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OXIDATION OF POLYAMINES BY PYRROLOQUINOLINE QUINONE (PQQ)

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Pyrroloquinoline quinone (PQQ) was found to oxidize the polyamines spermine and spermidine in stoichiometric amounts with diaminopropane as the main product identified by reversed phase high performance liquid chromatography and mass spectroscopy of the reaction products. Putrescine was oxidized much slower than the polyamines and cetyl-trimethyl-ammonium bromide did not promote oxidation. A model for the PQQ oxidation of polyamines is suggested to account for these observations.

INTRODUCTION

The polyamines spermine (SPM), spermidine (SPD) and putrescine (PUT) are necessary for cell growth and cell division (1-4). The cellular concentration of these molecules is regulated through biosynthesis and degradation. The degradation of polyamines is catalyzed by amine oxidases, which are enzymes catalyzing the oxidative degradation of amines. Amine oxidases can be divided into two classes: 1. FAD-dependent (mainly monoamine oxidases) and 2. PQQ-dependent (mainly diamine and polyamine oxidases, also classified as Cu-amine oxidases) (5,6). The organic prosthetic group of the second class, has only recently been identified as PQQ (7-10). Since PQQ has been reported to be capable of efficient catalytic oxidation of amino acids (11) and amines (12) we have investigated the ability of PQQ to oxidize polyamines.

ABBREVIATIONS

PQQ, pyrroloquinoline quinone; SPM, spermine; SPD, spermidine; PUT, putrescine; DAP, diaminopropane; FAD, flavin adenine dinucleotide; FAB-MS, fast atom bombardment - mass spectroscopy; TEAP, triethylammonium phosphate; CTAB, cetyltrimethylammoniumbromide; NMR, nuclear magnetic resonance.

MATERIALS AND METHODS

SPM·4HCl, SPD·3HCl, CTAB, and dansylchloride were from Sigma (St. Louis, USA). PUT·2HCl, triethylamine, diaminopropane, and PQQ were from FLUKA (Buchs, Switzerland). H_3PO_4, NaOH, NaCl, NaH_2PO_4 and Na_2HPO_4 were from Merck (Darmstadt, Germany). MeOH was from Rathburn (Walkerburn, Scotland). Reactions were performed at 37°C in phosphate buffer pH 7.2. Samples for HPLC were dansylated and analysed as described by Brossat et al. (13) using a linear gradient from 50% MeOH, 50% 50 mM TEAP pH 4, to 100% MeOH in 15 min (A) or from 60% MeOH to 100% MeOH in 30 min (B). HPLC was done on a Waters system with two model 510 pumps, a model 420-AC fluorescence detector, a 710 B WISP, a Waters Data Module, a Z-module with a 8 mm x 10 mm RP-8 column, and a Waters System Controller.

Samples from the HPLC were dried, extracted into MeOH, dried again and dissolved in glycerol for FAB-MS.

FAB-MS was done on a VG Masslab VG 20-250 Quadropole mass spectrometer fitted with a VG FAB source and probe. The primary beam of xenon atoms was produced from an ion gun, Ion Tech. Ltd., operating at 1.0 mA, 8 kV.

RESULTS

In contrast to its effect on primary amines PQQ in μmolar concentrations had no measurable effect on mmolar concentrations of polyamines, both in the presence or absence of CTAB (Table 1). However, in mmolar concentration PQQ exerted a time and concentration dependent oxidation of polyamines with the order of effectiveness: SPM > SPD > PUT. In contrast to its effect on oxidation of primary amines (12) CTAB had an inhibitory effect on the oxidation of SPM.

