INHIBITION BY AVIDIN OF THE ATP-Pi EXCHANGE ACTIVITIES ASSOCIATED WITH PREPARATIONS OF ENERGY TRANSFER FACTORS A AND A•D*

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Summary: Two water-soluble protein fractions were isolated from sonic extracts of beef heart mitochondria, which corresponded to the energy transfer factors A and A•D (ATP synthetase) described by Sanadi and coworkers. Both fractions augmented the activities of "urea" particles for ATP-Pi exchange and ATP-dependent DPN reduction by succinate. These activities were strongly inhibited by rutamycin. In the absence of added particles, both soluble fractions exhibited ATP-Pi exchange activities, which were not affected by rutamycin, but were strongly inhibited by avidin. The inhibitory effect of avidin was abolished when it was pretreated with biotin. The soluble fractions also exhibited avidin-sensitive propionyl-coenzyme A carboxylase activities, which were compatible with their ATP-Pi exchange activities.

In the course of our studies on the resolution and reconstitution of the mitochondrial electron transport-oxidative phosphorylation system, we became interested in the energy transfer factors A and A•D described by Sanadi and his colleagues (1-4). Factor A has been considered by these investigators to be the terminal ADP phosphorylating enzyme with latent ATPase activity, and factor A•D (subsequently referred to as ATP synthetase) has been reported to have oligomycin- and uncoupler-sensitive ATP-Pi exchange activity (4). The purpose of this communication is to show that materials corresponding to A and A•D, prepared in this laboratory, were both capable of catalyzing ATP-Pi exchange, but without rutamycin and uncoupler sensitivity. However, the ATP-Pi exchange activities of both preparations were inhibited by avidin, but not by biotin-treated avidin. Both preparations of A and A•D also exhibited propionyl-CoA carboxylase activities, which were similarly affected by avidin and biotin-treated avidin.

METHODS AND MATERIALS

Energy transfer factors A and A•D were prepared from extracts of sonicated

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beef heart mitochondria according to the procedures described by Sanadi and co-workers (1-4), except that dithiothreitol (DTT) was added throughout after the sonication step. The principal features of the isolation procedure were as follows. The sonic extract was adjusted to pH 5.7 by dropwise addition of 0.5 N H_2SO_4, and centrifuged. The supernatant was immediately neutralized to pH 7.5 with 0.5 N NaOH, and brought to 50% salt saturation by addition of solid ammonium sulfate while maintaining neutrality (pH 7.2) with dropwise addition of 0.5 N NaOH. The precipitate, referred to hereafter as fraction I, was taken up in 5 mM potassium phosphate, pH 7.5, containing 2 mM DTT, and filtered through a Sephadex G-25 column, which had been equilibrated with the same buffer medium, in order to remove ammonium sulfate. The effluent was diluted to a protein concentration of 7 mg/ml, fractionated with neutralized saturated ammonium sulfate, and the fraction precipitating between 39.8% and 49.8% salt saturation was collected by 5 min centrifugation at 22,000 g. The precipitate so obtained was dissolved in the aforementioned buffer mixture, desalted on Sephadex G-25 as before, and applied to a DEAE-52 column equilibrated with the same buffer. The column was eluted with 50, 80 and 150 mM potassium phosphate, pH 7.5, containing 2 mM DTT. Fractions eluted from the column with 80 and 150 mM phosphate correspond, respectively, to energy transfer factors A and A·D (2,4). They were collected by ammonium sulfate precipitation and centrifugation, taken up in a small volume of 10 mM Tris-H_2SO_4, pH 7.5, containing 2 mM DTT, and dialyzed for 2 hrs against the same buffer mixture. In this communication, we shall refer to these preparations as fractions II and III, respectively, to emphasize that they are not claimed to be the exact duplicates of Sanadi's A and A·D.

"Urea" particles were prepared according to Andreoli et al. (1); ATP-Pi exchange activity was measured by the method of Fisher et al. (4), using ^{33}Pi; ATP-dependent DPN reduction by succinate was assayed according to Lam et al. (5); and propionyl-CoA carboxylase assays were conducted according to Giorgio and Plaut (6). Protein was measured by the biuret method.

