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ROLE OF 3-MERCAPTOPYRUVATE SULFURTRANSFERASE IN THE FORMATION OF THE IRON–SULFUR CHROMOPHORE OF ADRENAL FERREDOXIN

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SUMMARY

1. Enzymatic activities responsible for H_2S production in various tissues were compared. The distribution of 3-mercaptopyruvate sulfurtransferase (EC 2.8.1.2) correlated with the estimated labile sulfur contents in each tissue, however, that of thiosulfate sulfurtransferase (EC 2.8.1.1) did not correlate at all, being extremely high in liver and kidney. Therefore, it is unlikely that thiosulfate sulfurtransferase is responsible for iron–sulfur chromophore formation.

2. The subcellular distribution of 3-mercaptopyruvate sulfurtransferase and transaminase activities shows that cysteine transamination occurs in cytosol and then 3-mercaptopyruvate so formed would be degraded to pyruvate and H_2S by cytosol 3-mercaptopyruvate sulfurtransferase. However, the possibility that mitochondrial 3-mercaptopyruvate sulfurtransferase is involved in the degradation of 3-mercaptopyruvate within mitochondria cannot be excluded.

3. Upon incubation of the apoprotein with 3-mercaptopyruvate and purified 3-mercaptopyruvate sulfurtransferase or cysteine, α-ketoglutarate, transaminase and 3-mercaptopyruvate sulfurtransferase in the presence of iron and thiol compounds, the reconstitution of iron–sulfur chromophore could be achieved.

4. From these results, it is concluded that 3-mercaptopyruvate sulfurtransferase plays a role in iron–sulfur chromophore formation in adrenal cortex.

INTRODUCTION

In 1966, Malkin and Rabinowitz [1] succeeded in the chemical reconstitution of the iron-sulfur chromophore of bacterial ferredoxin by reacting the apoprotein with iron and H_2S in the presence of 2-mercaptoethanol. Thus, when H_2S is produced in vivo and in the presence of iron and reducing agents, the iron–sulfur chromophore is formed. We have utilized this non-enzymatic procedure for the reconstitution of native adrenal iron–sulfur protein from its apoprotein and for the preparation of selenium derivatives of the adrenal protein [2]. Yet, the manner in which H_2S is produced in vivo remains to be answered. In higher animals, there are two major possibilities: one is the degradation of cysteine to pyruvate and H_2S [3], and the other is

thiosulfate sulfurtransferase reaction which produces H_2S in the presence of reduced thiol compounds [4].

In this context, in addition to the normal complement of iron–sulfur proteins associated with the respiratory chain, the adrenal cortex has a unique iron–sulfur protein, adrenal ferredoxin, which is an electron transfer component of the mitochondrial steroid hydroxylases, and of which the content in the mitochondria is quite high (2 nmoles adrenal ferredoxin per mg mitochondrial protein). The purpose of this investigation is (i) to compare the capacity of the adrenal cortex for H_2S production with those of other tissues, which have normal levels of iron–sulfur proteins, (ii) to examine by which pathway H_2S production in the adrenal cortex occurs predominantly, (iii) to gain some insight into the subcellular compartmentalization of the enzymes responsible for H_2S production, and (iv) to achieve the reconstitution of the iron–sulfur complex of adrenal ferredoxin by the use of its biological substrate and enzyme.

