THE OXIDATION OF RIBOSE 5-PHOSPHATE BY FERRICYANIDE IN THE PRESENCE OF CATALASE PREPARATIONS

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SUMMARY

1. The rate of oxidation of ribose 5-phosphate (R 5-P) by ferricyanide in the presence of purified preparations of catalase and alfalfa R 5-P isomerase was proportional to the R 5-P isomerase activity of the enzyme preparations and not to their catalatic activity.

(2) Anaerobic incubation of R 5-P with either type of enzyme preparation resulted in the accumulation of a transformation product of R 5-P which reduced ferricyanide or iodine non-enzymically. The rate of this reaction was proportional to the concentration of a product of R 5-P which had a definite u.v. spectrum with a maximum at 284 mμ.

3. Substances which inhibited the ferricyanide reduction (cyanide, borate, 5-phosphoribonic acid) prevented the increase in optical density at 284 mμ, while those which did not affect the oxidation reaction (azide, 3-amino-1:2:4-triazole) also did not affect the spectral change.

4. The oxidation of R 5-P in this system used up four equivalents of ferricyanide or iodine.

5. The curve relating the rate of reduction of ferricyanide to the pH had a broad maximum at about pH 9 and differed considerably from the pH-activity curves of isomerase and catalase.

6. It was concluded that the oxidation of R 5-P by ferricyanide occurring in the presence of recrystallized preparations of catalase was probably due to contamination of the enzyme preparations with R 5-P isomerase, and that the ferricyanide-reducing substance which accumulated was possibly a further transformation product of ribulose 5-phosphate.

INTRODUCTION

FRANCOEUR AND DENSTEDT[1] showed that red-blood-cell haemolysates as well as purified preparations of liver catalase were able to promote the oxidation of ribose 5-phosphate (R 5-P) by ferricyanide under anaerobic conditions. This effect was prevented by cyanide but not by azide; these authors concluded that catalase probably acted by accepting electrons from the substrate leading to a reduction of the catalase

References p. 139/140.

iron from Fe^{+++} to Fe^{++}, and that the ferro-catalase was then oxidized back to the usual ferri-catalase by ferricyanide.

Following the observation that preparations of catalase inhibited by 3-amino-1,2,4-triazole were fully active in the oxidation of R 5-P by ferricyanide[2] it was found that the rate of this reaction was proportional not to the catalatic activity of the enzyme preparation but to its R 5-P isomerase activity. The same reaction could be carried out with a plant R 5-P isomerase preparation showing no significant catalatic activity. These results as well as other characteristics of the reaction and the effect of various inhibitors are reported below.

MATERIALS AND METHODS

Enzymes

Catalase: A commercial, twice-crystallized beef-liver catalase preparation (Worthington Biochemical Corp., New Jersey; Kat. f. value: 26,000) and a crystallized human-erythrocyte catalase (Kat. f. value: 58,000) prepared according to HERBERT AND PINSENT[3] were used.

Isomerase. Alfalfa R 5-P isomerase was prepared according to AXELROD AND JANG[6], taking the purification to the 5th step. This preparation contained 10,600 isomerase units/mg protein N and had a low catalatic activity.

Enzymic activities

Catalatic activity was estimated according to FEINSTEIN[4] using sodium perborate as substrate at 37°, and Kat. f. values were determined according to VON EULER AND JOSEPHSON[5]. *Isomerase activity* was determined according to AXELROD AND JANG[6]. *The rate of oxidation of R-5-P* was estimated by following the reduction of ferricyanide colorimetrically at 37° in Thunberg tubes under N_2, and unless otherwise stated, at pH 7.0 (phosphate buffer 0.05 *M*), in a mixture containing 0.02 *M* R 5-P, the enzyme and 0.002 *M* $K_3Fe(CN)_6$. A Klett-Summerson photoelectric colorimeter (filter 42) into which the Thunberg tubes could fit directly was used. The results were plotted and the amount of reduction of ferricyanide per hour was calculated from the initial rate, determined graphically.