Fig. 1 shows oxidation of SPM at various concentrations of PQQ. Using a 15 min linear gradient from 50 to 100% MeOH for the HPLC determination of polyamines the major product eluted at a position corresponding to PUT/DAP and the minor product eluted at a position corresponding to SPD. Using a 30 min gradient from 60% to 100% MeOH the major product eluted at a position corresponding to DAP (Table 2). The identity of the peaks was further confirmed by FAB-MS of the collected dansylated compounds from the HPLC (Table 2). These two products were formed in a time dependent manner in the PQQ oxidation of SPM (Fig. 2). SPD was oxidized less effectively (Fig. 3) and PUT was oxidized only to a small extent (Fig. 4). In line with these results it was found that only SPM and SPD reacted rapidly with PQQ at pH 4 forming orange and yellow precipitates, whereas PUT did not react with PQQ to form a precipitate.

TABLE 1. Effect of PQQ on mM concentrations of SPM at various concentrations of CTAB. Samples were incubated for 20 hours in phosphate buffer pH 7.2 at 37°C.

SPM conc. (mM)	PQQ conc. (mM)	CTAB conc. (mM)	% Oxidation
1	0.001	0	0
1	0.001	2	0
1	0.001	20	0
10	0.01	0	0
10	0.01	2	0
10	0.01	20	0
1	1	0	80
1	1	2	46
1	1	20	20

TABLE 2. HPLC and FAB-MS analysis of the products from PQQ oxidation of SPM.

Peak nr.	Elution time[a] (min.)		Mass[b]	Compound[c]
1 (major)	14.66	0.06	540	DAP
2 (minor)	26.98	0.02	670	SPD
STD A	14.69	0.05	540	DAP
STD B	15.73	0.00	554	PUT
STD C	27.10	0.02	670	SPD
STD D	29.53	0.03	-	SPM

a) Gradient B
b) Mass of the dansylated compound
c) Name of the un-dansylated compound

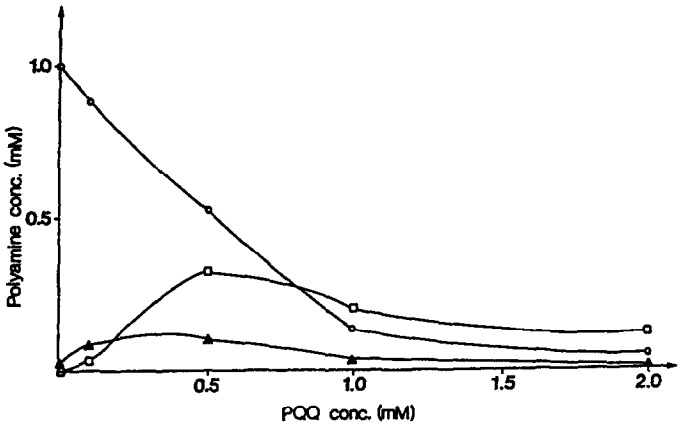

Fig. 1 Effect of PQQ concentration on oxidation of SPM. Samples were incubated in phosphate buffer pH 7.2 at 37°C for 20 hours. Polyamine concentrations were determined by HPLC.

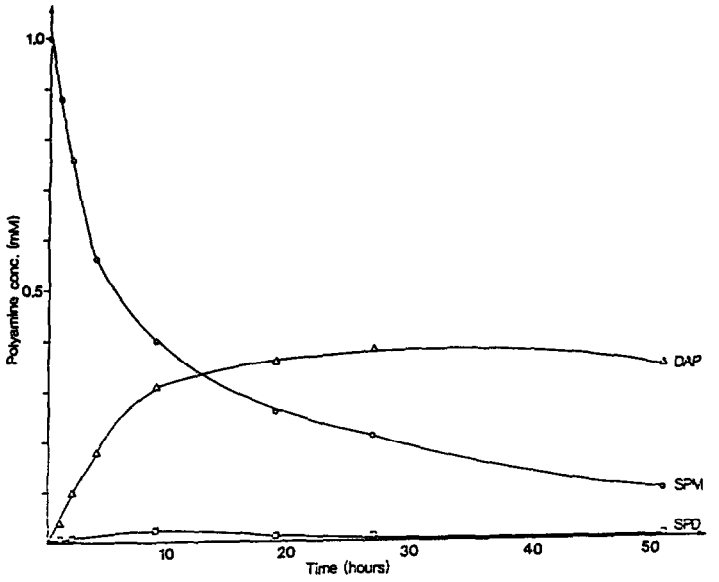

Fig. 2 Time course for the oxidation of SPM by PQQ. Samples were incubated in phosphate buffer pH 7.2. PQQ concentration was 1 mM.