METHODS AND MATERIALS

Enzymatic assay methods

3-Mercaptopyruvate sulfurtransferase (EC 2.8.1.2) assay method is based on the colorimetric determination of thiocyanate formed in the reaction. The reaction mixture contained 40 μmoles of Tris–HCl buffer, pH 9.0, 15 μmoles of 3-mercaptopyruvate, 25 μmoles of potassium cyanide, and the enzyme solution in a final volume of 0.50 ml. The reaction was started by addition of the enzyme solution. After 15 min. at 37 °C the reaction was terminated by the addition of 0.1 ml of 38% formaldehyde. The color developed with 0.50 ml of a ferric nitrate reagent [5]. 10 ml of distilled water was then added and after centrifugation, if necessary, the absorbance was read at 460 nm. A control containing no enzyme was always carried out because the mixture of 3-mercaptopyruvate and the ferric nitrate reagent gave a yellow–green complex with an absorbance at 460 nm. This absorbance was subtracted from that of the samples. Calculations were based on an absorbance value of $A_{460\,nm} = 0.223$ for 1 μmole of thiocyanate in the presence of 15 μmoles 3-mercaptopyruvate. One 3-mercaptopyruvate sulfurtransferase unit is defined as that amount of enzyme which forms 10 μmoles of thiocyanate under the above conditions. Specific activity is expressed as units per mg of protein.

Cysteine transaminase was assayed by using 3-mercaptopyruvate and glutamate as substrates coupled to a glutamate dehydrogenase reaction which required α-ketoglutarate formed in the reaction, NH_4^+, and NADH as substrates. Glutamate dehydrogenase (2.0 units) and extracts were added to a mixture preincubated at 37 °C and containing 300 μmoles Tris–HCl, pH 7.4, 30 μmoles glutamate, 200 μmoles ammonium acetate and 0.25 μmoles NADH in a final volume of 3.00 ml. The reaction was started by the addition of 3-mercaptopyruvate and the decrease in the absorbance at 340 nm was recorded. A blank without 3-mercaptopyruvate was always carried out.

Thiosulfate sulfurtransferase (EC 2.8.1.1) activity was determined by the method of Sorbo [5, 6]. Definition of thiosulfate sulfurtransferase unit (R.U.) and specific activity were used as defined by Sorbo [5, 6].

Glutamic-oxaloacetic transaminase was assayed by the method of Nisselsaum and Bodansky [7].

Succinate dehydrogenase was assayed as described by Veeger et al. [8]. $K_3Fe(CN)_6$ was used as the electron acceptor and the reduction of $K_3Fe(CN)_6$ was observed at 455 nm. The reaction was carried out in a volume of 3 ml at 25 °C.

Lactate dehydrogenase was assayed by the method of Kornberg [9].

Glucose-6-phosphatase was assayed as described by Swanson [10]. The phosphate produced was estimated by the method of Fiske and SubbaRow [11].

Measurement of H_2S-production

Unless otherwise stated, the standard assay mixture contained 50 mM Tris-HCl buffer, pH 7.4, 10 mM α-ketoglutarate, 1 mM pyridoxal HCl, 3 mM dithiothreitol, 10 mM cysteine, and enzyme solution in a final volume of 2.0 ml. The reaction mixture was placed in the main compartment of a Thunberg tube, and a mixture of 1.5 ml of 1% zinc acetate and 0.1 ml of 12% sodium hydroxyde to trap the H_2S produced, was placed in the side arm. This method is similar to that of Yamaguchi et al. [12]. H_2S was determined by the method reported by Massey et al. [13].

Preparation of bovine tissue homogenates

(a) Fresh adrenal glands, liver, heart, and kidney were obtained from a local slaughter house. Each tissue slice (in the case of the adrenal glands, the medulla was removed and only the adrenal cortex was weighed) was homogenized with 3 volumes of 0.25 M sucrose solution, and centrifuged at 750 g for 10 min. The supernatant solutions were used for the H_2S-production and glutamic-oxaloacetic transaminase activity measurement. (b) Fresh organs were stored at −20 °C overnight, thawed and 10 g of each organ were homogenized with 5 vol. of 10 mM Tris–HCl buffer, pH 7.6, by means of a Waring blender and teflon homogenizer. Each homogenate was centrifuged at 500 × *g* for 10 min and the cellular debris removed. The supernatants were used for the sulfurtransferases distribution experiment.

Protein determination

Protein determination was performed by the Biuret method [14] and the Lowry method [15] using bovine serum albumin as a standard.