R 5-P was obtained from a commercial preparation of the Ba salt by treatment with a cation exchanger (Dowex-50) in the H^+ form. 5-Phosphoribonic acid was prepared by bromine oxidation of R 5-P, essentially by the method of HORECKER[7] for the preparation of 6-phosphogluconate. Sugar phosphates were separated from incubation mixtures by the barium–ethanol fractionation technique[8], following deproteinization with trichloracetic acid.

The orcinol reaction for pentoses was carried out according to MEJBAUM[9]. Ketopentose was determined by the cysteine–carbazole reaction according to AXELROD AND JANG[6] and inorganic and total phosphorus by the method of FISKE AND SUBBAROW[10]. Nitrogen was determined according to KOCH AND MCMEEKIN[11] and haematin in the catalase preparations according to KEILIN AND HARTREE[12].

Localization of ferricyanide-reducing substance on paper chromatograms

Paper chromatograms thoroughly dried after development were lightly sprayed with 0.5 % $K_3Fe(CN)_6$ in 0.1 *M* phosphate buffer pH 6.8. After heating for 3–5 min at 80° the ferricyanide-reducing material was localized by lightly spraying with 1 % $FeCl_3$ in water. Under these conditions R 5-P did not react.

References p. 139/140.

U.V. spectra were measured with a Beckman D.U. spectrophotometer, and when anaerobic conditions were required quartz spectrophotometer cells fused onto quartz Thunberg tubes were used. N_2 was purified by passing through a column of activated copper, deposited on kieselguhr at 180°[13].

RESULTS

Effect of preincubation

When R 5-P was incubated at 37° under purified N_2 at pH 7.0 with preparations of either recrystallized beef-liver or human-erythrocyte catalase it was found that the rate of reduction of subsequently added ferricyanide increased with the time of incubation. The results of such an experiment are presented in Fig. 1 (see curve 2). In the control tubes which did not contain catalase or which contained boiled catalase the rate of ferricyanide reduction also increased but only very slightly (see Fig. 1, curve 4). It could further be shown that this effect was not a non-specific protein effect since preincubation with ten different purified proteins, at protein concentrations equal to that of the catalase, did not result in a significant increase of the rate of ferricyanide reduction above that of the control. The proteins used were: egg albumin (2 × cryst.), bovine plasma globulin, bovine plasma albumin, beef-heart cytochrome *c* (chromatographically purified), horse-heart myoglobin (2 × cryst.), egg-white lysozyme (2 × cryst.), pepsin (2 × cryst.), trypsin (2 × cryst.), chymotrypsin (2 × cryst.) and soya-bean trypsin inhibitor (5 × cryst.).

However, after a sufficient time of preincubation (6–10 h) the presence of the catalase was no longer required, since the protein could be centrifuged off after denaturation by boiling for 5 min, and the supernatant solution reduced ferricyanide at a rate equal to or above that of a control solution from which the catalase had not been removed. Deproteinization with cold 10 % trichloracetic acid gave the same results.

These experiments indicated that in the oxidation of R 5-P by ferricyanide, promoted by catalase preparations, the enzyme preparation did not itself react with ferricyanide, but was necessary to cause the transformation of the R 5-P into a substance or substances which reduced ferricyanide non-enzymically.

R 5-P isomerase activity of catalase and alfalfa isomerase preparations and rate of ferricyanide reduction

It was found that all the purified preparations of catalase tested had a small but definite R 5-P isomerase activity. Moreover, the rate of ferricyanide reduction was roughly proportional to the isomerase and not to the catalase activity of the preparations. This was best demonstrated with the alfalfa isomerase preparation which had a low catalatic activity and a high isomerase activity, resulting in a high rate of ferricyanide reduction. At a concentration of alfalfa isomerase in the test mixture which gave the same rate of R 5-P isomerization as the concentrations of catalase usually used, the rate of ferricyanide reduction was identical with both enzyme preparations, although the catalatic activity of the isomerase preparation was 215 times less than that of the beef-liver catalase. Typical results are given in Table I.

