POLYAMINE EFFECT ON NADPH-OXIDATION CATALYZED BY SPINACH AND ADRENAL FLAVOPROTEIN–IRON–SULFUR PROTEIN COMPLEXES AND A POSSIBLE REGULATORY MECHANISM *

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1. Introduction

For many years aliphatic polyamines have been well known as constituents of biological material. Although polyamines have been implicated in contributing to membrane stability and as a growth factor for certain microorganisms, their biochemical functions still remain unclear. In previous communications [1, 2] from this laboratory, we have reported that basic proteins such as cytochrome *c* and histones can stimulate markedly the rate of NADPH-oxidation catalyzed by spinach and adrenal flavoprotein–iron–sulfur protein in 1:1 molar complexes. Of interest was the fact that the reaction product of the cytochrome *c*-stimulated reaction is H_2O, while that of the histone-stimulated reaction is H_2O_2. Furthermore, the acidic phospholipid, cardiolipin, enhances the rate of oxygen uptake about 3-fold in the histone-stimulated reaction of the spinach system, but it completely inhibits the oxygen-uptake by the adrenal system. These findings urged us to investigate the effects of polyamines on the adrenal and spinach systems.

In this communication, we wish to report that cadaverine stimulated markedly the rate of NADPH-oxidation catalyzed by the spinach system, whereas under comparable conditions spermine enhanced that catalyzed by the adrenal system. Also, spermine inhibited adrenal steroid hydroxylation reactions but had no effect on the DCPIP† reduction catalyzed by adrenodoxin reductase. In this context, it is concluded that polyamines affect the interaction between the flavoprotein and the iron–sulfur protein.

2. Materials and methods

Spinach ferredoxin-$NADP^+$ reductase and ferredoxin were prepared by the methods of Shin et al. [3] and by Tagawa et al. [4], respectively.

Adrenodoxin reductase and adrenodoxin were prepared by the methods previously reported by us [5, 6]. Adrenal mitochondrial cytochrome *P*-450 was prepared by the method of Mitani and Horie [7] with some mod ifications.

Polyamines were obtained from Sigma and neutraliz to pH 7.3. Oxygen-uptake was measured by a Gilson-oxygraph, Model KM, equipped with a Clark type electrode. NADPH-oxidation and DCPIP-reduction were measured by following the decreases in absorbance at 340 nm and 600 nm, respectively. Procedures for the measurements of enzyme activities have been described elsewhere [6].

3. Results and discussion

Fig. 1 shows the effects of polyamines on the O_2 uptake catalyzed by the spinach ferredoxin-$NADPH^+$ reductase and ferredoxin system. Stimulatory effects were observed in the cases of spermidine, spermine, and cadaverine. Putrescine showed a small stimulatory effect. No oxygen uptake was observed in the absence

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† DCPIP: 2,6-dichlorophenolindophenol.

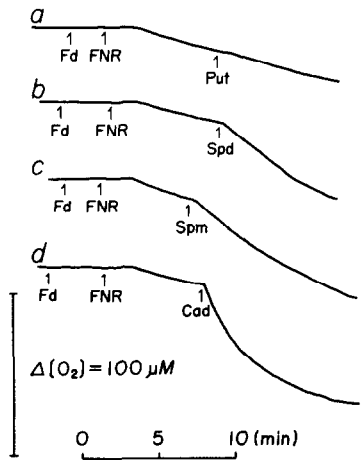

Fig. 1. Effects of polyamines on O_2-uptake by spinach ferredoxin-NADP$^+$ reductase and ferredoxin system. The reaction mixtures contained: ferredoxin-NADP$^+$ reductase (FNR), 5.65×10^{-7} M; ferredoxin (Fd), 1.3×10^{-6} M; NADPH, 1.27×10^{-4} M; EDTA, 2.9×10^{-4} M; putrescine (Put), spermidine (Spd), spermine (Spm), or cadaverine (Cad), 2.9×10^{-3} M. The reaction was carried out at 25° and pH 7.3 (0.01 M Tris-HCl buffer).

Table 1
Comparison of polyamine effects on the NADPH-oxidase activities of the spinach and adrenal systems.