Purification of 3-mercaptopyruvate sulfurtransferase from bovine adrenal glands

The following manipulations were performed at 4 °C: frozen adrenal glands were thawed and washed with tap water, and homogenized with 10 mM phosphate buffer, pH 7.4 in a Waring blender. The homogenate was centrifuged at 18 000 × *g* for 30 min and the supernatant solution was fractionated by adding ammonium sulfate at pH 6.0. The fraction between 25% and 70% saturation was collected by centrifugation. The precipitates were dissolved in a minimal amount of distilled water and dialyzed against 10 mM Tris–HCl buffer, pH 7.6, containing 10 mM $Na_2S_2O_3$ (Buffer A). The insoluble proteins formed during dialysis were removed by centrifugation. The supernatant solution was diluted twice by the addition of 20 mM 2-mercaptoethanol, and placed on a DEAE-cellulose column (3.4 cm × 36 cm), equilibrated with 5 mM Tris–HCl buffer, pH 7.6, containing 5 mM $Na_2S_2O_3$ and 10 mM 2-mercaptoethanol (Buffer B). After washing with Buffer B, the enzyme was eluted with a linear gradient from 0 to 0.20 M KCl in Buffer B. The active fractions were pooled and the enzyme was precipitated by addition of solid ammonium sulfate to

90% saturation, and collected by centrifugation. The precipitate was dissolved in a minimal amount of Buffer A, and dialyzed against Buffer A. The dialyzate was diluted with 20 mM 2-mercaptoethanol solution and placed on the second DEAE-cellulose column (3.0 cm × 32 cm). The same procedure, as described above, was performed. The active fractions were pooled and again precipitated by addition of ammonium sulfate to 90% saturation and collected by centrifugation. The precipitate was dissolved in a minimal amount of Buffer A and placed on a Sephadex G-75 column (5 cm × 40 cm) equilibrated with Buffer A. The enzyme was again precipitated by addition of ammonium sulfate to 90% saturation and collected by centrifugation. The precipitate was dissolved in 2.0 ml of Buffer A. This purified enzyme is quite unstable in the absence of high concentrations of 2-mercaptoethanol. The addition of 2-mercaptoethanol enhances the activity approximately three fold. Table I summa-

TABLE I

PURIFICATION PROCEDURE OF 3-MERCAPTOPYRUVATE SULFURTRANSFERASE FROM BOVINE ADRENAL GLAND

Procedure	Volume (ml)	Protein (mg)	3-Mercaptopyruvate sulfur transferase		Thiosulfate sulfur transferase	
			Total activity (units)	Specific activity (units/mg)	Total activity (Rhodanese units)	Specific activity (Rhodanese units/mg)
Crude extract	740	41 000	9500	0.232	1170	0.028
Ammonium sulfate fractionation	200	6200	1550	0.250	940	0.152
1st DEAE-cellulose chromatography	207	356	1060	2.97	101	0.285
2nd DEAE-cellulose chromatography	165	47.2	274	5.72	40.6	0.850
Sephadex gel filtration	27	2.36	78.8	33.4	4.89	2.07

1) 200 g of frozen adrenal gland was used.
2) Assay methods and definition of enzyme unit are described elsewhere in this paper.

rizes the purification procedures of 3-mercaptopyruvate sulfurtransferase together with the thiosulfate sulfurtransferase activity.

Materials

Adrenal ferredoxin ($A_{414}/A_{276} = 0.75$) was prepared from bovine adrenal glands as described elsewhere [16].

Adrenal apoferredoxin was prepared by treatment of adrenal ferredoxin with trichloroacetic acid as previously described [17].

Ammonium 3-mercaptopyruvate was prepared by the method of Kun [18]. The m.p. (178–183 °C) and infrared spectrum were in agreement with the previously published data [19].

Glucose 6-phosphate (Na^+ salt), glutathione (reduced form), lipoic acid (reduced form), bovine serum albumin, dithiothreitol, NADH, a glutamate dehydro-

TABLE II

H_2S PRODUCTION FROM CYSTEINE IN BOVINE ADRENAL CORTEX

Omissions	H_2S formed**
complete system	1.41
−Cysteine	0.01
−α-Ketoglutarate	0.03
−Dithiothreitol	1.05
−pyridoxal phosphate	1.21

* Preparation of the adrenal cortex homogenate and assay method are described elsewhere in this paper.