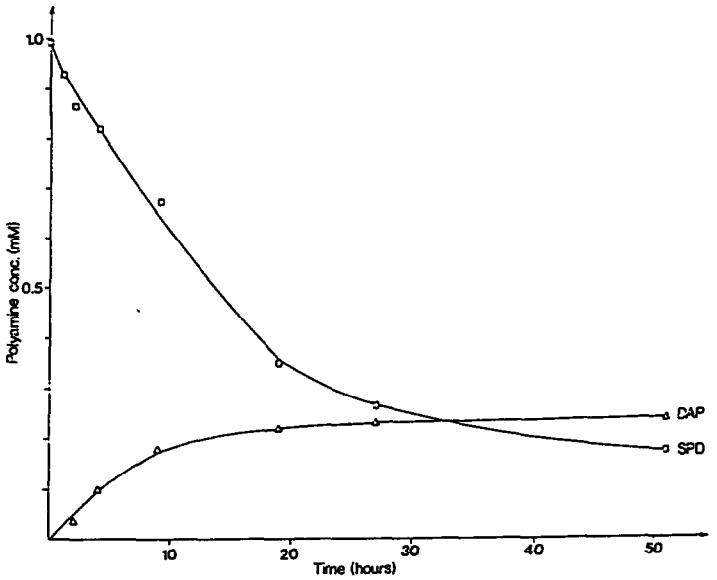

Fig. 3 Time course for the oxidation of SPD by PQQ. Samples were incubated in phosphate buffer pH 7.2 with 1 mM PQQ.

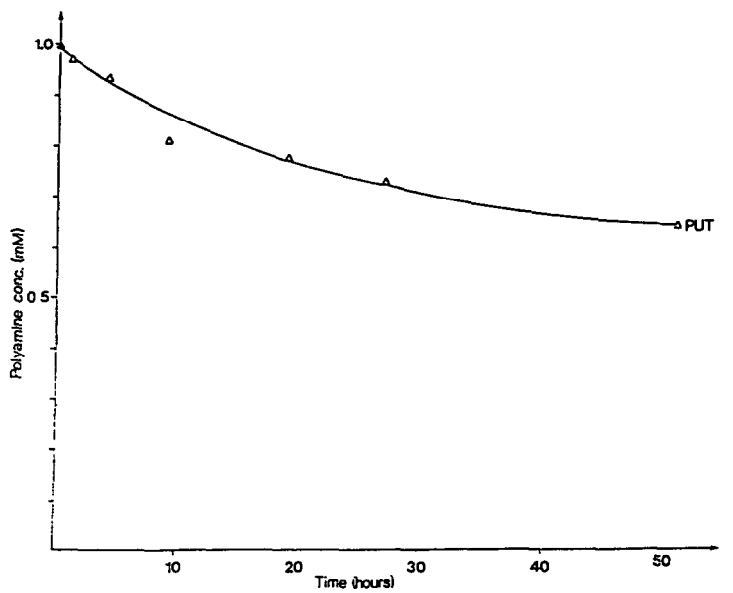

Fig. 4 Time course for the oxidation of PUT by PQQ. Samples were incubated in phosphate buffer pH 7.2 with 1 mM PQQ.

