J. Biosci., Vol. 2, Number 1, March 1980, pp. 23–27. © Printed in India.

Interactions of progesterone with D-amino acid oxidase–Different effects on apo- and holo-enzyme*

FAIZY AHMED and MAHATAB S. BAMJI
National Institute of Nutrition, Jamai-Osmania, Hyderabad 500 007

MS received 31 December 1979

Abstract. A simple method using charcoal treatment was developed for the preparation of apo-D-amino acid oxidase from rat kidney homogenates. This apo-D-amino acid oxidase was used to study the effect of progesterone on the apo- and holo-enzyme. Progesterone inhibited the activity of D-amino acid oxidase, when the apo- enzyme, preincubated with saturating amounts of FAD was used; this effect varied with FAD concentration. Progesterone did not inhibit the activity when added to a mixture of non-preincubated apo-enzyme and FAD; this suggests that progesterone has different effects on apo- and holo D-amino acid oxidase.

Keywords. Progesterone; D-amino acid oxidase; rat kidney; flavin adenine dinucleotide.

Introduction

Administration of steroidal oral contraceptives containing estrogen and progestrogen to adult female rats elevates the activity of some flavin enzymes such as D-amino acid oxidase (D-amino acid: O_2 oxidoreductase (deaminating) EC 1.4.3.3, Ahmed and Bamji, 1976). To elucidate the mechanism of this increase, the *in vitro* interactions of different steroids with highly purified hog kidney D-amino acid oxidase were studied. Tanaka *et al.,* 1978). Among several steroids tested, progesterone inhibited the enzyme markedly, while other compounds were only slighcy effective. Similar inhibition of the enzyme activity was observed when progesterone was added to rat liver and kidney homogenates (Ahmed and Bamji, 1977). Subsequent studies with kidney homogenates however showed that the inhibitory effect of progesterone on D-amino acid oxidase activity was abolished and sometimes even stimulation was observed when FAD was added to completely saturate the apoenzyme present in the homogenate. This observation indicated the possibility of two types of effects of progesterone on the enzyme– an inhibitory effect on the holoenzyme but a stimulatory effect on the apoenzyme. Tissue homogenates contain both holo- and apo-enzymes, and in the presence of added FAD, the stimulatory and inhibitory effect may therefore tend to cancel out each other

The results of a study to test this possiblity presented here suggest that progesterone has different effects on apo- and holo-D-amino acid oxidase.

Materials and Methods

Progesterone, FAD and dextran-T80 were obtained from Sigma Chemical Company, St. Louis, Missouri, USA.

---

* Preliminary report presented at the V International Congress of Hormonal Steroids, 29 Oct–4 Nov. 1978, New Delhi. *J. Steroid Biochem.* **9,** 832, (abstract 94) 1978.

Charcoal obtained from Merck Chemicals was activated by refluxing with 1N HCl for 8 h. It was then washed several times with water to remove the acid completely and dried. To obtain dextran-coated charcoal, 10 *g* of charcoal was mixed with 1 g dextran-T80.

Preparation of crude apo-D-amino acid oxidase from kidney homogenates

Adult female rats of Wistar strain were sacrified by decapitation, the kidneys removed and homogenised to 20% in glass distilled water.

The following conditions were found to be optimum to obtain apo-D-amino acid oxidase with minimum residual activity and maximum stimulation with added FAD; 2% kidney homogenate (diluted from 20%) was allowed to stand at 0°C for 15 min. Dextran-T80-coated charcoal (5 mg/ml) was added to the homogenate, stirred and immediately centrifuged at 4°C and 1200 *g* for 30 min. The supernatant was used as a source of the crude apo-D-amino acid oxidase.

