The Mechanism of the Reversible Aerobic Inhibition of Glycolysis in a Pea-Seed Extract

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Previous studies established that a partially purified extract from pea seeds contained a complete Embden-Meyerhof-Parnas glycolytic system (Turner, 1954, 1957; Hatch & Turner, 1958). Glycolytic activity was inhibited by aerobic conditions (Turner & Mapson, 1958; Hatch & Turner, 1959) and some properties of this effect were similar to those of the Pasteur effect of intact tissues. Further investigations (Hatch & Turner, 1959) showed that the aerobic inhibition of glycolysis in the pea-seed extracts was due to the reversible inactivation of glyceraldehyde 3-phosphate dehydrogenase. Balazs & Richter (1958*a, b*) found that a similar mechanism was partly responsible for the aerobic inhibition of glycolysis by a brain-mitochondrial preparation.

The present investigation is concerned with the mechanism of the reversible aerobic inhibition of glyceraldehyde 3-phosphate dehydrogenase and the oxidation of protein sulphhydryl groups and reduction of protein disulphide groups in the pea-seed extracts.

EXPERIMENTAL

Preparation of the pea-seed extract

The partially purified pea-seed extract was prepared as described by Turner (1957), except that extracts used for glycolytic studies were dialysed against 25 mM-phosphate buffer (KH_2PO_4 - Na_2HPO_4) at pH 6.5. The extract contained approx. 45 mg. of protein/ml. Extracts used for the study of protein disulphide (S-S)-group reduction or the activation of glyceraldehyde 3-phosphate dehydrogenase were incubated in air for 2 hr. at 30° after dialysis. This treatment decreased the protein sulphhydryl (SH)-group content from approx. 25 μg .equiv. of SH/g. of protein to approx. 15 μg .equiv. of SH/g. of protein.

Materials

Fructose, glucose 6-phosphate (G 6-P), adenosine triphosphate (ATP), diphosphopyridine nucleotide (DPN), reduced diphosphopyridine nucleotide (DPNH), triphosphopyridine nucleotide (TPN), reduced glutathione (GSH), cysteine hydrochloride and *p*-chloromercuribenzoate were commercial products as described previously (Hatch & Turner, 1958, 1959). Oxidized glutathione (GSSG) was obtained from C. F. Boehringer and Söhne, Mannheim, Germany, and cystine and fructose 1:6-diphosphate (FDP) were from L. Light and Co. Ltd.

Glucose 1-phosphate (G 1-P) was prepared by an unpublished method of Professor C. S. Hanes and Dr R. Hill and pea-seed glyceraldehyde 3-phosphate dehydrogenase was prepared according to Hageman & Arnon (1955). Solutions of G 1-P, G 6-P and FDP were standardized as described previously (Hatch & Turner, 1958).

Analytical methods

Glyceraldehyde 3-phosphate dehydrogenase activity. Glyceraldehyde 3-phosphate dehydrogenase activity in the pea-seed extract was determined as described previously (Hatch & Turner, 1959). The activity of the pea-seed glyceraldehyde 3-phosphate dehydrogenase preparation was determined by the method of Warburg & Christian (1939).

Cystine reductase and oxidized glutathione reductase activity. Cystine reductase and GSSG reductase were assayed as described by Romano & Nickerson (1954) and Racker (1955) respectively.

Pyridine nucleotide transhydrogenase activity. Two spectrophotometric methods were used to determine whether pyridine nucleotide transhydrogenase was present in the pea-seed extract. The extract contained a DPN-specific alcohol dehydrogenase and a TPN-specific GSSG reductase. TPN reduction was measured in a reaction mixture containing 0.3 ml. of the pea-seed extract (containing alcohol dehydrogenase); Na_2HPO_4 - KH_2PO_4 buffer (pH 6.8), 50 μmoles ; ethanol, 0.1 ml.; DPN, 0.05 μmole ; TPN, 0.6 μmole ; total vol., 2.8 ml. DPNH oxidation was measured in a reaction mixture containing 0.3 ml. of the pea-seed extract (containing GSSG reductase); Na_2HPO_4 - KH_2PO_4 buffer (pH 7.0), 50 μmoles ; GSSG, 12 μmoles ; TPN, 0.05 μmole ; DPNH, 0.65 μmole ; total vol., 2.8 ml. Both assays were carried out at 26°. No evidence was obtained for pyridine nucleotide transhydrogenase activity.