Effect of inhibitors

FRANCOEUR AND DENSTEDT[1] showed that cyanide inhibited the oxidation of

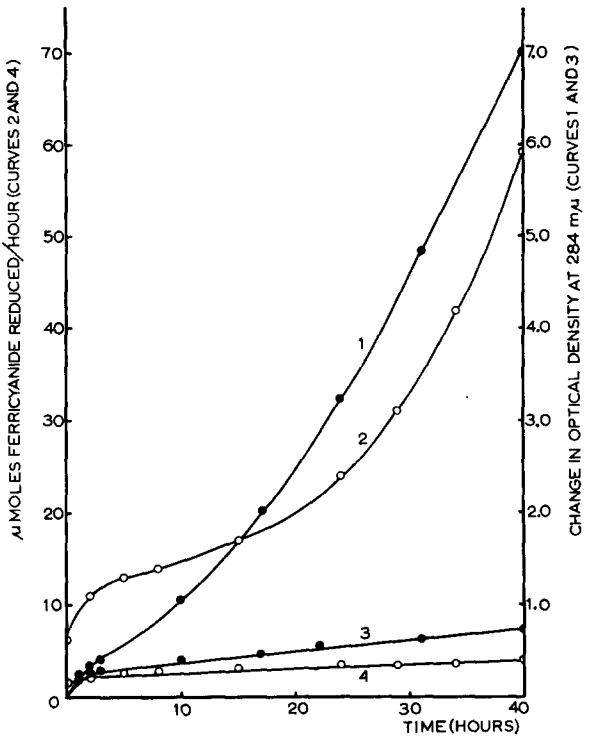

Fig. 1. Effect of incubation of a recrystallized beef-liver catalase preparation with R 5-P, on the rate of reduction of subsequently added ferricyanide, and on the optical density at 284 mμ. Final concentrations in mixtures used for ferricyanide-reduction tests: recrystallized beef-liver catalase haematin, $0.51 \cdot 10^{-5}$ *M*, containing 0.6 R 5-P isomerase units/ml; R 5-P, 0.024 *M*; phosphate buffer, pH 7.0, 0.05 *M*. Total vol., 30 ml. Incubated at 37° under purified N_2. At intervals 5.0 ml of mixture removed to Thunberg tube, 1 ml of 0.012 *M* $K_3Fe(CN)_6$ added at zero time under N_2, and rate of reduction at 37° estimated as given under METHODS (Curve No. 2). Control mixture not containing catalase preparation was treated similarly (Curve No. 4). Final concentrations in mixtures used for estimation of optical density change at 284 mμ: recrystallized beef-liver catalase haematin, $0.21 \cdot 10^{-5}$ *M* containing 0.25 R 5-P isomerase units/ml; R 5-P, 0.02 *M*; phosphate buffer, pH 7.0, 0.05 *M*. Total vol., 6.0 ml. Incubated at 37° under purified N_2 in quartz Thunberg tubes sealed onto quartz 1-cm spectrophotometer cells. When optical density at 284 mμ increased above 1.0, suitably diluted samples were read (Curve No. 1). Control mixture did not contain catalase preparation (Curve No. 3).

TABLE I

CATALATIC AND R 5-P ISOMERASE ACTIVITIES OF PREPARATIONS OF BEEF-LIVER AND HUMAN-ERYTHROCYTE CATALASE AND OF ALFALFA R 5-P ISOMERASE

Catalatic activity determined by the method of FEINSTEIN[4]. Rate of reduction of ferricyanide estimated as given under METHODS, with the concentrations of the enzyme preparations adjusted so as to give the same rate of ferricyanide reduction for the 3 preparations (about 6.4 μmoles/h). R 5-P isomerase activity determined according to AXELROD AND JANG[6].

Preparation	*Catalatic activity moles perborate/mg protein N*	*Ferricyanide reduction rate μmoles ferricyanide/h/mg protein N*	*R 5-P isomerase activity units/mg protein N*
Beef-liver catalase	1.29	16.5	7.8
Human-erythrocyte catalase	2.80	2,520	1,200
Alfalfa R 5-P isomerase	0.006	19,800	10,600

References p. 139/140.

R 5-P by catalase preparations while azide did not, although both are strong catalase inhibitors. MARGOLIASH AND NOVOGRODSKY[2] found that catalase fully and irreversibly inhibited by 3-amino-1,2,4-triazole still retained its entire activity in the oxidation of R 5-P by ferricyanide.