Two types of experiments were carried out. System- 1 in which apo-D-amino acid oxidase was preincubated with FAD for 10 min to regenerate the holoenzyme, and progesterone added at the time of starting the reaction with D-alanine. System-2 in which FAD and progesterone were added to the apoenzyme simultaneously, at the time of starting the reaction. A third system involving pre-incubation of apo-enzyme with progesterone and then starting the reactions with FAD and D-alanine was also tried but not pursued since the apo-enzyme was rapidly degraded at 37°C in the absence of FAD. Effects of different concentrations of FAD, D-alanine and progesterone were tested in both the systems.

D-amino acid oxidase activity was assayed by measuring the pyruvate formed from D-alanine (Seifter *et al.,* 1948). Protein was estimated by the method of Lowry *et al.* (1951). The enzyme activity was expressed as μ mol pyruvate formed/mg protein/h.

Results

The reconstitution of D-amino acid oxidase activity by incubation of apo-enzyme with increasing concentrations of FAD (4 to 100 μM) in the absence and presence of progesterone (60 μM) in systems 1 and 2 are presented in figures 1a and 1b. Progesterone inhibited the enzyme activity in competition with the coenzyme, FAD in system 1. K_m for FAD in the absence and presence of progesterone (60 μM) being 7.6 μM and 10.1 μM respectively (figure 1a inset). However, at FAD concentrations less than 1 μM progesterone tended to activate the enzyme marginally in system 1 (data not shown).

In system 2, where progesterone and FAD (4-100 μM) were added together to the apo-enzyme, the inhibitory effect seen in system 1 was abolished (figure 1b).

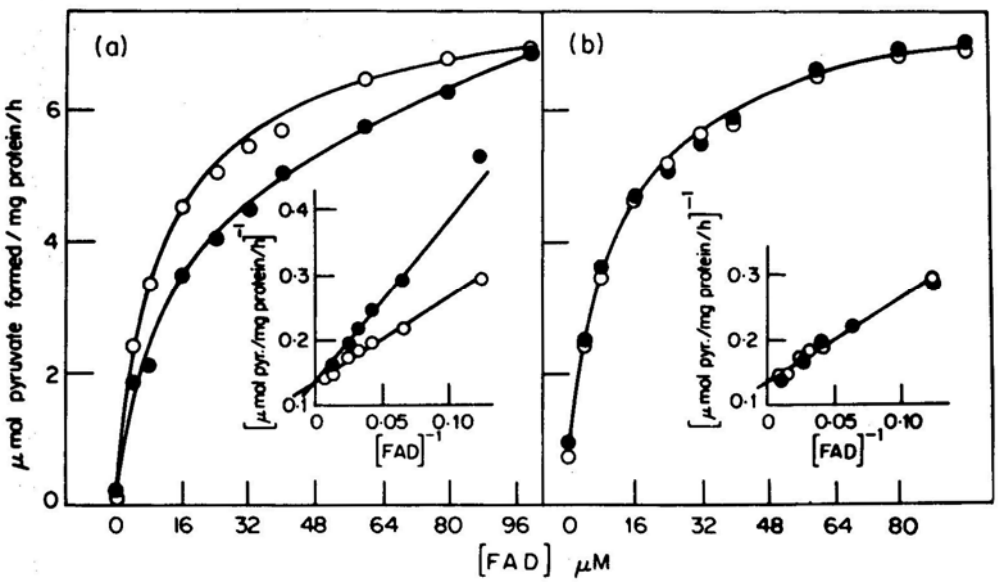

Figure 1. Reconstitution of rat kidney D-amino acid oxidase from crude apo-enzyme and FAD and the effect of progesterone, (a) Progesterone effect in system 1. The apoenzyme was preincubated for 10 min with FAD (4-10 μM) and added before starting the reactions with D-alanine (—O —) no progesterone (–●–)plus progesterone (60 μM), (b) Progesterone effect in system 2. Progesterone (60 μM) and FAD (4-100 μM) were added together before starting the reaction with D-alanine. The inset shows 1/v vs 1/(FAD) plot with and without progesterone.