Addition	Relative activity	
	FNR-Fd system	AR-Ad system
None	1.00	1.00
Putrescine	1.33	1.77
Cadaverine	8.70	0.51
Spermidine	3.48	3.80
Spermine	3.66	5.01
Agmatine	2.08	2.38
Tyramine	0.69	1.18
Tryptamine	1.55	2.46
Histamine	1.44	1.55
Asparagine	0.93	1.07
Glutamine	1.14	1.17

The reactions were carried out by similar methods to those shown in fig. 1. FNR, ferredoxin-NADP$^+$ reductase; Fd, ferredoxin; AR, adrenodoxin reductase; Ad, adrenodoxin. Polyamine concentrations were 2.9×10^{-3} M.

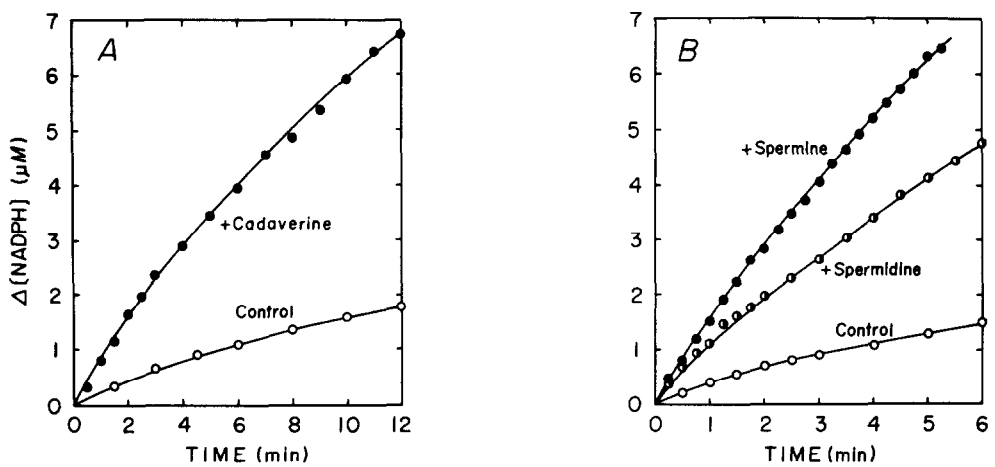

Fig. 2. Effects of polyamines on NADPH-oxidation by the spinach (A) and adrenal (B) systems. The reaction mixtures contained: (A) NADPH, 2.2×10^{-5} M; EDTA, 2×10^{-4} M; ferredoxin-NADP$^+$ reductase, 9.6×10^{-8} M; ferredoxin, 2.24×10^{-7} M; polyamine: 1×10^{-3} M. (B) NADPH, 2.2×10^{-5} M; EDTA, 2×10^{-4} M; adrenodoxin reductase, 2.5×10^{-8} M; adrenodoxin, 6.8×10^{-7} M; polyamines, 1×10^{-3} M. The reactions were carried out at 10° and pH 7.3 (0.01 M Tris-HCl buffer).

of NADPH, thereby excluding the possibility that the amines used are oxidized by the highly purified enzymes.

As shown in fig. 2, NADPH-oxidation was measured by the decrease in absorbance at 340 nm with the use of a stopped-flow spectrophotometer. In agreement with the stimulatory effects of polyamines obtained in fig. 1, the rates of disappearance of NADPH by both spinach and adrenal systems were markedly increased by the presence of cadaverine in the case of the spinach system, and by the presence of spermine or spermidine in the case of the adrenal system. Therefore, it is concluded that the polyamines stimulate the rate of oxygen-uptake by the NADPH-dependent flavoprotein and iron–sulfur system.

Table 1 summarizes the effects of various amines and amides on both the spinach and adrenal systems through similar experiments to those shown in fig. 1. It is clear from this table that cadaverine is most effective in the spinach system, whereas it is slightly inhibitory in the adrenal system. Spermine shows the most stimulatory effect on the adrenal system, being also effective in the spinach system. From this data it appears that the length of the carbon chain and the number of nitrogen atoms of the polyamines are important determinants for the degree of stimulation of the respective systems.

Table 2 shows the effects of various concentrations of cadaverine on the NADPH-oxidase stimulation of the spinach system.

Table 2
Effects of various concentrations of cadaverine on NADPH-oxidase activity of the spinach system.

