Oxidation of D-Glucose by Coenzyme PQQ: 1,2-Enediolates as Substrates for PQQ Oxidation

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Studies on the oxidation of D-glucose and related substrates with coenzyme PQQ have established that 1,2-enediolates are efficient substrates for PQQ oxidation.

PQQ (Pyrroloquinoline quinone, methoxatin) has received much attention as a novel coenzyme of several important oxidoreductases (quinoproteins).[1] Recently, glucose dehydrogenases in various micro-organisms have been recognized to be quinoproteins in which PQQ acts as a coenzyme catalysing the direct oxidation of D-glucose.[2–5] However, the detailed mechanism of this enzymatic oxidation is not yet clear. We now report the first example of the non-enzymatic oxidation of D-glucose by PQQ and discuss its mechanism.

When D-glucose (0.061 mmol) was treated with an equimolar amount of PQQ in 0.3 M carbonate buffer solution (2 ml, pH 10.4) at room temperature for 2 weeks under anaerobic conditions, gluconic acid was formed in 51% yield, equation (1).† This reaction was followed spectrophotometrically by monitoring the appearance of reduced PQQ ($PQQH_2$) at 320 nm under pseudo-first order conditions ([PQQ] = 4.0×10^{-5} M, [D-glucose] = 8.0×10^{-2} M, 0.05 M carbonate buffer, pH 10.4, $\mu = 0.2$ with KCl, 30 °C, anaerobic conditions). The appearance of $PQQH_2$ was found to be zero order up to 70% conversion. Introduction of oxygen into the final reaction mixture regenerated PQQ quantitatively, and within a few minutes of closing the reaction cell, the zero order appearance of $PQQH_2$ started again. This phenomenon occurred repeatedly showing that PQQ can act as a turnover catalyst, as in the case of PQQ oxidation of amines,[6] amino acids,[7] and thiols.[8]

The reaction was first order in D-glucose concentration ($k_{2app} = k_{obs.}/[\text{D-glucose}][PQQ] = 8.3 \times 10^{-3}$ mol^{-1} dm^3 s^{-1}). Furthermore, general base catalysis (first order in [OH^-]) was observed over the pH range 8.9—10.6. A linear plot was obtained of the rate constant k_0 (the intercept of the plot of $k_{obs.}$ *vs.* [buffer]) *vs.* pH. These results accord with the mechanism of equation (2) and the kinetic expressions in equations (3) and (4). Namely, the active species in the

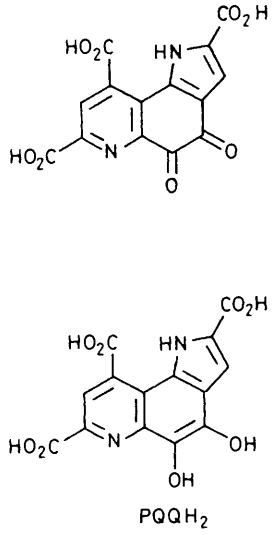
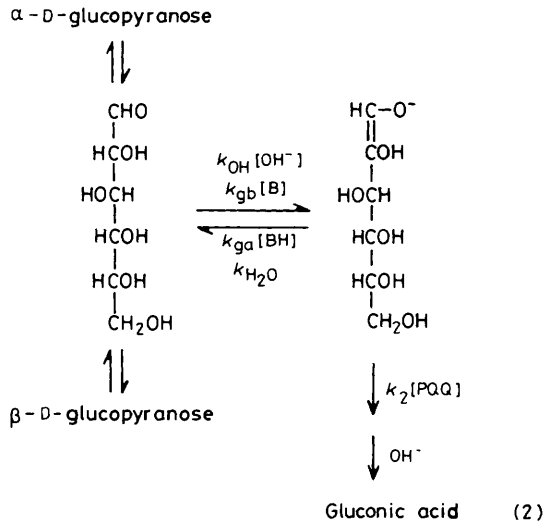


Table 1. Relative reactivity of sugars towards PQQ-oxidation.[a]

Sugar	pH[3]	k_{2app} /mol^{-1} dm^3 s^{-1}	Relative rate of oxidation[b]
D-glucose	11.21	3.6×10^{-2}	1.0 (1.0)
D-fructose	11.15	3.0×10^{-1}	8.3 (10.7)
D-arabinose	11.23	1.5×10^{-1}	4.2 (4.1)
D-mannose	11.13	3.7×10^{-2}	1.0 (0.5)
D,L-glyceraldehyde	11.20	11.8	328 (–)

[a] [PQQ] = 4.0×10^{-5} M, [sugar] = 8.0×10^{-2} M, 0.2 M Na_2CO_3, 25 °C, anaerobic conditions. [b] Relative rate of tritium uptake[10] is shown in parentheses.

