BBA 43190

Photoreduction of amino-acid oxidases in the presence of free flavin and the effect of urea

The reduction of flavin by illumination in the presence of potential electron donors under anaerobic conditions has been demonstrated[1] as has the similar photoreduction of FAD-dependent flavoproteins with EDTA[2]. In a recent investigation on the nature of such photoreductions of flavins and flavoproteins by several photoreductants, it was shown that addition of trace quantities of free flavin enhances the rate of photoreduction of certain of the flavoproteins[3]. Both D-amino-acid oxidase (D-amino-acid:oxygen oxidoreductase (deaminating), EC 1.4.3.3) and L-amino-acid oxidase (L-amino-acid:oxygen oxidoreductase (deaminating), EC 1.4.3.2) were found to be photoreduced several times faster when light was shone on an anaerobic, buffered solution which contained FAD or FMN in addition to flavoprotein and EDTA. Addition of urea above 2 M to D-amino-acid oxidase with EDTA also leads to a photoreduction rate as great as that of free flavin[3].

Since FMN does not bind and function well with the amino-acid oxidases[4,5], but can serve at least as effectively as FAD in enhancing the rate of photoreduction of these enzymes[3], it is unlikely that usual binding of the additional flavin at the coenzyme site is prerequisite for photoreduction of these flavoproteins. The main sequence of reactions in these cases must be photoreduction of free flavin by EDTA followed by reduction of flavoprotein by the photoreduced flavin. Whether the free flavin, which directly reduces the flavoprotein, is the half-reduced semiquinone or fully reduced hydroquinone was not determined, as illumination was done with all reactants together in solution. Reduction of flavins to the hydroquinone level proceeds *via* the semiquinone which disproportionates rapidly and nearly completely to oxidized and hydroquinone species at slightly alkaline pH (ref. 6) used for the photoreduction of the amino-acid oxidases. Thus, if reduction of flavoprotein is induced by flavin hydroquinone, the free flavin could be separately photoreduced with EDTA and then added to the oxidized flavin–enzyme to generate bound flavin semiquinone.

The effect of urea in enhancing the rate of photoreduction of D-amino-acid oxidase could be due to disruption of subunit structure and unfolding or to additional uncoiling of the α-helix. If the concentration of urea needed to break the α-helical structure of this protein is greater than that which allows the marked increase in photoreduction rate, the changes in optical rotation at a wavelength characteristic of α-helix should not occur at the lower concentrations of urea.

D-Amino-acid oxidase from pig kidney was isolated as the benzoate complex[7] and then freed from benzoate[8]. L-Amino-acid oxidase was isolated from snake venom[5].

Solutions in Thunberg cuvettes were deoxygenated by repeated evacuation followed by readmission of O_2-free nitrogen. Illumination was done as before[3] with light from a 500-W xenon lamp unit selected at 450 nm by the monochromator of a Zeiss spectrophotometer. Contents of the cuvette were stirred and temperature was maintained near 5°. Absorption spectra were registered with a Cary Model 14 recording spectrophotometer with the cell holder also thermostated. Optical rotatory dispersion data were obtained with the Jasco Model ORD/UV-5. The depth of the Cotton effect at 233 nm was measured with solutions at 14° containing varying amounts of urea and the calculated reduced mean residue rotation, $[m']^{14^\circ}_{233\,nm}$, taken

as representative of the helical content[9]. The values of a_0 and b_0 were calculated with the MOFFITT–YANG equation[10].

The separate addition of oxidized D-amino-acid oxidase, but not the addition of apoenzyme to reduced FAD (Fig. 1) or reduced FMN, leads to formation of a

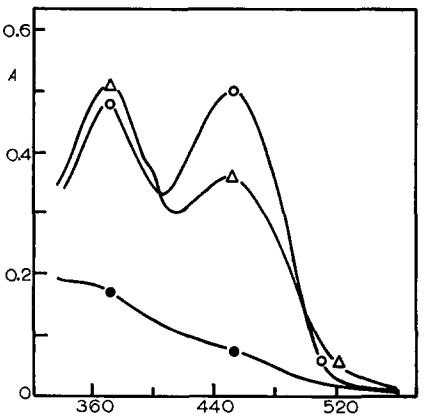

Fig. 1. The influence of photoreduced FAD on D-amino-acid oxidase. Spectra of FAD photoreduced with EDTA (●); after anaerobic addition of D-amino-acid oxidase (△); and upon reoxidation of the FAD–oxidase mixture (○). Final concentrations of reactants in 4 ml were 25 μM FAD, 25 mM EDTA, and 3 mg oxidase in 0.1 M sodium pyrophosphate buffer (pH 8.3). Temp., 5°.