Assay of SH groups. The method used to estimate protein SH groups was described previously (Hatch & Turner, 1958).

The SH groups of glutathione were determined by amperometric titration with AgNO_3 (Benesch & Benesch, 1945). Cysteine SH groups were determined by the method of MacDonnell, Silva & Feeney (1951).

Determination of protein. The protein content of the pea-seed extract and the pea-seed glyceraldehyde 3-phosphate dehydrogenase preparation was estimated by the spectrophotometric method of Warburg & Christian (1941).

Enzyme studies

Glycolytic activity was determined as described previously (Hatch & Turner, 1958). Other reaction mixtures were incubated at 30° in Thunberg tubes and anaerobic

conditions were established by alternately evacuating and flushing with O_2 -free N_2 . Spectrophotometric studies were conducted at 26°.

RESULTS

Relationship of protein SH-group concentration and glycolytic activity

It was shown previously that glycolytic activity in the pea-seed extract was related to the extent of oxidation of the constituent protein SH groups (Hatch & Turner, 1959). When the reaction mixtures were incubated in air the concentration of protein SH groups either increased or decreased (compared to the initial level of 25 μ g. equiv. of SH/g. of protein) depending on the amount of DPN added. When the aerobic glycolytic activity was plotted against protein SH-group content a linear relationship was obtained (Fig. 1). These experiments indicated that mechanisms for the oxidation of protein SH groups and the reduction of protein S-S groups were operating in the enzyme digests.

Oxidation of protein SH groups and the inhibition of glyceraldehyde 3-phosphate dehydrogenase

When the pea-seed extract was incubated in air there was a decrease in the protein SH-group content (Table 1). The addition of ethylenediaminetetra-acetic acid (EDTA) (final concn. 0.15 mM) inhibited this aerobic oxidation by 80%. However, concentrations of DPN which prevented

the aerobic inhibition of glycolysis in the pea-seed extracts (Hatch & Turner, 1959) had little influence on the decrease in protein SH groups. Glyceraldehyde 3-phosphate dehydrogenase activity in the pea-seed extract was affected in a similar manner probably through the oxidation of essential SH groups on the enzyme molecule (Table 2). EDTA (0.3 mM) reduced the rate of aerobic inactivation of glyceraldehyde 3-phosphate dehydrogenase activity but DPN (0.5 mM) had no effect.

Mandels (1956) reported the direct enzymic oxidation by molecular O_2 of the SH groups of cysteine and GSH by an extract from fungi. In the present investigation it was found that the addition of the pea-seed extract to solutions of cysteine or GSH in

Table 1. *Effect of ethylenediaminetetra-acetic acid and diphosphopyridine nucleotide on the aerobic oxidation of protein SH groups in the pea-seed extract*

Reaction mixtures contained: Expt. 1: pea-seed extract, 1.5 ml.; Na_2HPO_4 - KH_2PO_4 buffer (pH 7.0), 37 μ moles; $MgCl_2$, 18 μ moles; total vol., 2.3 ml. Expt. 2: pea-seed extract, 3.5 ml.; Na_2HPO_4 - KH_2PO_4 buffer (pH 7.0), 87 μ moles; $MgCl_2$, 36 μ moles; total vol., 7.2 ml. EDTA (0.15 mM) and DPN (0.4 mM) were added as shown. Gas phase, air.