When these inhibitors were tested for their effect on the isomerase activity of the catalase preparations it was found that azide and 3-amino-1,2,4-triazole did not have any effect, whereas cyanide at the concentration used caused a partial inhibition (Table II). Similarly, azide and 3-amino-1,2,4-triazole did not affect the rate of ferricyanide reduction whereas cyanide caused a partial inhibition of this process. Borate, on the other hand, which did not affect the catalatic activity of the preparations prevented their isomerase activity only partially, presumably by complexing with the sugar, but completely inhibited the ferricyanide reduction. 5-phosphoribonic acid also caused no significant inhibition of catalatic activity but completely inhibited both isomerase activity and ferricyanide reduction.

In conclusion, it may be stated that the inhibition of ferricyanide reduction bore no relation to the inhibition of catalatic activity, that complete or partial inhibition of isomerase activity resulted in complete or partial inhibition of ferricyanide reduction respectively but that partial inhibition of isomerase activity could also be associated with complete inhibition of ferricyanide reduction. These observations may be interpreted to show that isomerase activity was required for the ferricyanide reduction, but that at least one intermediate other than ribulose 5-phosphate (Ru 5-P) and derived from it was involved in the ferricyanide reduction.

TABLE II

EFFECT OF INHIBITORS ON THE RATE OF REDUCTION OF FERRICYANIDE BY R 5-P IN THE PRESENCE OF RECRYSTALLIZED BEEF-LIVER CATALASE, AND ON THE CATALATIC AND R 5-P ISOMERASE ACTIVITY OF THIS ENZYME PREPARATION.

Catalatic activity determined according to FEINSTEIN[4], isomerase activity according to AXELROD AND JANG[6], and rate of ferricyanide reduction as given under METHODS, except that when 5-phosphoribonic acid was used as inhibitor the R 5-P concentration was decreased to 0.01 *M*, and the ferricyanide concentration to 0.0014 *M*. Interference of cyanide and azide in the development of the ketopentose colour was corrected for by using standard curves determined in the presence of these inhibitors. 3-Amino-1,2,4-triazole inhibited catalase prepared according to MARGOLIASH AND NOVOGRODSKY[2].

Inhibitor	*Inhibition of*		
	Catalatic activity %	*R 5-P isomerase activity* %	*Rate of ferricyanide reduction* %
Azide, 0.005 *M*	100	0.5	0
3-Amino-1,2,4-triazole	97	0	0
Cyanide, 0.005 *M*	100	65	83
Borate, 0.02 *M*	2	46	99
5-Phosphoribonic acid 0.02 *M*	8	91	100

Spectral change in incubation mixtures containing R 5-P and catalase or isomerase preparations

When R 5-P was incubated with catalase or isomerase preparations at 37° under N_2 or air in the absence of ferricyanide, there was a rapid increase in the absorbance in the mixture in the region of 250–300 mμ. By 16–20 h the absorbance

References p. 139/140.

at 284 mμ was about 10 times the original value and a new spectrum was obtained with a maximum at 284 mμ and a minimum at 240 mμ. Since there was no way of determining the actual concentration of this new compound its spectrum given in Fig. 2 is in relative values only. Changing the pH from 4.0 to 9.0 caused only minor changes in this spectrum. Fig. 1 shows that the increase in the absorbance at 284 mμ representing the increase in the concentration of this compound paralleled the increase in the rate of reduction of ferricyanide, tested by adding ferricyanide to aliquots of the incubation mixture, under the usual conditions, after various times of incubation. It is thus possible that the compound having the spectrum given in Fig. 2 was the compound oxidized by ferricyanide.

The increase in absorbance at 284 mμ could also be produced with the alfalfa isomerase preparation, and the initial rate of this change increased with the number of isomerase units in the incubation mixture (Fig. 3). The increase in absorbance at 284 mμ was prevented by cyanide and borate, but not by azide, with either the catalase or the alfalfa isomerase preparations (Fig. 3). It occurred also with catalase preparations that had been completely inactivated with respect to catalatic activity by 3-amino-1,2,4-triazole[2].