† The formation of gluconic acid was confirmed by h.p.l.c.; YMC PA-03 column (Yamamura Chemical Laboratories Co., Ltd.), solvent 0.1 M acetate buffer (pH 4.5)–MeCN (75 : 25, v/v).



(2)

reaction is the 1,2-enediolate of D-glucose and its formation is the rate-determining step ($k_{ga}[BH] + k_{H_2O} \ll k_2[PQQ]$).

$$V = \frac{k_2(k_{gb}[B] + k_{OH}[OH^-])[\text{glucose}][PQQ]}{(k_{ga}[BH] + k_{H_2O}) + k_2[PQQ]} \quad (3)$$

$$k_{ga}[BH] + k_{H_2O} \ll k_2[PQQ]$$
$$k_{obs.} = (k_{gb}[B] + k_{OH}[OH^-])[\text{glucose}] \quad (4)$$

The same kinetic observations were made for the oxidation of benzoin (α-hydroxy ketone) by PQQ ($k_{2app} = 5.1 \times 10^{-2}$ mol^{-1} dm^3 s^{-1}, 30 °C, pH 9.9). The reaction was zero order in [PQQ] and first order in [benzoin]. General base catalysis was observed over the pH range 9.7—11.1, and the intercept k_0 of the plot of $k_{obs.}$ *vs.* [buffer] was proportional to [OH$^-$]. It is well known that the 1,2-enediolate is the important intermediate in the oxidation of α-ketols with several oxidants.[9]

The rates of 1,2-enediol formation for various sugars have been estimated by monitoring hydrogen–tritium exchange rates at 25 °C in alkaline solution.[10] The oxidations of D-glucose, D-fructose, D-arabinose, D-mannose, and D,L-glyceraldehyde by PQQ were investigated under the same conditions (25 °C, pH 11.15—11.23, Table 1). The relative rates of the oxidation were comparable to those of tritium uptake (1,2-enediol formation), and an acyclic substrate (glyceraldehyde) was much more reactive than the other sugars. The difference in reactivity is attributed mainly to the difference in the concentration of the acyclic form from which the 1,2-enedidate intermediate is derived.

The mechanism of the oxidation of 1,2-enediolates by PQQ is an important problem. Kinetic behaviour indicating the possibility of an electron transfer mechanism was observed in the oxidation of L-ascorbic acid by PQQ (first-order in [PQQ], $k_{2app} = k_{obs.}/[\text{ascorbic acid}] = 59.3$ mol^{-1} dm^3 s^{-1}, pH 4.5, 30 °C).

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References

1 J. A. Duine and J. Frank, Jun., *Trends Biochem. Sci.*, 1981, **6**, 278.
2 J. A. Duine, J. Frank, Jun., and J. K. Van Zeeland, *FEBS Lett.*, 1979, **108**, 443.
3 M. Ameyama, K. Matsushita, Y. Ohno, E. Shinagawa, and O. Adachi, *FEBS Lett.*, 1981, **130**, 179.
4 B. J. Van Schie, K. J. Hellingwerf, J. P. Van Dijken, M. G. L. Elferink, J. M. Van Dijl., J. G. Kuenen, and W. N. Konings, *J. Bacteriol.*, 1985, **163**, 493.
5 M. Ameyama, M. Nonobe, E. Shinagawa, K. Matsushita, K. Takimoto, and O. Adachi, *Agric. Biol. Chem.*, 1986, **50**, 49.
6 Y. Ohshiro, S. Itoh, K. Kurokawa, J. Kato, T. Hirao, and T. Agawa, *Tetrahedron Lett.*, 1983, **24**, 3465.
7 S. Itoh, N. Kato, Y. Ohshiro, and T. Agawa, *Tetrahedron Lett.*, 1984, **25**, 4753.
8 S. Itoh, N. Kato, Y. Ohshiro, and T. Agawa, *Chem. Lett.*, 1985, 135.
9 S. Shinkai, T. Kunitake, and T. C. Bruice, *J. Am. Chem. Soc.*, 1974, **96**, 7140.
10 H. S. Isbell, H. L. Frush, C. W. R. Wade, and C. E. Hunter, *Carbohydr. Res.*, 1969, **9**, 163.