** Enzyme activity is expressed as n-moles of product per 30 min per mg protein.

genase, a glutamic-oxaloacetic transaminase and malate dehydrogenase were purchased from Sigma Chemical Company.

RESULTS

H_2S formation from cysteine in adrenal cortex homogenate

We have examined the conditions under which H_2S is produced from cysteine in the adrenal cortex homogenate. Table II shows the absolute requirement of α-ketoglutarate for this reaction, while the addition of dithiothreitol and pyridoxal phosphate are not necessary. These results strongly suggest that the reaction is catalyzed by two enzymes, transaminase and 3-mercaptopyruvate sulfurtransferase, as is the case in other tissues:

Cysteine transaminase reaction:

α-ketoglutarate + cysteine → glutamate + 3-mercaptopyruvate

3-Mercaptopyruvate sulfurtransferase reaction:

3-mercaptopyruvate ——→ H_2S + pyruvate

H_2S production, glutamic-oxaloacetic transaminase and sulfurtransferases in comparison with labile sulfur content

In order to estimate the H_2S production capacity of each tissue, enzymatic activities related to H_2S production were determined (Table III). H_2S production from cysteine in adrenal cortex was relatively high compared with other tissues, although H_2S production activity did not correlate well with the labile sulfur contents in each tissue (Table IV). H_2S production seems to correlate with transaminase activity, rather than 3-mercaptopyruvate sulfurtransferase activity. In the case of 3-mercaptopyruvate sulfurtransferase, the distribution appears to be related to the estimated labile sulfur content in each tissue. However, the distribution of thiosulfate sulfurtransferase activity did not correlate with the estimated labile sulfur for each tissue, being extremely high in liver and kidney, but low in adrenal and heart. Our distribution of thiosulfate sulfurtransferase activity is in agreement with Villarejo and Westley [20].

From these results, it is concluded that 3-mercaptopyruvate sulfurtransferase is responsible for the iron–sulfur chromophore formation.

TABLE III

H_2S PRODUCTION, GLUTAMIC-OXALOACETIC TRANSAMINASE, AND SULFURTRANSFERASES IN BOVINE TISSUE HOMOGENATES

Assay methods and preparation procedure of bovine tissue homogenates are mentioned elsewhere in this paper.

Bovine tissue	Adrenal cortex	Liver	Kidney	Heart
H_2S production (n moles/30 min/mg)	1.40	0.96	0.61	—
Glutamic-oxaloacetic transaminase (μ moles/min/mg)	0.863	0.830	0.333	1.19
3-Mercaptopyruvate sulfurtransferase (μmoles/min/mg)	0.193	0.102	0.101	0.237
Thiosulfate sulfurtransferase (μmoles/min/mg)	0.132	1.05	0.528	0.140

Subcellular distribution of sulfurtransferase and cysteine transaminase in adrenal cortex

The subcellular distribution of enzymes related to H_2S production was studied in order to examine whether or not these enzymes are compartmentalized in particular subcellular fractions. The contamination due to other subcellular fractions was checked by the use of marker enzymes for each fraction. These results are summarized in Table V. Thiosulfate sulfurtransferase activity was observed specifically in the mitochondrial fraction, which is in good agreement with a previous observation [25]. 3-Mercaptopyruvate sulfurtransferase activity was localized in both mitochondrial

TABLE IV

ESTIMATION OF LABILE SULFUR CONTENTS IN BOVINE TISSUES BASED ON PREVIOUSLY PUBLISHED DATA [21, 22]

The labile sulfur contents were estimated based on the following assumption: (i) 5(Fe–S) centers are associated with NADH dehydrogenase, 2 with succinate dehydrogenase, and 1 with cytochrome *b–c* complex [23, 24], (ii) each Fe–S center consists of 2 iron atoms and 2 labile sulfur atoms, (iii) the ratio of cytochrome to respective flavoprotein is 1:1.