Comparing these results with those presented in the previous section, it can be seen that only those substances which prevented the increase in absorbance at 284 mμ were also inhibitors of the oxidation reaction; indeed, in all cases tested, it was found that the rate of ferricyanide reduction of any mixture containing R 5-P which had been incubated for 6 h or longer paralleled the increase in absorbance at 284 mμ which had occurred during the incubation. This relationship held not only when the absorbance change was unimpeded (see Fig. 1), but also when partial or complete inhibition of this change had taken place. Fig. 3 also shows that the increase in absorbance at 284 mμ did not occur when the incubation was carried out at 0°.

The ten purified proteins found to be ineffective in the promoting of ferricyanide reduction by R 5-P were also found to be ineffective in causing the spectral change.

Oxidizing agents other than ferricyanide

It was found that iodine could replace the ferricyanide in the enzymically promoted oxidation of R 5-P. The rate of reduction of iodine was approximately the same as that of ferricyanide under the same conditions. Cytochrome *c* could also be reduced by the R 5-P-enzyme system, but at a considerably slower rate; with 0.02 *M* R 5-P and 0.17 isomerase units/ml, at pH 6.8 and 37°, $0.33 \cdot 10^{-4}$ *M* cytochrome *c* was reduced by 70 % in 1 h, while in the control solution which did not contain the enzyme, the cytochrome *c* was 41 % reduced.

Stoichiometry of oxidation of R 5-P

Mixtures containing catalase preparations, R 5-P and ferricyanide with molar ratios of ferricyanide to R 5-P of 2, 3, 4 and 5 respectively were incubated at pH 7.0 and 37° under purified N_2 until no further reduction of the ferricyanide occurred. The ferricyanide remaining was determined colorimetrically. The results given in Table III show that the complete oxidation of R 5-P in these systems required 4 equivalents of ferricyanide.

Similar experiments using iodine as oxidizing agent showed that 4 equiv. of iodine were used up in the oxidation of R 5-P by this system.

References p. 139/140.

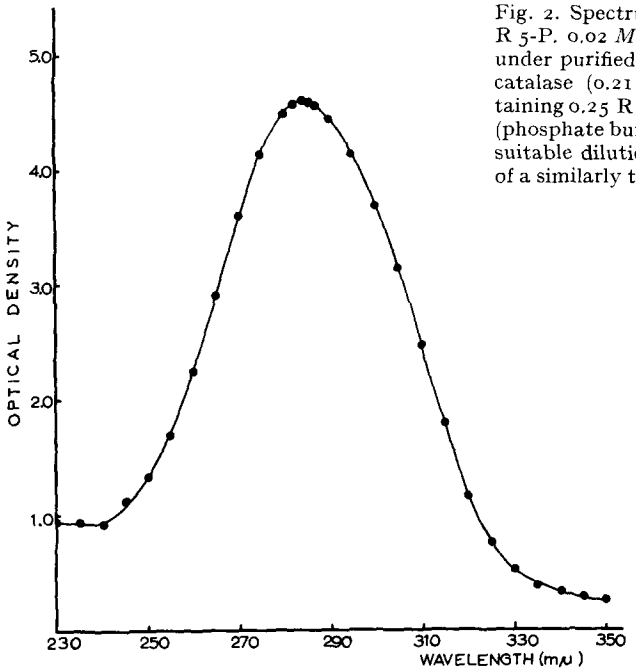

Fig. 2. Spectrum of transformation product of R 5-P. 0.02 *M* R 5-P incubated for 28 h at 27° under purified N_2 with recrystallized beef-liver catalase ($0.21 \cdot 10^{-5}$ *M* catalase haematin, containing 0.25 R 5-P isomerase units/ml) at pH 7.0 (phosphate buffer, 0.05 *M*). Spectrum taken after suitable dilution. Values corrected for spectrum of a similarly treated mixture containing catalase but no R 5-P.