Cadaverine concentration ($\times 10^3$ M)	nmoles/min	Relative activity
0	7.26	1.0
1.3	21.77	3.0
2.6	38.58	5.3
3.3	73.55	10.1

The reaction mixtures contained: NADPH, 1.69×10^{-4} M; spinach ferredoxin, 1.012×10^{-6} M; spinach ferredoxin-NADP$^+$ reductase, 5.8×10^{-7} M; EDTA, 2×10^{-4} M. The reactions were carried out at 25° and pH 7.3 (10 mM Tris-HCl buffer). The enzymatic activity was measured by decrease in the absorbance at 340 nm.

Table 3
Effects of polyamines on enzymatic activities of the spinach and adrenal systems.

Polyamine concentration ($\times 10^3$ M)	Adrenal system				Spinach system	
	11-β Hydroxylase		DCPIP Reductase		DCPIP Reductase	
	nmoles/30 min	Relative activity	nmoles/min	Relative activity	nmoles/min	Relative activity
0	1.57	1.00	20.9	1.00	124.4	1.00
0.5	0.56	0.36	----	----	----	----
1.0	0.19	0.12	20.9	1.00	124.4	1.00
2.0	----	----	20.9	1.00	124.4	1.00
3.0	----	----	20.9	1.00	124.4	1.00

Steroid 11 β-hydroxylase activity was measured as described elsewhere [6]. The reaction mixture contained: *P*-450, 0.7×10^{-6} M; adrenodoxin reductase, 0.53×10^{-7} M; adrenodoxin, 0.78×10^{-5} M; deoxycorticosterone, 4.0×10^{-3} M; NADP$^+$, 2.4×10^{-4} M; glucose-6-phosphate, 4.0×10^{-2} M; and glucose-6-phosphate dehydrogenase, 5 units. The reaction was carried out at 37° and pH 7.4 for 30 min. NADPH-DCPIP reductase activity was measured at 25° by the rate of reduction of DCPIP at 600 nm (ϵ = 21,000). The reaction mixture for the adrenal system contained: DCPIP, 2.4×10^{-5} M; adrenodoxin reductase, 4.12×10^{-7} M; NADPH, 3.12×10^{-5} M; spermine, 8.3×10^{-4} M at pH 7.4. The reaction mixture for the spinach system contained: DCPIP, 1.00×10^{-5} M; ferredoxin-NADP$^+$ reductase, 7.15×10^{-9} M; NADPH, 1.17×10^{-5} M. The polyamines used were spermine and cadaverine for the adrenal and spinach systems, respectively, at the concentrations as indicated in the table. All reactions were carried out in 10 mM Tris-HCl buffer, pH 7.4.

In table 3, the effects of polyamines on various enzymatic activities of the spinach and adrenal systems are shown. Cadaverine showed neither inhibitory nor stimulatory effect on the DCPIP reductase activity of the spinach system. Additionally, the DCPIP reductase activity of the adrenal system remained unchanged by the addition of spermine. From this data, it is likely that upon the addition of polyamines the auto-oxidizability of reduced iron–sulfur protein by O_2 increases markedly in some manner.

Together with the fact that polyamines serve as the inhibitor for steroid hydroxylation reactions, the leak of reducing equivalents from the flavoprotein–iron–sulfur protein complex by the addition of polyamine might suggest a possible regulatory mechanism of the hydroxylation reactions of adrenal mitochondria by polyamines. The accessibility of basic compounds such as polyamines and histones to mitochondiral membranes, where adrenodoxin reductase, adrenodoxin, and *P*-450 are localized, would stop the steroid hydroxylation reactions even under the conditions of continuous supply of steroids and NADPH.

Tabor [8] reported that low concentrations (10^{-3}–10^{-4} M) of spermine or spermidine inhibit mitochondrial swelling. The mechanism of the protection of mitochondria against induced swelling has been interpreted as the binding of polyamines to fatty acids released by mitochondria. The effects of polyamines on single enzymes have been investigated by several workers. Polyamine protects against the loss of activities of prostatic acid phosphatase [9] and β-gluconidase [10] upon dilution. Polyamines also inhibit the activities of RNAase and DNAase [11]. This effect may not be attributable to the enzymes, but rather to binding of the substrate by the polyamines.

The function of polyamines proposed in this study is the direct effects on the protein–protein interaction. In this sense, our postulated mechanism of the physiological function of polyamines would be unique. Our present results might be a clue to test the effects of polyamines on biologically important protein assemblies, such as in ribosomes, mitochondria, microsomes, chloroplasts, and protoplasts.

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