The reaction mixture, taken in 15 ml tubes contained: sodium arsenite (0.04M in 0.11 M Nacl) –0.3 ml; Krebs-Ringer buffer, pH 8.3–1.0 ml; enzyme-0.5 ml; FAD-0.1 ml (4-100 μM). The reaction was started by the addition of 0.1 ml D-alanine (15 μM). Oxygen was passed into each tube for 10 sec at a pressure of 50 kg/cm^2 and the tubes closed with rubber stoppers. The reaction was stopped at the end of 1 h with 0.5 ml of 20% trichloroacetic acid. Pyruvate was estimated in the protem-free supernatant, as its 2:4 dinitrophenylhydrozone dirivative by a colorimetric method (Seifter *et al.,* 1948). The inset shows double reciprocal plot with and without progesterone.

Figure 2 describes the effects of different fixed concentrations of progesterone on 1/v vs 1/ [*S*] plot of D-amino acid oxidase activity of various concentrations of D-alanine. In system 1 (figure 2a) progesterone produced significant inhibition of the enzyme in competition with its substrate D-alanine. The K_m for D-alanine was found to be 8.0 mM in the absence of progesterone. The K_i for progesterone was 1.25 μM.

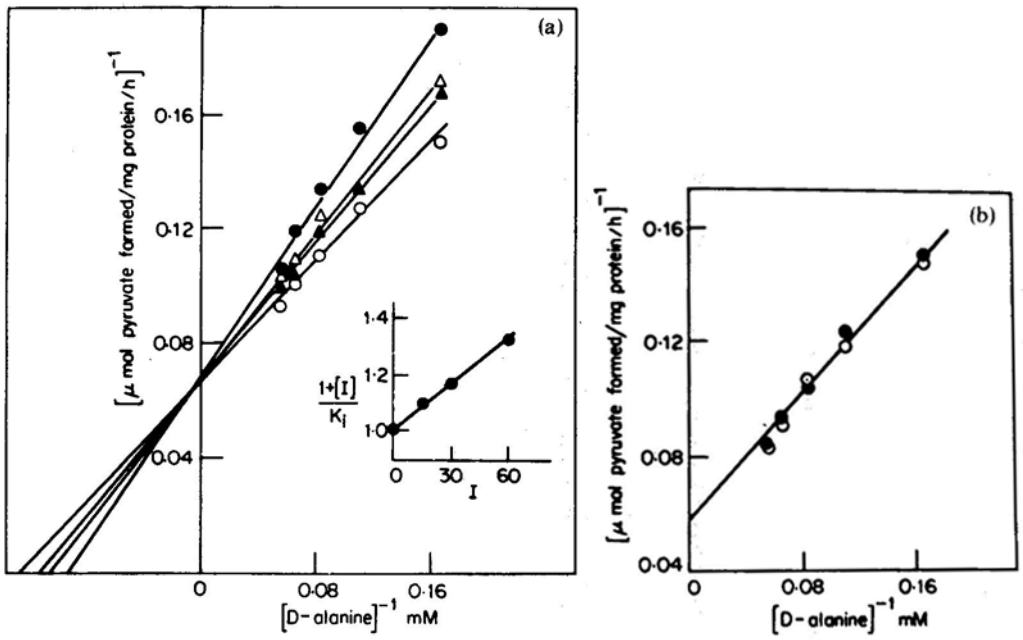

Figure 2. Effect of progesterone on the double reciprocal plot of catalytic oxidation of D-lanine by rat kidney D-amino acid oxidase reconstituted from the apo-enzyme and FAD (60 μM). in presence of varying concentrations of D-alanine.

The concenntrations of progesterone used were, none (O), 15 μM (▲) 30 μM (Δ) and 60 μM (●). Progesterone was added in 10 μ 1 of alcohol to obtain the desired concentration. Same amount of alcohol was added to control tubes. Linear best fit pots were obtained by the method of least squares.