Bovine tissue	Adrenal cortex	Liver	Kidney	Heart
Cytochrome $a + a_3$ (nmoles/mg of mit. protein)	0.25	0.09	0.23	1.30
Estimated labile sulfur contents for respiratory chain system (ngatoms/mg of mit. protein)	4.00	0.96	3.68	20.8
Ferredoxin (nmoles/mg of mit. protein)	2.00	0	0	0
Estimated labile sulfur contents for hydroxylation system (ngatoms/mg of mit. protein)	4.00	0	0	0
Total estimated labile sulfur contents in tissue (ngatoms/mg of mit. protein)	8.00	0.96	3.68	20.8

V

BCELLULAR DISTRIBUTION OF SULFURTRANSFERASES AND CYSTEINE TRANSAMINASE IN BOVINE ADRE

ethods are described elsewhere in this paper. Differential centrifugation of adrenal cortex homogenate in 0.25 M sucrose solution bed by Hogeboom [26]. The mitochondrial and microsomal fractions were suspended separately in 10 mM Tris–HCl buffer, pH 7. nd used for the experiments.

	Succinate dehydrogenase (nmoles per mg per min)	Glucose--6-phosphatase (nmoles per mg per min)	Lactate dehydrogenase (nmoles per mg per min)	Thiosulfate sulfurtransferase		3-Mercaptopyruvate sulfurtransferase		Cysteine t
				Specific activity (μmoles per min per mg)	Total activity (μmoles per min	Specific activity (μmoles per min per mg)	Total activity (μmoles per min)	Specific activity (nmoles per mg per min)
ndrial	55.0	5.93	0.045	0.640	615	0.386	372	0.0204
mal	2.86	13.8	0.061	0.085	31.2	0.169	60.9	0.0238
	0	5.53	0.404	0.085	88.5	0.160	168	0.163

and soluble fractions. The specific activity in the mitochondrial fraction, however, was approximately twice as much as in the soluble fraction. Cysteine transaminase activity was located mainly in the soluble fraction, and a small amount of the activity was also found in both mitochondrial and microsomal fractions.

Subcellular distribution of capacities for H_2S production in adrenal cortex

We have examined the activity of H_2S production from cysteine in the mitochondrial, post-mitochondrial, and the combined fractions. The results (Table VI)

TABLE VI

THE SUBCELLULAR DISTRIBUTION OF H_2S PRODUCTION IN BOVINE ADRENAL CORTEX

Activity is expressed as total H_2S production in 10 g of adrenal cortex. Dithiothreitol and pyridoxal HCl were omitted from the standard assay mixture. The subcellular fraction was carried out as in Table V.

Fraction	H_2S formed (μmoles/30 min)
Homogenate	0.81
Mitochondrial	0.04
Post-mitochondrial	0.62
Mitochondrial + post-mitochondrial	0.72

indicate that the mitochondrial fraction did not produce H_2S from cysteine. The post-mitochondrial fraction produced H_2S to extent of 80% of that produced by the homogenate. The combination of both fractions slightly increased the amount of H_2S produced. From these results, it is concluded that the post-mitochondrial fraction alone is capable of producing H_2S, without the participation of the mitochondrial enzymes.

Enzymatic reconstitution of adrenal iron–sulfur protein

From the results described above, we are in favor of the possibility that the conversion of cysteine to pyruvate via 3-mercaptopyruvate is predominant pathway for H_2S production in adrenal cortex. The enzymes responsible for this reaction are located in the post-mitochondrial fraction. In order to confirm our finding, we have studied the enzymatic reconstitution of the iron–sulfur chromophore by the use of 3-mercaptopyruvate in the presence of 3-mercaptopyruvate sulfurtransferase, or cysteine and α-ketoglutarate in the presence of both transaminase and 3-mercaptopyruvate sulfurtransferase. From these experiments, we expect to know how effectively H_2S produced by enzymes will be incorporated into the apoprotein, relative to the chemical procedure where an excess amount of H_2S is utilized.