Expt. no.	Additions	Time (hr.)	Concn. of protein SH groups (μ g.equiv./g. of protein)
1	—	0	23.6
	—	1	16.3
	EDTA	1	22.1
2	—	0	26.0
	—	2	16.9
	DPN	2	18.5

Table 2. *Effect of ethylenediaminetetra-acetic acid and diphosphopyridine nucleotide on the aerobic inhibition of glyceraldehyde 3-phosphate dehydrogenase in the pea-seed extract*

Reaction mixture contained: pea-seed extract, 2.5 ml.; Na_2HPO_4 - KH_2PO_4 buffer (pH 7.0), 92 μ moles; total vol., 6.1 ml. EDTA (0.3 mM) and DPN (0.5 mM) were added as indicated. Gas phase, air.

Additions	Time (min.)	Glyceraldehyde 3-phosphate dehydrogenase activity (ΔE /min.)
None	0	0.142
EDTA	0	0.170*
None	70	0.043
EDTA	70	0.114
DPN	70	0.046

* EDTA caused some activation of glyceraldehyde 3-phosphate dehydrogenase.

Fig. 1. Relationship of the protein SH-group concentration to aerobic glycolytic activity. Reaction mixtures contained: pea-seed extract, 1.3 ml.; Na_2HPO_4 - KH_2PO_4 buffer (pH 6.5), 25 μ moles; G 1-P, 22 μ moles; fructose, 66 μ moles; $MgCl_2$, 18 μ moles; ATP, 0.23 μ mole; total vol., 2.3 ml. DPN concentration was varied from 0 to 0.31 mM to give different steady rate glycolytic activities. Gas phase, air.

phosphate buffer brought about no increase in the rate of oxidation of the SH groups of these compounds.

Reduction of protein S-S groups

The observation that protein S-S groups were reduced during glycolysis suggested that DPNH (generated during glycolysis) may have been responsible for the reduction. When substrate levels of DPNH were added the protein SH-group concentration in the pea-seed extract increased from 14.0 $\mu\text{g. equiv.}$ to 27.2 $\mu\text{g. equiv./g.}$ of protein in 2 hr. (Table 3). Protein S-S groups were also reduced when DPNH was produced in the reaction mixtures. DPNH was generated by glyceraldehyde 3-phosphate dehydrogenase by adding either fructose plus G 1-P or FDP together with a catalytic amount of DPN; these treatments increased the protein SH-group concentration from 15.5 $\mu\text{g.}$

equiv. to 30.3 (in 4 hr.) and 28.0 (in 2 hr.) $\mu\text{g. equiv./g.}$ of protein respectively. Fluoride was added to inhibit glycolysis at the 3-phosphoglyceric acid stage. The alcohol dehydrogenase present in the pea-seed extract was also used to generate DPNH and, on the addition of ethanol, there was an increase in the protein SH-group content. TPNH, produced by the G 6-P dehydrogenase present in the pea-seed extract, was also effective in reducing the protein S-S groups. As pyridine nucleotide transhydrogenase activity could not be detected in the pea-seed extract it is probable that the protein S-S reducing system utilized DPNH or TPNH directly.

There was no reduction of protein S-S groups when a pea-seed glyceraldehyde 3-phosphate dehydrogenase preparation was incubated with DPNH (Table 4). When a small quantity (equivalent to 8 mg. of protein) of the partially purified

Table 3. *Increase of protein SH groups in pea-seed extract by reduced diphosphopyridine nucleotide or by systems producing reduced di- or tri-phosphopyridine nucleotide*

Reaction mixtures contained: Expt. 1: pea-seed extract, 1.5 ml.; $\text{Na}_2\text{HPO}_4\text{--KH}_2\text{PO}_4$ buffer (pH 7.0), 37 μmoles ; MgCl_2 , 18 μmoles ; ATP, 0.75 μmole ; DPNH as indicated; total vol., 2.5 ml. Expt. 2: pea-seed extract, 1 ml.; $\text{Na}_2\text{HPO}_4\text{--KH}_2\text{PO}_4$ buffer (pH 7.0), 25 μmoles ; MgCl_2 , 18 μmoles ; ATP, 0.75 μmole ; total vol., 2.2 ml. Additions to produce DPNH or TPNH are shown in the table. Gas phase, N_2 .