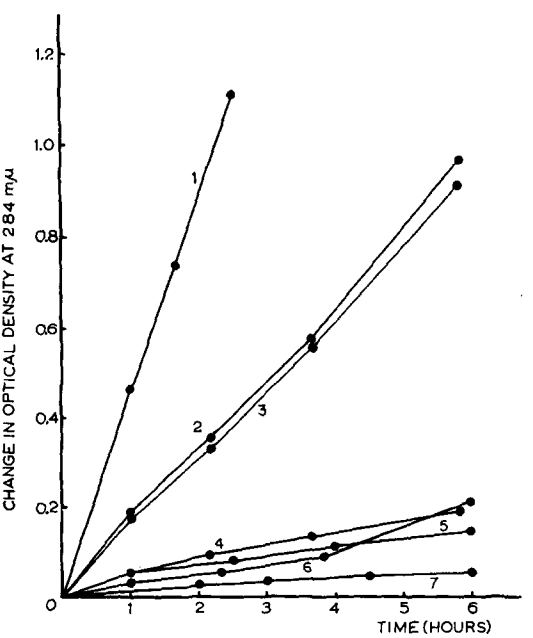

Fig. 3. Effect of inhibitors on change in optical density at 284 mμ in incubation mixtures containing R 5-P and catalase or isomerase preparations. Final concentrations: beef-liver catalase, R 5-P and buffer same as for Fig. 2; 0.005 *M* KCN; 0.02 *M* borax; 0.005 *M* NaN_3; 4.1 units/ml alfalfa isomerase. Total vol., 3.0 ml. Incubated, unless otherwise stated, at 37° in 1-cm spectrophotometer cells in air. Curve 1, alfalfa isomerase and R 5-P; curve 2, beef-liver catalase and R 5-P; curve 3, catalase, R 5-P and azide; curve 4, R 5-P alone; curve 5, catalase, R 5-P and borate; curve 6, catalase, R 5-P and cyanide; curve 7, catalase and R 5-P at 0°.

References p. 139/140.

TABLE III

STOICHIOMETRY OF OXIDATION OF R 5-P BY FERRICYANIDE IN THE PRESENCE OF RECRYSTALLIZED BEEF-LIVER CATALASE

Final concentrations in incubation mixtures: R 5-P, 0.002 *M*; $K_3Fe(CN)_6$, from 0.004 to 0.01 *M*; recrystallized beef-liver catalase haematin, $0.86 \cdot 10^{-5}$ *M*, containing 1.0 R 5-P isomerase units/ml; phosphate buffer, pH 7.0, 0.05 *M*. Total vol., 6.0 ml. Incubated at 37°, in Thunberg tubes under purified N_2. Reaction ended at 22 h after which no further reduction of ferricyanide could be observed. Excess ferricyanide determined colorimetrically. Colorimetric control mixtures contained catalase and R 5-P, but no ferricyanide.

Tube No.	*R 5-P at start of reaction µmoles*	*Ferricyanide at start of reaction µmoles*	*Ferricyanide at end of reaction µmoles*	*Equiv. of ferricyanide reduced*
1	12.0	24.0	0	2
2	12.0	36.0	0	3
3	12.0	48.0	0.24	3.98
4	12.0	60.0	13.44	3.88

Effect of pH on the rate of R 5-P oxidation by ferricyanide in the presence of catalase or alfalfa isomerase preparations

The effect of changing the pH on the rate of reduction of ferricyanide by R 5-P in the presence of the catalase preparation and the alfalfa isomerase was determined. The results presented in Fig. 4 show that for both the catalase and alfalfa isomerase preparations the same curve was obtained. The decrease of the rate of ferricyanide reduction on the alkaline side of the maximum was not due to an actual decrease, but rather to the fact that the rate of reduction in the control not containing the enzyme preparation gradually caught up with the rate of reduction in the experimental mixtures, although both increased rapidly as the solutions were made more alkaline. Moreover, this pH curve differed grossly from the pH-isomerase activity and pH-catalase activity curves, having a broad maximum at about pH 9.0, while the pH-activity curve of catalase[14] is flat till pH 6.8 and decreases above this pH, and the pH-activity curve of isomerase[6] has a sharp maximum at pH 7.0.