Preliminary measurements of the reconstitution of adrenal ferredoxin from the apo-protein, iron, and an H_2S-generating system were made by following the increase in absorbance of the reaction mixture at 414 nm. As shown in Fig. 1A, the absorbance at 414 nm increased proportionally to the amount of the 3-mercaptopyruvate sulfurtransferase added in the presence of both dithiothreitol and DL-dihydrolipoate (Fig. 1A, Curves a and b). No increase was observed in the absence of

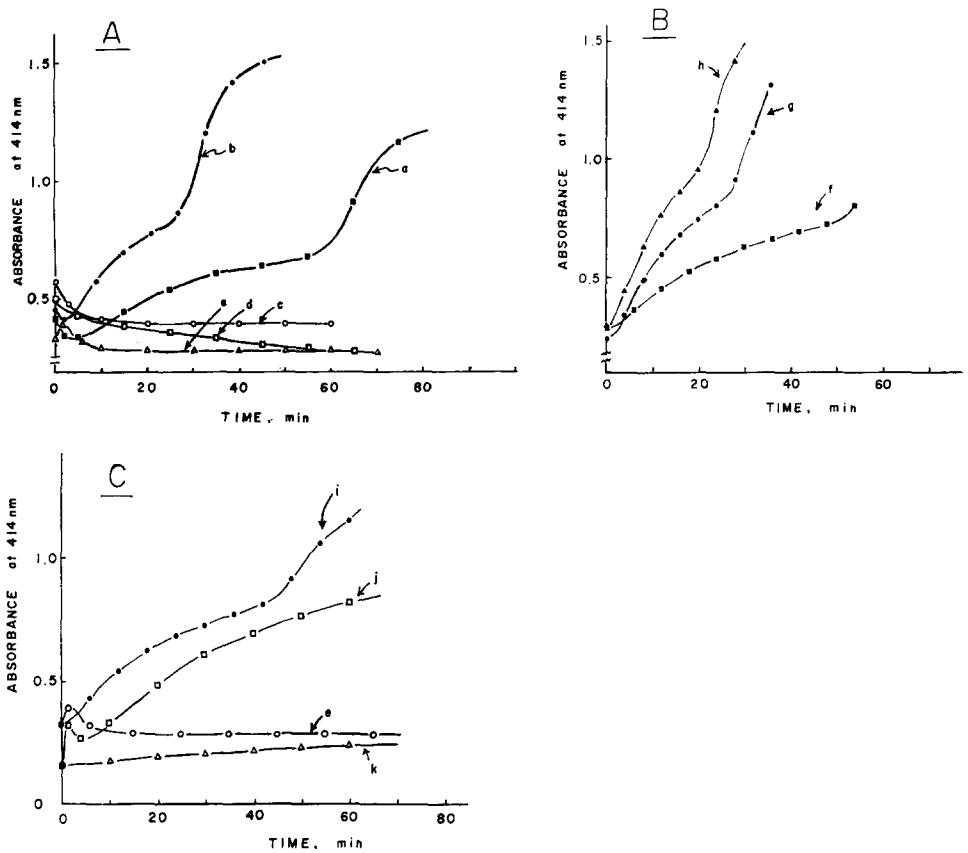

Fig. 1. Change in absorbance at 414 nm as a function of time. (A) The reaction mixture contained in 1.0 ml: 100 μmoles Tris–HCl buffer, pH 7.4, 0.3 μmoles adrenal apo-ferredoxin, 1.0 μmole ferrous ammonium sulfate, 6.8 μmoles dithiothreitol, 4.6 μmoles DL-dihydrolipoate, 2.0 μmoles 3-mercaptopyruvate and varying amounts of enzyme solution. a: 0.49 units of enzyme solution; b: 1.23 units of enzyme solution; c. no enzyme; d: no 3-mercaptopyruvate; e: no dithiothreitol and no DL-dihydrolipoate. An optical light path was 5 mm, and the reactions were carried out at 37 °C. (B) The same reaction mixture except that DL-dihydrolipoate was omitted. The enzyme amounts added are as follows: f: 0.49 units; g: 1.23 units; h: 2.47 units. (C) The same reaction mixture except that dithiothreitol was omitted. Enzyme amounts were 1.23 units in each case. i: 7.0 μmoles DL-dihydrolipoate; j: 7.0 μmoles of reduced glutathion added instead of dithiothreitol and DL-dihydrolipoate; K: control of j (no enzyme).