Expt. no.	Additions	Time (hr.)	Concn. of protein SH groups* ($\mu\text{g. equiv./g.}$ of protein)
1	DPNH (5 mm)	0	14.0
	DPNH (5 mm)	2	27.2
2	None	0	15.5
	None	2	15.5
	Fructose (22 mm); G 1-P (13 mm); DPN (mm); fluoride (4.5 mm)	4	30.3
	FDP (25 mm); fluoride (4.5 mm); DPN (mm)	2	28.0
	Ethanol (0.24 M); DPN (mm)	2	22.3
	G 6-P (22 mm); TPN (mm)	2	26.4

* There was no significant increase in concentration of SH groups when either substrate or pyridine nucleotide was omitted.

Table 4. *Enzymic reduction of protein S-S groups in a pea-seed glyceraldehyde 3-phosphate dehydrogenase preparation*

Reaction mixtures contained: pea-seed glyceraldehyde 3-phosphate dehydrogenase preparation (containing 112 mg. of protein); $\text{Na}_2\text{HPO}_4\text{--KH}_2\text{PO}_4$ buffer (pH 7.0), 26 μmoles ; MgCl_2 , 18 μmoles ; ATP, 1.5 μmoles ; total vol., 3.0 ml. Pea-seed extract (0.25 ml., containing 8 mg. of protein) and DPNH (12 μmoles) were added as indicated. Gas phase, N_2 .

Additions	Time (hr.)	Concn. of SH groups in glyceraldehyde 3-phosphate dehydrogenase preparation* ($\mu\text{g. equiv./g.}$ of protein)
DPNH	0	0.45
DPNH	1.5	0.45
Pea-seed extract + DPNH	0	0.67
Pea-seed extract + DPNH	1.5	5.02
Boiled pea-seed extract + DPNH	0	0.48
Boiled pea-seed extract + DPNH	1.5	0.72
Pea-seed extract	0	0.65
Pea-seed extract	1.5	0.83

* Corrected for the protein SH of the pea-seed extract.

pea-seed extract was added to the system there was a marked increase in the protein SH-group content of the glyceraldehyde 3-phosphate dehydrogenase preparation. There was very little increase when DPNH was omitted or when the partially purified pea-seed extract was boiled. It was concluded that a heat-labile component, presumably an enzyme which catalysed the reduction of protein S-S groups, was present in the pea-seed extract.

The rate of reduction by DPNH of protein S-S groups in the pea-seed extract was only slightly decreased when the extract was dialysed for 20 hr. instead of 2.5 hr. (Fig. 2). A DPNH-specific cystine reductase (Romano & Nickerson, 1954) and a TPN-specific GSSG reductase (Mapson & Goddard, 1951; Conn & Vennesland, 1951) have been identified in pea-seed extracts. The preparation used in the present studies contained both enzymes and their possible role in the reduction of protein S-S groups is discussed subsequently.

Activation of glyceraldehyde 3-phosphate dehydrogenase by reduced diphosphopyridine nucleotide

When the pea-seed extract was incubated in air there was a decrease in glyceraldehyde 3-phosphate dehydrogenase activity (Fig. 3). This decrease was largely prevented if the extract was incubated under anaerobic conditions (Hatch & Turner, 1959). In the presence of a system which generated DPNH, glyceraldehyde 3-phosphate dehydro-

Fig. 3. Reversal of the aerobic inhibition of glyceraldehyde 3-phosphate dehydrogenase in the pea-seed extract by a DPNH-producing system and cysteine. Reaction mixtures of the following composition were incubated in air: pea-seed extract, 1.5 ml.; Na₂HPO₄-KH₂PO₄ buffer (pH 6.5), 38 μmoles; MgCl₂, 36 μmoles; ATP, 1.5 μmoles; total vol., 6 ml. Additions (as shown) were: FDP, 10 μmoles; DPN, 1.5 μmoles; fluoride, 36 μmoles. Samples of the reaction mixture to which no additions had been made were used to determine glyceraldehyde 3-phosphate dehydrogenase activity in the presence of cysteine (final concn. 5 mM).