Attempts at isolation of the ferricyanide-reducing substance

During the formation of the ferricyanide-reducing substance from R 5-P in the presence of catalase preparations or alfalfa isomerase no inorganic phosphate was liberated. Similarly no inorganic phosphate was formed during the oxidation of this substance by ferricyanide. It was, moreover, found that the ferricyanide-reducing substances appeared together with R 5-P and Ru 5-P in the barium–ethanol-insoluble fraction of deproteinized incubation mixtures that had contained no oxidizing agent and had been kept strictly anaerobic. Such precipitates were dissolved in 0.1 *N* HCl, the barium removed on a Dowex-50 column (H^+ form) and the solution freeze dried. Deproteinization was carried out with cold 5 % trichloracetic acid.

Attempts to separate the ferricyanide-reducing substance from the other components of the mixture by paper chromatography using 4 different solvents failed.

The solvents used for developing the paper chromatograms were: butanol–acetic acid–water[15], butyric acid–NaOH[16], methanol–ammonia–water[18], and butanol–propionic acid–water[18]. In each case the ferricyanide-reducing substance, which could be readily localized as described under METHODS, moved together with the R 5-P.

References p. 139/140.

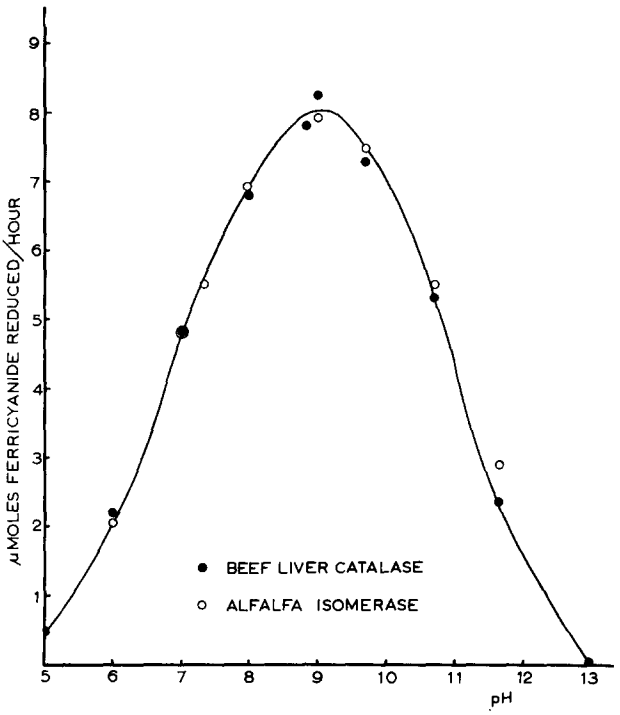

Fig. 4. Effect of pH on the rate of reduction of ferricyanide by R-5-P in the presence of beef-liver catalase and alfalfa isomerase preparations. Rate of reduction estimated as given under METHODS. Final concentrations of both enzyme preparations adjusted so as to give the same rate of ferricyanide reduction at pH 7.0. Buffers used: acetate for pH 5.0, phosphate for pH 6.0–7.6, 2-amino-2-hydroxymethylpropane-1,3-diol-HCl for pH 7.5–9.0, carbonate–bicarbonate for pH 9.2–10.7 carbonate–NaOH for pH 10.7–12.0, and 0.1 *M* NaOH for pH 13.0. Beef-liver catalase (●); alfalfa isomerase (○). Every value represents the difference between the rate of ferricyanide reduction in the presence of the enzyme and in its absence.

Fresh control samples of R 5-P did not show any ferricyanide reduction when chromatographed together with the enzymic incubation products. Reducing sugars were localized by the phloroglucinol reaction[19] and organic phosphate according to HANES AND ISHERWOOD[20].

DISCUSSION

The experiments described above indicate that the oxidation of ribose 5-phosphate by ferricyanide in the presence of blood haemolysates and crystalline catalase preparations described by FRANCOEUR AND DENSTEDT[1], and attributed by these authors to catalase, is the result of the ribose 5-phosphate isomerase activity of these preparations.