ATP and DPN were obtained from P-L Biochemicals, propionyl-coenzyme A and

TABLE I

Effects of Avidin, Rutamycin, and Uncouplers on the ATP-^{33}Pi Exchange Activities of Fractions I, II and III

Additions	Specific Activity		
	I	II	III
None	45	887	90
PCP	--	903	86
Cl-CCP	--	1012	82
Rutamycin	43	966	107
Avidin	9	101	19
Biotin-treated avidin	45	985	89

Conditions: The reaction mixture (1 ml) contained 50 mM Tris-H_2SO_4, pH 7.5, 10 mM ATP, 5 mM ADP, 10 mM potassium phosphate-^{33}P (1-6 x 10^4 cpm/μmole), 10 mM $MgCl_2$, and 1 mM DTT. The reaction was initiated by addition of ATP and terminated by addition of 0.1 ml of 35% trichloroacetic acid. ^{33}Pi incorporation was estimated according to Pullman (8). The amounts of fractions I, II and III were, respectively, 2.2, 0.67, and 2.42 mg protein. Duration of each experiment was 2 hrs and the temperature was 38°. When added, avidin (150 μg) was preincubated with each 1 mg of fraction I, II or III for 10 min at 38°. Biotin-treated avidin was prepared by incubating avidin with 2 to 5 times overdose of biotin (per unit of avidin) for 10 min at 38° before combining it with the soluble fractions. The amounts of other inhibitors used were per mg of soluble fractions, 8 nmoles PCP or Cl-CCP and 15 μg rutamycin. Specific activity is expresses as *nano*moles ^{33}Pi incorporated/hr/mg protein of fraction I, II or III.

avidin from Sigma Chemical Co., biotin from Nutritional Biochemicals, DTT and *m*-chlorocarbonylcyanide phenylhydrazone (Cl-CCP) from Calbiochem, ^{33}Pi from International Chemical and Nuclear Corporation, ^{14}C-sodium bicarbonate from New England Nuclear, and pentachlorophenol (PCP) from J.T. Baker. Rutamycin was a gift from Eli Lilly and Co.

RESULTS

Table I shows the ATP-Pi exchange activities of fractions I, II and III in the absence and presence of uncouplers, rutamycin, avidin, and biotin-treated avidin. It is seen that rutamycin, PCP and Cl-CCP do not inhibit the ATP-Pi exchange activities of fractions I, II and III, whereas avidin results in strong

TABLE II

Effects of Rutamycin and Avidin on the ATP-^{33}Pi Exchange Activity of "Urea" Particles Before and After Stimulation by Fractions II and III

Additions	Specific Activity		
	Particle	Particle + II	Particle + III
None	1200	4220	2052
Rutamycin	16	587	232
Avidin	1214	3803	1916
Biotin-treated avidin	----	3913	2162

Conditions: "Urea" particle, 0.5 mg; fraction II, 0.27 mg; and fraction III, 1.21 mg. All other conditions were the same as in Table I, except that the duration of experiments was 30 min. The ATP-Pi exchange activities of fractions II and III in the amounts used were, respectively, 124 and 48 *nano*moles/hr. Specific activity is expressed as *nano*moles ^{33}Pi incorporated/hr/mg particle protein.

inhibition. The lack of inhibition by biotin-treated avidin indicates that avidin inhibition of ATP-Pi exchange activity is associated with the biotin binding activity of avidin. Fractions I, II and III increased the activity of "urea" particles for rutamycin-sensitive ATP-Pi exchange and ATP-dependent DPN reduction by succinate. Representative results are shown for fractions II and III in Table II, and for fraction I in Table III. It is seen that the activities of "urea" particle alone and the increased activities in the presence of fraction I, II or III were strongly inhibited by rutamycin, but unaffected by avidin. In agreement with the work of Sanadi and his colleagues (1,3.4), these results indicate that fractions I, II and III contain factors which appear to be required for certain energy transfer activities of mitochondria (e.g., rutamycin sensitive ATP-Pi exchange, and energization of the respiratory chain by ATP). However, as suggested by the results of Table I, a major portion of the ATP-Pi exchange activity of the soluble factors may not be related to oxidative phosphorylation.

