BRIEF COMMUNICATION

EFFECTS OF PRESSURE AND TEMPERATURE ON THE REACTIONS OF HORSERADISH PEROXIDASE WITH HYDROGEN CYANIDE AND HYDROGEN PEROXIDE

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ABSTRACT Reactions of ferric horseradish peroxidase with hydrogen cyanide and hydrogen peroxide were studied as a function of pressure. Activation volumes are small and differ in sign ($\Delta V^{\ddagger}$ = 1.7 ± 0.5 ml/mol for peroxidase + HCN and −1.5 ± 0.5 ml/mol for peroxidase + H_2O_2). The temperature dependence of cyanide binding to horseradish peroxidase was also determined. A comparison is made of relevant parameters for cyanide binding and compound I formation.

INTRODUCTION

Effects of pressure on equilibrium and NMR properties of native horseradish peroxidase have been investigated (Ogunmola et al., 1977; Morishima et al., 1980). However, the potential of high-pressure stopped flow experiments in this field has not been previously exploited (Asano and Le Noble, 1978). We have chosen cyanide binding to the ferric enzyme as an example of a ligand-binding reaction. The formation of compound I is the initial obligatory step in the enzymatic cycle. We have determined the volumes and energies of activation for these processes and compared them.

EXPERIMENTAL

Materials

Horseradish peroxidase (E.C. 1.11.1.7 donor H_2O_2 oxidoreductase) was purchased from Boehringer-Mannheim Biochemicals, Indianapolis, Ind. (lot 1399140) as an ammonium sulfate suspension and dialyzed extensively against multiply distilled water (Hewson and Dunford, 1976). The purity number (ratio of absorbances at 403 and 280 nm) was 3.22. Enzyme solution concentrations were measured at 403 nm using a molar absorbance of $1.02 \times 10^5\ M^{-1}cm^{-1}$ (Ohlsson and Paul, 1976). Potassium cyanide was analytical grade Merck brand (Merck, Darmstadt, F.R.G.) for the pressure study and Fisher brand (Fisher Scientific Co., Pittsburgh, Pa.) for the temperature study. Cyanide solutions were prepared daily and their concentrations found by weight. Hydrogen peroxide was bought from Fluka A.G. (Buchs, Switzerland) and its concentration was determined as described by Cotton and Dunford (1973).

Apparatus and Methods

All high-pressure kinetic experiments were performed at 25.0 ± 0.2°C on a stopped flow apparatus specifically designed for this purpose (Heremans et al., 1980). The reaction of the ferric enzyme with cyanide was monitored at 420 nm whereas compound I formation was followed at 403 nm. Absorbance measurements were performed on a Cary 118 spectrophotometer (Cary Instruments, Monrovia, Calif.) and the pH was measured with a Radiometer pH meter (model 26; Copenhagen, Denmark) in conjunction with a Radiometer/Copenhagen electrode. All solutions were 0.10 ionic strength in potassium nitrate and 0.01 ionic strength in phosphate buffer of pH 7.0. The observation chamber enzyme concentrations were 2.5 and 1.0 μM for cyanide binding and compound I formation experiments, respectively. Excess substrate, always at least tenfold, ensured pseudo–first-order conditions and prevented the influence of the dissociation reaction in the case of cyanide binding. Average first-order rate constants were calculated from the results of three or four traces recorded for each reaction under a specific set of conditions.

Experiments to obtain the temperature dependence of cyanide binding to horseradish peroxidase at one atmospheric pressure were performed using the stopped flow mode of a Union Giken (model 601; Osaka, Japan) rapid-reaction analyzer which has previously been described (Palcic and Dunford, 1980). Temperatures were accurate to ±0.3°C. The concentrations of enzyme and cyanide in the observation chamber were always 1.0 μM and 9.35×10^{-5} M. Both enzyme and cyanide solutions were of total ionic strength 0.11 (0.10 because of potassium nitrate and 0.01 because of phosphate buffer of pH 6.9). Between four and nine traces were recorded at each temperature. The mean values have a precision of ±5%.

RESULTS

Second-order rate constants obtained at 1 atm on the high-pressure apparatus are 9.2×10^4 $M^{-1}s^{-1}$ for cyanide binding to the ferric enzyme and 1.2×10^7 $M^{-1}s^{-1}$ for compound I formation in good agreement with the literature (Marklund et al., 1974; Dunford et al., 1978). The dependences on pressure of the rate constants for the two reactions are shown in Fig. 1. Activation volumes were calculated from the slopes of the linear plots of ln k vs. p (Asano and Le Noble, 1978). The Arrhenius equation $k = A \exp(-E_a/RT)$ was used to analyze temperature-dependent data (Fig. 2). The pressure- and temperature-dependent results are summarized in Table I.

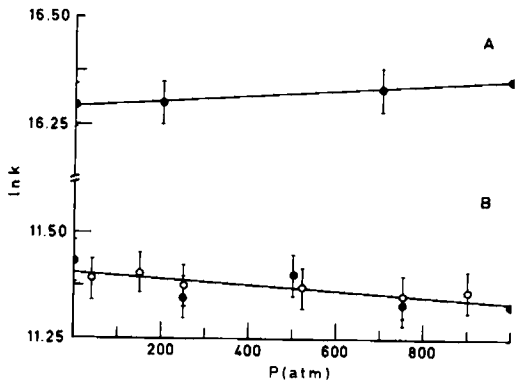

FIGURE 1 Dependence of ln k on pressure for the reaction of horseradish peroxidase (HRP) (A) with H_2O_2 and (B) HCN. $[H_2O_2] = 10$ μM in all cases. ●, [HCN] = 1.0×10^{-4} M; ○, [HCN] = 5.0×10^{-5} M.