spectrum characteristic of the flavin semiquinone–enzyme. Thus, it seems that the latter is produced by binding the flavin semiquinone produced by disproportionation between dissociated oxidized enzyme–flavin and the free hydroquinone. However, the direct transfer of 1 equivalent by an aspecifically bound hydroquinone to the oxidized enzyme–flavin cannot be completely excluded. Similar results were obtained with L-amino-acid oxidase.

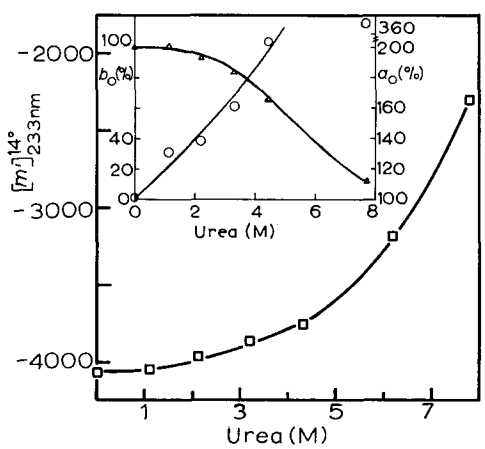

Fig. 2. Change in reduced mean residue rotation of D-amino-acid oxidase as a function of urea concentration. Solutions contained 1.67 mg oxidase in 3 ml of 0.1 M sodium pyrophosphate (pH 8.3) with urea as indicated. Insert shows the influence of urea on the a_0 and b_0 values, calculated according to ref. 10. ○—○, a_0; △—△, b_0. Temp., 14°.

The effect of urea on the reduced mean residue rotation of D-amino-acid oxidase is shown in Fig. 2. More than 4 M urea is required to elicit a large change in the values and an approach to that reported for random coil[9]. As concentrations lower than this are quite effective in increasing the rate of photoreduction of the oxidase with EDTA[3], it would seem that such enhancement may be due to an unfolding of the enzyme with greater exposure to the photoreductant. This is supported by the finding that the a_0 value increases at low urea concentrations. The b_0 value declines above 4 M urea concentrations.

This work was supported by The Netherlands Foundation for Chemical Research (S.O.N.) with financial aid from The Netherlands Organization for the Advancement of Pure Research Z.W.O.).

Department of Biochemistry,
Agricultural University,
Wageningen (The Netherlands)
J. F. KOSTER
C. VEEGER

Graduate School of Nutrition and Biochemistry Section,
Cornell University,
Ithaca, N.Y. (U.S.A.)
D. B. MCCORMICK

1 W. R. FRISELL, C. W. CHUNG AND C. G. MACKENZIE, *J. Biol. Chem.*, 234 (1959) 1297.
2 V. MASSEY AND G. PALMER, *Biochemistry*, 5 (1966) 3181.
3 D. B. MCCORMICK, J. F. KOSTER AND C. VEEGER, *European J. Biochem.*, 2 (1967) 387.
4 T. P. SINGER AND E. B. KEARNEY, *Arch. Biochem.*, 27 (1950) 348.
5 D. WELLNER AND A. MEISTER, *J. Biol. Chem.*, 235 (1960) 2013.
6 A. EHRENBERG, *Arkiv Kemi*, 19 (1962) 97.
7 V. MASSEY, G. PALMER AND R. BENNETT, *Biochim. Biophys. Acta*, 48 (1961) 1.
8 K. YAGI AND T. OZAWA, *Biochem. Z.*, 338 (1963) 330.
9 G. D. FASMAN, in S. P. COLOWICK AND N. O. KAPLAN, *Methods in Enzymology*, Vol. VI, Academic Press, New York, 1963, p. 942.
10 W. MOFFITT AND J. T. YANG, *Proc. Natl. Acad. Sci. U.S.*, 42 (1956) 596.

Received January 4th, 1968