The inhibitory effect of avidin on the ATP-Pi exchange activities of the soluble factors suggested the presence of acyl-CoA carboxylase activity (most

TABLE III

Effects of Rutamycin and Avidin on the ATP-dependent Reduction of DPN by Succinate as Catalyzed by "Urea" Particles Plus Fraction I

Additions	*nano*moles DPNH formed/min
Fraction I (0.55 mg)	< 0.5
Particle (0.92 mg)	13.5
Particle + I	41.5
Particle + I + avidin	43.5
Particle + I + rutamycin	0.0

Conditions were the same as those described by Lam et al. (5). The appearance of DPNH was monitored at 340 nm. Temperature was 38°, and additions of avidin and rutamycin were made as in Table I.

TABLE IV

Propionyl-Coenzyme A Carboxylase Activities of Mitochondrial Sonic Extract and Fractions II and III

Additions	*micro*moles $^{14}CO_2$ fixed/mg/hr		
	Sonic Extract	II	III
None	2.6	94.7	2.7
Avidin	0.06	1.8	0.04
Biotin-treated avidin	----	90.2	2.5

Conditions were the same as in ref. 6. Temperature was 38°, and additions of avidin and biotin-treated avidin were made as in Table I.

likely propionyl-CoA carboxylase) in these preparations. The results shown in Table IV confirmed this possibility. It is seen that both fractions II and III contain propionyl-CoA carboxylase activity as evidenced from $^{14}CO_2$ fixation in experiments carried out according to Giorgio and Plaut (6). As expected, this activity was inhibited by avidin, but not by biotin-treated avidin.

CONCLUSIONS

The data reported above permit of the following conclusions:

1. Water-soluble preparations made from sonic extracts of mitochondria, according to the procedures described by Sanadi and coworkers for the preparation of energy transfer factors A and A•D, are capable of increasing the activity of "urea" particles for ATP-Pi exchange and ATP-dependent reduction of DPN by succinate. These activities, in the "urea" particles and in the factor-supplemented systems, are inhibited by rutamycin. To this extent fractions II and III described above are comparable to energy transfer factors A and A•D (ATP synthetase)

2. Both fractions II and III exhibit ATP-Pi exchange activity, which has been reported also by Sanadi and coworkers (4) for factor A•D (corresponding to fraction III), but not for factor A (corresponding to fraction II) (1). Indeed, the specific activity of fraction II for ATP-Pi exchange was found to be considerably higher than that of fraction III (see Table I). Fraction III differs, however, from factor A•D (ATP synthetase) with respect to the effect of oligomycin and uncouplers on its ATP-Pi exchange activity. The former preparation showed no sensitivity toward these reagents, whereas the ATP-Pi exchange activity of ATP synthetase has been reported to be strongly inhibited by oligomycin and uncouplers (4).

3. The ATP-Pi exchange activities of fractions II and III were strongly inhibited by avidin, but not by biotin-treated avidin. These fractions also exhibited concordant specific activities and avidin sensitivities for CO_2 fixation in the presence of propionyl-CoA. We find the presence of propionyl-CoA carboxylase in fractions II and III not surprising, because the procedure for isolation of factors A and A•D from mitochondria would not be expected to purify away propionyl-CoA carboxylase (see ref. 7).

4. While avidin inhibited the ATP-Pi exchange activities of fractions I, II and III, it had no effect on the augmented ATP-Pi exchange activity of "urea" particles supplemented with these fractions. By contrast, rutamycin inhibited the latter reaction, but not the former. In view of the presence of propionyl-CoA carboxylase in fractions I, II and III, it might be concluded that the components responsible for the ATP-Pi exchange activity of the soluble preparations

are not the same as those responsible for stimulation of the ATP-Pi exchange activity of "urea" particles.

5. Because of the differences indicated in part 2 above, fractions II and III cannot be equated with factors A and A·D, respectively. Therefore, we do not exclude the possibility that dissimilarities might also exist in the nature of ATP-Pi exchange reactions found by us and Sanadi's group in our respective preparations.

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