enzyme (Curve c), substrate (Curve d), or both thiol compounds (Curve e). It is clear from the results in Fig. 1B that in the absence of DL-dihydrolipoate, dithiothreitol acts not only as a reductant of the disulfide bonds of the apo-protein but also as a sulfur acceptor from 3-mercaptopyruvate. Experiments shown in Fig. 1C were carried out without addition of dithiothreitol, and the results indicate that DL-dihydrolipoate serves as well as dithiothreitol both as a reductant and a sulfur acceptor (Curve i).

Ferrous ions in the reconstitution reaction form colored iron complexes with the thiol compounds, which, together with the iron sulfide formed, interfere with the 414 nm absorbance maxima of adrenal ferredoxin. Therefore, in order to verify the

formation of adrenal ferredoxin the reaction mixtures were placed on small DEAE-cellulose columns (1.0 cm × 2.0 cm), washed with 0.17 M KCl in 10 mM phosphate buffer, pH 7.4, and the adrenal ferredoxin was eluted with 0.50 m KCl in the same buffer. Absorbance spectra obtained from the isolated samples with dithiothreitol or DL-dihydrolipoate are shown in Fig. 2. These spectra displayed absorption charac-

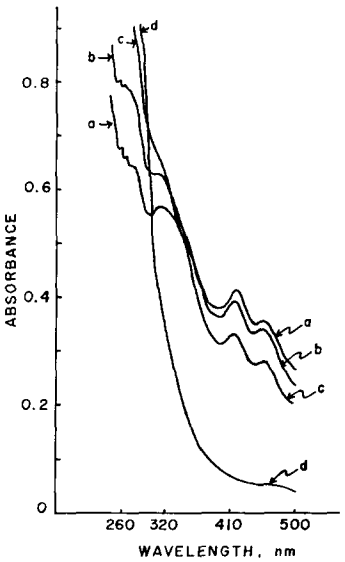

Fig. 2. Absorbance spectra obtained from the isolated samples. a: chemically reconstituted adrenal ferredoxin; b: in the presence of dithiothreitol, corresponding to Fig. 1B, Curve g; c: in the presence of DL-dihydrolipoate, corresponding to Fig. 1C, Curve i; d: in the presence of reduced glutathione, corresponding to Fig. 1C, Curve j. Each reaction mixture was explained in the legend to Fig. 1.

teristics of adrenal ferredoxin and were similar to the spectrum of a chemically reconstituted sample. The samples reconstituted with dithiothreitol, DL-dihydrolipoate, and H_2S had ratios (A_{414}/A_{276}) of 0.50, 0.36, and 0.63, respectively. The yields of reconstituted and isolated adrenal ferredoxin from the apo-protein were approximately 70%, 50%, and 85% with dithiothreitol, DL-dihydrolipoate, and H_2S, respectively. In the presence of adrenodoxin reductase, the NADPH–cytochrome *c* reductase activity of the enzymatically reconstituted adrenal ferredoxin was measured [26]. The sample was found to be fully active in terms of iron content compared with that of native adrenal ferredoxin.