Fig. 2. Effect of dialysis on the protein S-S-group reducing activity of the pea-seed extract. Reaction mixtures contained: pea-seed extract, 1.5 ml. (O, dialysed for 2.5 hr.; ●, dialysed for 10 hr.; Δ, dialysed for 20 hr.); Na₂HPO₄-KH₂PO₄ buffer (pH 7.0), 36 μmoles; MgCl₂, 18 μmoles; ATP, 1.2 μmoles; DPNH, 10 μmoles; total vol., 2.5 ml. Experiments were conducted under anaerobic conditions.

Table 5. Enzyme-mediated activation of pea-seed glyceraldehyde 3-phosphate dehydrogenase by reduced diphosphopyridine nucleotide

Reaction mixtures contained: Na₂HPO₄-KH₂PO₄ buffer (pH 7.0), 35 μmoles; MgCl₂, 18 μmoles; ATP, 1.1 μmoles; DPNH, 11 μmoles; total vol., 2.2 ml. Pea-seed extract (0.15 ml. containing 5.5 mg. of protein) and the pea-seed glyceraldehyde 3-phosphate dehydrogenase preparation (containing 5.1 mg. of protein) were added as indicated. Gas phase, N₂.

Additions	Glyceraldehyde 3-phosphate dehydrogenase activity* (10 ³ ΔE/min.)	
	0 hr.	2 hr.
Glyceraldehyde 3-phosphate dehydrogenase prepn.	2 (110)	0 (116)
Pea-seed extract	3 (24)	16 (27)
Glyceraldehyde 3-phosphate dehydrogenase prepn. + pea-seed extract	0 (136)	66 (146)
Glyceraldehyde 3-phosphate dehydrogenase prepn. + boiled pea-seed extract	4 (120)	3 (124)

* Maximum glyceraldehyde 3-phosphate dehydrogenase activity (assayed with cysteine, final concn. 5 mM) is quoted in parentheses.

genase activity in the pea-seed extract increased rather than decreased when the enzyme digests were incubated in air. When compared with control assays conducted in the presence of cysteine, glyceraldehyde 3-phosphate dehydrogenase was completely activated after approx. 2 hr. DPNH was generated by the addition of FDP, DPN and fluoride. Glyceraldehyde 3-phosphate dehydrogenase was not activated when either FDP or DPN was omitted.

The pea-seed glyceraldehyde 3-phosphate dehydrogenase preparation was substantially inactive unless cysteine was added. When DPNH alone was added to the glyceraldehyde 3-phosphate dehydrogenase preparation there was no increase in activity (Table 5). The figures for the maximum glyceraldehyde 3-phosphate dehydrogenase activity obtained when the assay was conducted in the presence of cysteine are also shown in Table 5. On the addition of a small quantity of the pea-seed extract (equivalent to 5.5 mg. of protein) there was a substantial increase in glyceraldehyde 3-phosphate dehydrogenase activity above the increase due to activation of the enzyme present in the pea-seed extract. The boiled pea extract was ineffective. No activation of the enzyme was observed when DPNH was omitted. It appeared that the reactivation of glyceraldehyde 3-phosphate dehydrogenase was catalysed by an enzyme contained in the pea-seed extract with DPNH functioning as hydrogen donor.

DISCUSSION

The present investigation has shown that mechanisms exist in the pea-seed extract for the oxidation of protein SH groups and for the enzymic reduction of protein S-S groups. Similarly glyceraldehyde 3-phosphate dehydrogenase was oxidatively inactivated and the oxidized form of the enzyme was also reactivated, presumably through enzymic reduction of S-S groups. On the basis of the observed activity of glyceraldehyde 3-phosphate dehydrogenase in the pea-seed extract, it was calculated that the essential SH groups of the enzyme probably amounted to less than 2% of the protein SH-group fraction which was directly related to the rate of glycolysis. This figure was derived from data of the activity of pea-seed glyceraldehyde phosphate dehydrogenase (Hageman & Arnon, 1955), assuming that the number of essential SH groups per molecule of the pea-seed enzyme was similar to that of the mammalian enzyme (Velick, 1953). The relationship obtained between glycolytic activity and the fraction of protein SH groups oxidized and reduced under aerobic conditions suggested that both the glyceraldehyde 3-phosphate dehydrogenase and non-glyceraldehyde 3-phosphate dehydrogenase SH

groups contained in this fraction had similar oxidation-reduction properties.