TABLE I

ACTIVATION PARAMETERS FOR HCN BINDING AND COMPOUND I FORMATION USING H_2O_2 WITH HRP*

	Activation volume	Activation energy
	(*ml/mol*)	(*J/mol*)
HCN	$+1.7 \pm 0.5$	$(3 \pm 1) \times 10^4$
H_2O_2	-1.5 ± 0.5	9×10^3‡

*HRP, horseradish peroxidasec

‡Data from Marklund et al. (1974).

DISCUSSION

For both cyanide binding and compound I formation, the electrically neutral un-ionized ligand or substrate reacts (Dunford and Stillman, 1976; Dunford et al., 1978). Each small molecule must lose a proton before (or in concert with) bond formation to iron. For HCN ionization ΔV is -7 ml/mol and for H_2O_2 we assume a value similar to phenol, -19 ml/mol, based on similar entropy changes (Hamann, 1974). There is a volume change of 10–12 ml/mol when a proton is added to a carboxyl group, and the catalytic effect of such a group, perhaps the side chain of a distal aspartate residue, has been demonstrated by Dunford and Araiso (1979). Thus compensating effects can lead to the results observed by us. The small activation volumes also indicate that no large contributions from bond formation, spin-state conversions, or electron transfer occur, unless they compensate.

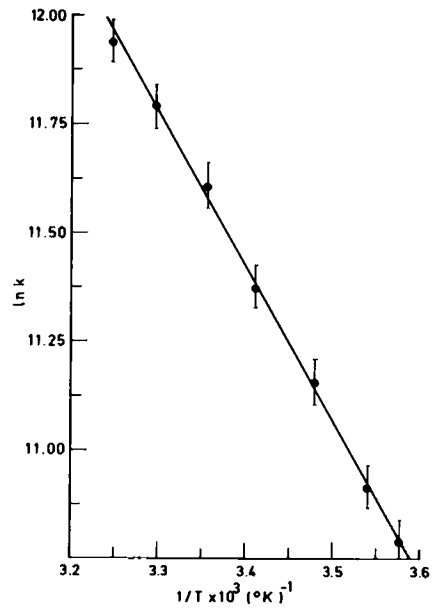

FIGURE 2 Plot of ln k vs. $1/T$ to determine the Arrhenius activation energy for the binding of cyanide to HRP.

Turnip peroxidase isoenzyme P1 is very similar to horseradish peroxidase isoenzyme C: the activation energy for compound I formation from the turnip isoenzyme is in agreement with the results in Table I (Job et al., 1978).

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REFERENCES

Asano, T., and W. J. Le Noble. 1978. Activation and reaction volumes in solution. *Chem. Rev.* 78:407–489.

Cotton, M. L., and H. B. Dunford. 1973. Horseradish peroxidase. XI. On the nature of compounds I and II as determined from the kinetics of the oxidation of ferrocyanide. *Can. J. Chem.* 51:582–587.

Dunford, H. B., and J. S. Stillman. 1976. On the function and mechanism of action of peroxidase. *Coord. Chem. Rev.* 19:187–251.

Dunford, H. B., W. D. Hewson, and H. Steiner. 1978. Horseradish peroxidase. XXIX. Reactions in water and deuterium oxide: cyanide binding, compound I formation and reactions of compounds I and II with ferrocyanide. *Can. J. Chem.* 56:2844–2852.

Dunford, H. B., and T. Araiso. 1979. Horseradish peroxidase. XXXVI. On the difference between peroxidase and metmyoglobin. *Biochem. Biophys. Res. Commun.* 89:764–768.

Hamann, S. D. 1974. Electrolyte solutions at high pressure. *Mod. Aspects Electrochem.* 9:47–158.

Heremans, K., J. Snauwaert, and J. Rijkenberg. 1980. Stopped-flow apparatus for the study of fast reactions in solution under high pressure. *Rev. Sci. Instrum.*, 51:806–808.

Hewson, W. D., and H. B. Dunford. 1976. Oxidation of *p*-cresol by horseradish peroxidase compound I. *J. Biol. Chem.* 251:6036–6042.

Job, D., J. Ricard, and H. B. Dunford. 1978. Kinetics of formation of the primary compound from hydrogen peroxide and turnip peroxidases. *Can. J. Biochem.* 56:702–707.

Marklund, S., P.-I. Ohlsson, A. Opara, and K.-G. Paul. 1974. The substrate profiles of the acidic and slightly basic horseradish peroxidases. *Biochim. Biophys. Acta.* 350:304–313.

Morishima, I., S. Ogawa, and H. Yamada. 1980. High pressure proton nuclear magnetic resonance studies of hemoproteins. Pressure-induced structural change in heme environments of myoglobin, hemoglobin and horseradish peroxidase. *Biochemistry.* 19:1569–1575.

Ogunmola, G. B., A. Zipp, F. Chen, and W. Kauzmann. 1977. Effects of pressure on visible spectra of complexes of myoglobin, hemoglobin, cytochrome *c*, and horseradish peroxidase. *Proc. Natl. Acad. Sci. U. S. A.* 74:1–4.

Ohlsson, P.-I., and K.-G. Paul. 1976. The molar absorptivity of horseradish peroxidase. *Acta Chem. Scand.* B30:373–375.

Palcic, M. M., and H. B. Dunford. 1980. The reaction of human erythrocyte catalase with hydroperoxides to form compound I. *J. Biol. Chem.* 255:6128–6132.