In the case of reduced glutathion an increase in absorbance at 414 nm was also observed (Fig. 1C, Curve j). As shown in Fig. 2, Curve d, the absorbance spectrum of the isolated sample was that of adrenal apo-ferredoxin. This result indicates that glutathion (reduced form) can serve as a sulfur acceptor for 3-mercaptopyruvate in the sulfurtransfer reaction, but it cannot reduce the disulfide bonds present in the apo-protein. Instead, the formation of an iron–sulfur complex with glutathion (reduced form) might take place [27]. Therefore, DL-dihydrolipoate is the most likely in vivo candidate to serve as both a reducing agent and sulfur acceptor.

Upon reaction of the apoprotein with cysteine, α-ketoglutarate, glutamic-

oxaloacetic transaminase and 3-mercaptopyruvate sulfurtransferase together with iron and thiol compounds, the reconstitution of the iron–sulfur chromophore was successfully demonstrated. The reaction was carried out in vacuo at 37 °C for 1 h. The spectrum obtained in the isolated sample was similar to those of the samples from the non-enzymatic reconstitution.

DISCUSSION

In higher animals, biological production of H_2S occurs upon degradation of cysteine to pyruvate and by a sulfur transfer reaction from thiosulfate. The former reaction is catalyzed by a transaminase and 3-mercaptopyruvate sulfurtransferase and the latter is catalyzed by thiosulfate sulfurtransferase (Rhodanese). In addition to the pyruvate pathway, cysteine is oxidized to taurine via cysteinesulfinate, without H_2S evolution (taurine pathway). In rat liver, it was estimated by Yamaguchi et al. [12] that 31% and 69% of cysteine are metabolized through the pyruvate and taurine pathways respectively. Our results favor the interpretation that the H_2S required for iron–sulfur chromophore formation in adrenal cortex is formed from cysteine, by coupling a cytosol transaminase with 3-mercaptopyruvate sulfurtransferase.

Among the tissues examined, the 3-mercaptopyruvate sulfurtransferase and transaminase activities were highest in the adrenal cortex. In fact, when the crude homogenate was used in place of the purified enzymes, H_2S evolution with high activity was observed with either 3-mercaptopyruvate or cysteine as a substrate. Utilizing transaminase and 3-mercaptopyruvate sulfurtransferase, the reconstitution of native adrenal ferredoxin could be achieved with an efficiency comparable to the chemical reconstitution procedure.

The mitochondrial membranes are known to be impermeable to cysteine [29], and the equilibrium of the mitochondrial transaminase reaction is shifted toward the formation of α-ketoglutarate from glutamate [30]. Thus, the deamination reaction of cysteine to 3-mercaptopyruvate is unfavorable in the mitochondria. Although it is likely from our results (Table V) that the mitochondrial transaminase and 3-mercaptopyruvate sulfurtransferase would not be responsible for the biological formation of the iron–sulfur chromophore, the possibility that mitochondrial 3-mercaptopyruvate sulfurtransferase is involved in iron–sulfur chromophore formation within the mitochondria still cannot be excluded.

An alternative to be considered is that thiosulfate sulfurtransferase might be involved in the formation of the iron-sulfur chromophore. A system consisting of thiosulfate, iron, the adrenal apoprotein, and thiosulfate sulfurtransferase, was able to reconstitute the iron–sulfur chromophore, which agrees with the observation of Finazzigo et al. [31]. As reported by Baxter et al. [32], thiosulfate is an oxidation product of H_2S, which is formed from 3-mercaptopyruvate by 3-mercaptopyruvate sulfurtransferase. However, in adrenal cortex, thiosulfate sulfurtransferase activity which is located exclusively in the mitochondria, is low compared with liver and kidney (Table III), and also the distribution of thiosulfate sulfurtransferase in each tissue did not correlate to the estimated labile sulfur content (Table IV). Accordingly, this alternative appears to be unlikely for thiosulfate sulfurtransferase in the reconstitution reaction.

In higher animals, transaminase is an inducible enzyme, the activity of which

is controlled by hormones such as anterior pituitary hormones, cortisone, and thyroxine, and also by diet [33]. Since H_2S production in the adrenal cortex seems to be limited by the activity of the transaminase, the regulation of the levels of iron–sulfur protein may in turn be under hormonal influence.

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