There was no evidence in the present investigation that the oxidation of protein SH groups was enzymic. The inhibition by EDTA of the oxidation of both protein SH groups and glyceraldehyde 3-phosphate dehydrogenase indicated that the oxidation may have been a non-enzymic, metal-catalysed process. Fromageot (1951) showed that the non-enzymic oxidation of SH groups by oxygen was accelerated by traces of heavy-metal ions. Haugaard, Hess & Itskovitz (1957) found that this oxidation was inhibited by EDTA. The direct enzymic oxidation of non-protein SH groups by molecular oxygen has been reported by Mandels (1956) with extracts of fungal spores and by Neufeld, Green, Latterell & Weintraub (1958) with culture filtrates of fungi. Similar enzymes could mediate the oxidation of protein SH groups either directly or indirectly in the presence of catalytic amounts of cysteine, GSH or other SH-group compounds, but activity of this type could not be detected in the pea-seed extract.

Previous experiments (Hatch & Turner, 1959) and those reported in the present investigation showed that protein S-S groups may be reduced during glycolysis under both anaerobic and aerobic conditions. The reduction was effected by systems producing DPNH or TPNH or by the addition of DPNH alone; the addition of TPNH was not tried. The effect of the pea-seed extract in reducing the protein S-S groups of the pea-seed glyceraldehyde 3-phosphate dehydrogenase preparation and reactivating the enzyme indicated that this was an enzymic reduction. There was no evidence that cystine reductase or GSSG reductase (which were both present in the pea-seed extract) was involved in this process. Prolonged dialysis, which would lower the concentration of any cysteine or GSH present, did not markedly decrease the rate of reduction of protein S-S groups. Whereas the cystine reductase (Romano & Nickerson, 1954) and the GSSG reductase (Mapson & Goddard, 1951; Conn & Vennesland, 1951) of pea seeds are nucleotide specific, both DPNH and TPNH functioned in reduction of protein S-S groups. The ability of the protein S-S-group-reducing system to utilize both DPNH and TPNH did not appear to be due to pyridine nucleotide transhydrogenase activity.

The present investigation has indicated the probable mechanisms responsible for the reversible aerobic inhibition of glycolysis in pea-seed extracts. It is suggested that glyceraldehyde 3-phosphate dehydrogenase is inhibited by the oxidation of essential SH groups and activated by a DPNH-mediated reduction of the constituent S-S groups (Fig. 4), DPNH being generated through the action of glyceraldehyde 3-phosphate dehydrogenase in

Fig. 4. Suggested mechanism for the reversible aerobic inhibition of glycolysis in the pea-seed extract. GPD, glyceraldehyde 3-phosphate dehydrogenase in the active (SH) and inactive (S-S) forms.

glycolysis. It appears that a balance between these two processes determined the ratio between the SH and S-S groups of glyceraldehyde 3-phosphate dehydrogenase and, through this, the glycolytic activity. It has been calculated (Hatch & Turner, 1959) that not more than 5% of the DPNH produced during maximum-rate aerobic glycolysis would be necessary to maintain the protein SH-group content at the optimum level for glycolysis. The possible relationship between the aerobic inhibition of glycolysis in the pea-seed extract and the Pasteur effect was discussed previously (Hatch & Turner, 1959).

