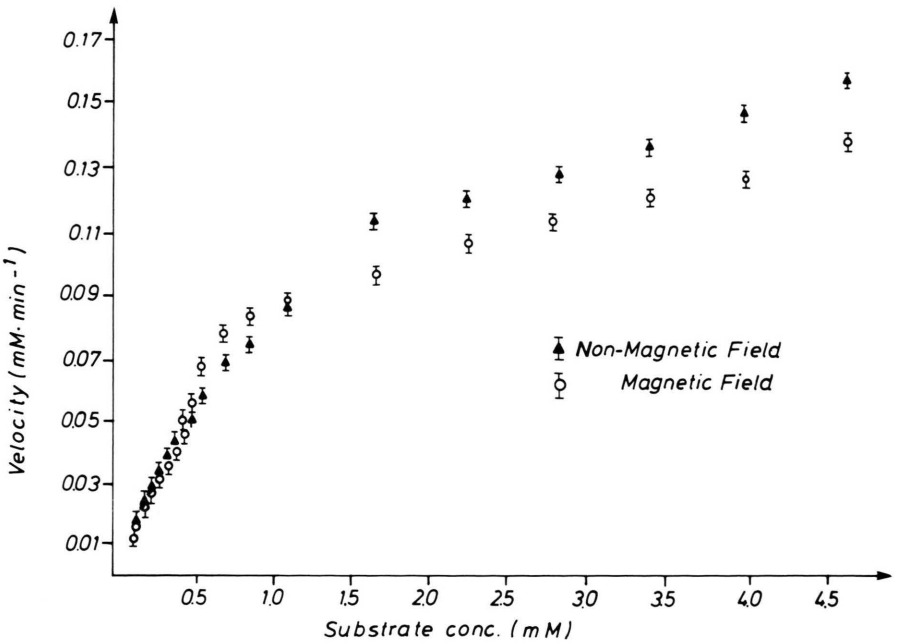

Fig. 1. The rate of oxidation of ascorbic acid by the enzyme is plotted *vs.* the concentration of the substrate. Each point – both control and sample exposed to 1.1 T magnetic field – represents the arithmetic mean of a triplicate experiment.

When exposed to the magnetic field the formation and dissociation of the ES complex is affected in such a way that the modified reaction may be described by equation (2):

$$E + S \underset{}{\overset{\alpha K_s}{\rightleftharpoons}} ES^* \overset{\beta K_p}{\rightleftharpoons} E + P, \qquad (2)$$

where ES* is defined as the magnetic-field-modified complex, α is a factor which changes K_s and β is a factor which changes K_p under the magnetic field applied. In accordance with Segel [7] this behavior is termed the "mixed-type inhibitory" effect: The magnetic field increases the affinity of the enzyme for the substrate but decreases the rate of formation of the products, as long as $\alpha < 1$ and $\beta < 1$. In other words both dissociations constants K_s as well as K_p decrease under the magnetic field. Fig. 1 shows that up to an intersection point of about 1 mM substrate concentration the rate of reaction increases due to the increased affinity of the enzyme for the substrate, while on the other hand from the intersection point upward to larger substrate concentrations the rate of reaction is decreased due to an inhibition of the dissociation of the complex into the free enzyme and the product as compared to the control. Thus the effect of the magnetic field upon the oxidation of ascorbic acid depends on the concentration of the ascorbic acid: at larger concentration inhibition finally exceeds the favorisation of the initial formation of the enzyme substrate complex. Fig. 2 gives the reciprocal Lineweaver-Burk plot which allows the quantification of the above defined kinetic parameters.

Table I. Kinetic parameters as described in text.

	Control - outside magnetic field	Sample exposed to 1.1 T magnetic field
K_s [mM]	1.21 ± .06	0.96 ± 0.05
slope [S]/V [min]	6.94 ± .35	6.25 ± 0.31
V_{max} [mM min^{-1}]	0.175 ± .008	0.154 ± 0.007
α	1.0	0.69
β	1.0	0.84

It must be concluded that the enzyme ascorbic acid oxidase which contains copper as cofactor at the active site [8] is affected by the exposure to a magnetic

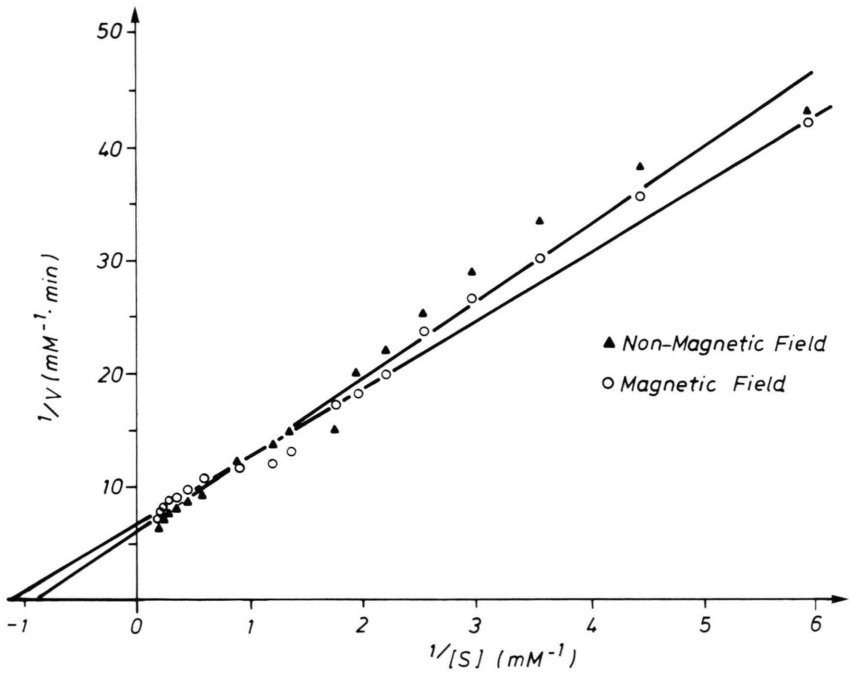

Fig. 2. The same data as in Fig. 1 plotted as the reciprocal values of reaction rate *vs.* substrate concentration in the usual Lineweaver-Burk manner, from which the kinetic parameters in Table I are calculated.

field of 1.1 T strength to a significant degree: at low concentration of substrates the enzyme is activated while at large concentrations it is deactivated. The magnetic field does not affect the enzyme nor the substrate isolated from each other; the effect under consideration is operating only in the presence of the field, the original activity is restored when the field is removed.

Acknowledgements

The authors are indebted to Dr. P. N. Joshi, Professor of Biochemistry Division, University of Poona, Dr. M. V. Hegde, Reader in Biochemistry, University of Poona, and Ms. S. R. Joshi, post-graduate student, University of Poona, for their encouragement, valuable suggestions, and active help throughout the work.

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