The question as to whether this activity is a property of the catalase molecule *per se* or whether crystalline catalase preparations are commonly contaminated by small amounts of ribose 5-phosphate isomerase, is difficult to decide. However, since both catalase and isomerase have rather similar precipitation properties and ribose 5-phosphate isomerase is an extremely active enzyme it is not unreasonable to assume

References p. 139/140.

that the second possibility is the more probable one. Indeed it could be calculated that a contamination of liver catalase by isomerase to the extent of about 0.01 % and of the blood catalase by 0.8 % in terms of protein, was enough to account for the activity of the catalase preparations in the oxidation of ribose 5-phosphate by ferricyanide, taking the activity of the purest fraction of alfalfa isomerase obtained by AXELROD AND JANG[6] as the specific activity of isomerase.

The experiments described in the present paper show that the enzyme did not react with the oxidizing agents but that its function in the overall reaction was to transform ribose 5-phosphate into a substance or substances that could reduce the oxidizing agents non-enzymically. Whether this intermediate reducing compound was ribulose 5-phosphate itself or a further enzymic or spontaneous transformation product of it, or possibly an intermediate in the ribose 5-phosphate to ribulose 5-phosphate reaction could not be ascertained. However, since it could be calculated that under the conditions used, the isomerization of ribose 5-phosphate was complete within less than 2 h, if no other reaction occurred, and that the rate of ferricyanide reduction continued to increase for very much longer, it is probable that the ferricyanide-reducing substance was neither ribulose 5-phosphate nor an intermediate in the ribose 5-phosphate to ribulose 5-phosphate reaction, but a further transformation product of ribulose 5-phosphate. A study of the effect of five inhibitors on the isomerase activity of the catalase preparations and on the rate of ferricyanide reduction in the presence of such preparations led to the same conclusion. Moreover, since the spectrum of the product obtained in incubation mixtures containing ribose 5-phosphate and the enzyme preparations was not very different from that of ascorbic acid[21], and that the accumulation of the product showing this spectrum ran parallel to the increase in the rate of ferricyanide reduction, it is possible that the ferricyanide-reducing substance was an endiol, similar to the one observed by DISCHE AND SHIGEURA[22] in incubation mixtures containing ribose 5-phosphate and blood haemolysates. It should be noted that ribulose 5-phosphate has been shown to be unstable in slightly alkaline solution[6,23], so that it is possible that the ferricyanide-reducing substance observed may be a non-enzymic transformation product of ribulose 5-phosphate, particularly since relatively long periods of incubation were necessary for its accumulation.

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PROPERTIES OF A PARTICULATE NITRATE REDUCTASE FROM THE NODULES OF THE SOYBEAN PLANT*

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SUMMARY

A particulate fraction has been isolated from *Rhizobium japonicum* cells of soybean nodules which catalyzes the reduction of nitrate to nitrite with either DPNH or succinate. The properties of the complex have been studied with each of these electron donors.

When succinate is used as an electron donor for the nitrate reductase complex, the system exhibits many of the properties of succinic dehydrogenase and succinoxidase which have been reported. These include competitive inhibition by malonate and pyrophosphate, inhibition by fluoride and an activation by phosphate.

The nitrate reductase complex, with either succinate or DPNH as the electron donor was inhibited by Antimycin A, dicumarol, DNP and *p*-chloromercuribenzoate. Inhibition by the latter compound was prevented by either glutathione or cysteine. No evidence has been obtained for the involvement of a flavin in electron transport from either DPNH or succinate to nitrate; however, it is concluded that the failure to demonstrate a flavin requirement is associated with the difficulties in dealing with the particulate system.

From studies of the inhibition of the nodule nitrate reductase by metal chelating agents and from kinetic studies of the cyanide inactivation of the system it is concluded that metal ions are involved in electron transport at two cyanide-sensitive sites with either DPNH or succinate as the electron donor.

When succinate is used as the electron donor for the complex it is suggested that succinic dehydrogenase, a cytochrome, an Antimycin A-sensitive site, two cyanide sensitive sites, and nitrate reductase are involved in the electron transport to nitrate. When DPNH is used as a source of electrons for the system, the evidence indicates involvement of a vitamin K, or a related quinone, a cytochrome, an Antimycin A-sensitive site, two cyanide-sensitive sites, and nitrate reductase.

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References p. 153.