DPN protects the essential SH groups of mammalian glyceraldehyde 3-phosphate dehydrogenase from oxidation by cystine or GSSG (Rapkin, Rapkin & Trpinac, 1939; Labeyrie, 1949). It was suggested previously that this may explain the effect of DPN concentration on aerobic glycolytic activity in the pea-seed extract (Hatch & Turner, 1959). In the present investigation it was shown that added DPN had little effect on the rate of inactivation of glyceraldehyde 3-phosphate dehydrogenase or the oxidation of protein SH groups in air, although if sufficient DPN was added to aerobic glycolytic digests the concentration of protein SH groups increased. As this effect was probably due to the enzymic reduction of protein S-S groups by DPNH, the rate of reduction may have been controlled by the absolute concentration of DPNH, which at equilibrium may depend upon the level of DPN added. When the DPN concentration was approximately $100 \mu\text{M}$ or more, glycolytic activity in air was not inhibited (Hatch & Turner, 1959). It appears that at this critical concentration of DPN the reduction of protein S-S groups was more rapid than the oxidation of protein SH groups.

SUMMARY

1. Processes responsible for the oxidation of sulphydryl groups and the reduction of disulphide groups of glyceraldehyde 3-phosphate dehydrogenase in a pea-seed extract were examined.

2. The inhibition of glyceraldehyde 3-phosphate dehydrogenase in aerobic glycolytic digests was due to the oxidation of the essential sulphydryl groups of the enzyme. Evidence indicated that the oxidation of protein sulphydryl groups may have been a non-enzymic process catalysed by traces of heavy metals.

3. Reduction of protein disulphide groups in the pea-seed extract occurred in the presence of reduced diphosphopyridine nucleotide or of systems which generated reduced di- or tri-phosphopyridine nucleotide. Under these conditions glyceraldehyde 3-phosphate dehydrogenase in the extract was activated.

4. In the presence of reduced diphosphopyridine nucleotide and a heat-labile component of the pea-seed extract, enzyme activity and the protein sulphydryl group concentration of a pea-seed glyceraldehyde 3-phosphate dehydrogenase preparation were increased.

5. It is suggested that aerobic glycolytic activity in the pea-seed extract was controlled by processes which mediated the oxidation of sulphydryl groups and the reduction of disulphide groups of glyceraldehyde 3-phosphate dehydrogenase.

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Lactic Dehydrogenase and Cytochrome b_2 of Baker's Yeast

THE AMINO ACID COMPOSITION OF THE CRYSTALLINE ENZYME

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Appleby & Morton (1954, 1959*a, b*, 1960) have described the purification and crystallization of yeast lactic dehydrogenase, and the enzymic, chemical and physical properties of the crystalline chromoprotein, which was shown to be identical with cytochrome b_2 . This paper describes the amino acid composition as determined qualitatively by paper chromatography and quantitatively with ion-exchange-resin columns. An account of this work was given to the Australian Biochemical Society in August, 1957 (Simmonds, Appleby & Morton, 1957*a, b*; Morton, 1958).

MATERIALS

Yeast lactic dehydrogenase (cytochrome b_2). The crystalline cytochrome was prepared as described by Appleby & Morton (1959*a*). Unless otherwise stated, the material was

recrystallized three times from a solution of the combined first crystals from a number of preparations.

Gelatin. The tyrosine-free protein was a gift from Davis Gelatine Co. Ltd., Sydney, N.S.W.

β -Lactoglobulin. Three-times crystallized material was a gift from Dr F. J. R. Hird.

Organic solvents. These were purified for chromatography by distillation at reduced pressure.

EXPERIMENTAL AND RESULTS

Amino acid composition by paper chromatography

Approx. 3 mg. of once-crystallized enzyme was hydrolysed with 0.1 ml. of 5*N*-HCl in a sealed ampoule at 105° for 20 hr. After removal of HCl, the residue was dissolved in about 70 μ l. of propan-2-ol (acidified with HCl). Suitable portions (4–10 μ l.) were applied to the appropriate grade of Whatman chromatography paper. The amino acids were separated by two-dimensional chromatography as described by Dent (1947). The ninhydrin-reactive compounds of the enzyme hydrolysate were identified by comparison with similar chromatograms of a reference mixture of known

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