(a). Progesterone effect in system 1 (as in legend to figure 1)

(b). Progesterone effect in system 2 (as in legend to figure 1)

–O– no progesterone;– ●–+ progesterone (60 μM)

Inset in figure 'a' shows plot of $1 + [I]\ K_i$ vs $[I]$ where K_i is the dissociation constant for the enzyme-inhibitor complex, and $[I]$ is the concentration of progesterone.

Discussion

The effects of progesterone on the regenerated holo-D-amino acid oxidase (system 1) at various D-alanine concentrations (figure 2a) are analogous to the observations of Tanaka *et al.* (1978) with the highly purified hog kidney enzyme. The K_i (1.25 μM) observed in the present study is lower than (18 μM) observed by Tanaka *et al.* (11978). This difference may probably be due to the difference in the sources of the enzyme or due to the environment provided in the crude system.

Tanaka *et al.* (1978) postulated that inhibition of D-amino acid oxidase by progesterone may be due to a steric block of the substrate binding site by progesterone. Since progesterone increased fluorescence emission as well as fluorescence polarization of the enzyme, the authors ruled out the possibility that progesterone was causing dissociation of FAD from the enzyme. However, the data presented in figure 1a

show that progesterone increased K_m for FAD in system 1, suggesting that the hormone does affect FAD binding at FAD concentrations greater than 4 μM. In view of the fact that in the enzyme, the binding sites for substrate and coenzyme are in close proximity it is possible that progesterone produces a steric block of FAD binding site in the holo-enzyme and thus prevents reassociation of FAD which is removed during enzyme catalysis.

The opposite effects of progesterone at high concentrations of FAD in system 1 and at all concentration of FAD in system 2 (where FAD, progesterone and substrate were added simultaneously to apoenzyme) are difficult to explain. It is possible that progesterone binds to different sites on apoenzyme and holoenzyme, producing different effects. In the holoenzyme, progesterone may produce a steric block of substrate and FAD binding sites and inhibit the activity. Binding of progesterone to the apoenzyme may produce changes which facilitate association with FAD and eliminate the steric block. Preliminary studies of Tanaka and Yagi (personal communication) with purified hog kidney D-amino acid oxidase also suggest that progesterone may increase the association of FAD with the apo-enzyme. The possibility of these two different types of effects of progesterone an apo- and holo-enzymes derives support from the observation that when FAD concentration was very low (permitting most of the enzyme to be in apoenzyme form) progesterone produced slight activation of the enzyme even in system 1.

The physiological significance of the dual effects of progesterone on D-amino acid oxidase is not clear, since it is an enzyme of limited biological significance and the observed changes are of a small magnitude. The latter however is not surprising in view of the opposite effects. Such hormonal effects if observed for other enzymes may have significance in the regulation of enzyme activities at low and high concentrations of coenzyme.

Acknowledgements

The authors are grateful to Dr. S. G. Srikantia, Director, National Institute of Nutrition, Hyderabad and to Dr. N. Appaji Rao, Department of Biochemistry, Indian Institute of Science, Bangalore for the benefit of useful discussions.

References

Ahmed, F. and Bamji, M. S. (1976) *Contraception,* **14,** 297.
Ahmed, F. and Bamji, M. S. (1977) *Indian J. Biochem. Biophys.,* **14,** (abstract 69).
Lowry, O. H., Rosenbrough, N. J., Farr, A. L. and Randall, R. J. (1951) *J. Biol. Chem.,* **193,** 265.
Mason, M., Ford, J. and Wu, H. L. C. (1969) *Ann. N. Y. Acad. Sci.,* **166,** 170.
Seifter, S., Harkness, D, M. Rabin, L. and Muntwyler, E. (1948) *J. Biol. Chem.,* **176,**137 1.
Southgate, J. (1972) *Adv. Biochem. Psychopharmacol.,* **5,** 263.
Tanaka, F., Bamji, M. S. and Yagi, K. (1978) *Biochem. Biophys. Acta.* **522,** 43.