DISCUSSION

The inability of CTAB to promote oxidation of polyamines may be explained by assuming a repulsion between the CTAB micelles and the polyamines or by an inability of CTAB to form micelles in the presence of polyamines. In any case, PQQ is not an effective autorecycling catalyst for the oxidation of polyamines. At equimolar concentrations, however, PQQ oxidized polyamines with the order of effectiveness: SPM > SPD > PUT. Also surprising is the identification of DAP as the main product from the PQQ oxidation of SPM and SPD, since the main product from the PQQ-dependent bovine plasma amine oxidase catalysed oxidation of SPM and SPD is PUT (G. Houen et al., unpublished results). This may be explained by assuming a reaction mechanism, where the polyamines preferentially makes a nucleophilic attack on the cofactor carbonyl group with the more nucleophilic secondary nitrogen atom (Fig. 5). In analogy with the PQQ catalyzed oxidation of amino acids, the adduct may then split to an aminoaldehyde, which easily cyclizes, and PQQ bound DAP. DAP may then be released and PQQ regenerated by the action of O_2 and H_2O with the concomitant generation of H_2O_2. In the enzyme catalyzed reaction it can be assumed that steric constraints imposed by the enzyme on the reaction, forces cleavage of the C-N bond to take place on the other side of the secondary N-atom with the formation of PUT from SPD and SPD from SPM with concomitant formation of 3-amino-propionaldehyde. This was found to be the case by 500 MHz ^{1}H-NMR studies of the enzyme reaction (G. Houen et al., unpublished results). In light of these results, the role of the enzyme in the plasma amine oxidase catalyzed oxidation of polyamines can be considered to be threefold: 1. Steric control of the site of cleavage to assure formation of PUT instead of DAP. 2. Efficient regeneration of the catalyst. 3. Lowering of the energy of activation, i.e. "catalysis", of the reaction.

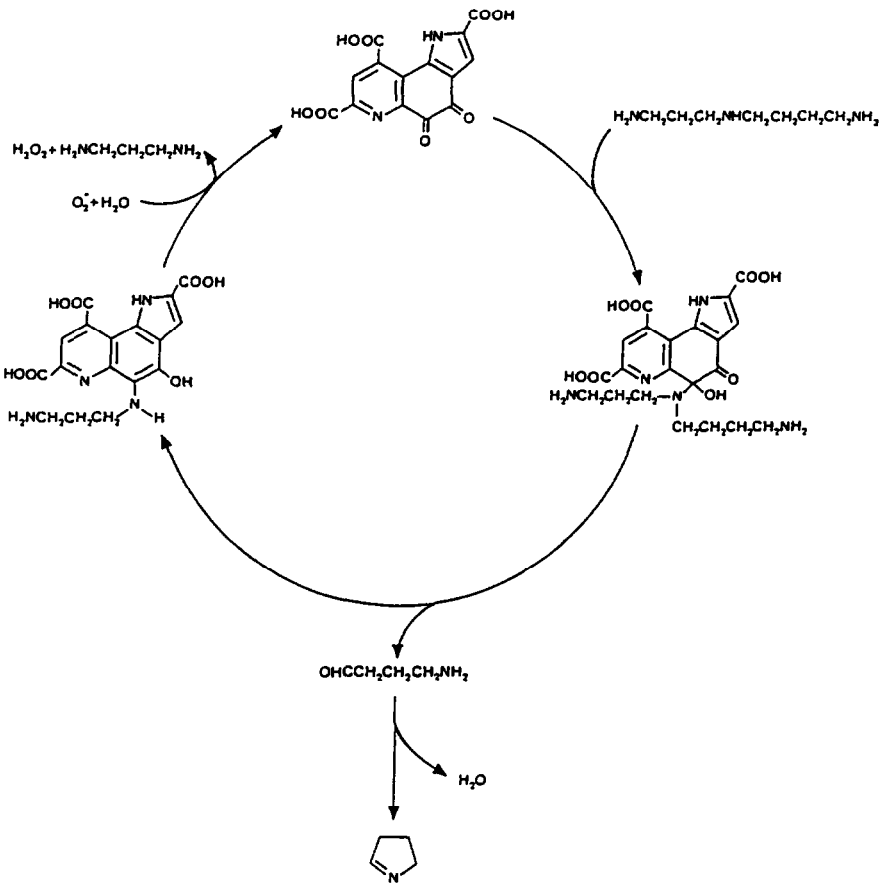

Fig. 5 Possible reaction mechanism for the PQQ oxidation of polyamines.

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