The Biochemistry of the Nitrifying Organisms

6. THE EFFECT OF OXYGEN CONCENTRATION ON NITRITE OXIDATION IN THE PRESENCE OF DIFFERENT INORGANIC IONS*

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Lees & Simpson (1957) reported that the cyanate ion was a strong inhibitor of nitrite oxidation by *Nitrobacter*. We have now found that this inhibition by cyanate, and by certain other inorganic ions, may be eliminated or even replaced by a stimulation if the oxygen concentration in the *Nitrobacter* suspension medium is decreased. A preliminary account of some of this work has already appeared (Butt & Lees, 1958).

MATERIALS AND METHODS

Culture methods. *Nitrobacter* cells were grown and harvested by methods essentially similar to those described by Lees & Simpson (1957). The main differences between the original techniques and those used in the present work were: (a) the 10 l. culture bottles were replaced by a 100 l. stainless-steel tank fitted with a bubbler tube to supply sterilized air and a 6 in. stainless-steel paddle rotating at 1450 rev./min. to ensure adequate aeration and mixing of the culture; (b) the concentration of inorganic salts (apart

from nitrite) in the medium was decreased to one-tenth of the original value since it has been found (L. Herzenberg, personal communication) that *Nitrobacter* grows more readily and is more active in oxidizing nitrite when it is cultured in the more dilute medium.

Measurement of nitrite oxidation. In each experiment 0.5 ml. of a washed suspension of *Nitrobacter* cells was placed in the side arm of a Warburg flask; after temperature equilibration for 15 min. at 30°, the cells were tipped into the main chamber containing the other reactants in 2.5 ml. of 0.03 M-phosphate buffer, pH 7.5, and the oxygen uptake was measured over a suitable period.

The required oxygen concentrations were obtained by flushing with mixtures of oxygen and nitrogen prepared by displacement of water from a graduated reservoir.

Reagents. Sodium nitrite used for culturing *Nitrobacter* was of technical grade; sodium arsenite was A.R. grade; all other reagents were as those used by Lees & Simpson (1957).

RESULTS

Nitrate, at a concentration of 33 mM, inhibited nitrite oxidation at high oxygen concentrations but stimulated it at lower oxygen concentrations (Table 1); this result is typical of several examples obtained with different nitrite and nitrate concentrations. Similar results were obtained with

Table 1. *Effect of nitrate, arsenite and cyanate on nitrite oxidation by Nitrobacter at different oxygen concentrations*

In each Warburg vessel there was 2.5 ml. of 0.03 M-phosphate buffer, pH 7.5, containing nitrite and nitrate, arsenite or cyanate. The gas phase in each vessel was changed by flushing with the appropriate mixture of oxygen and nitrogen and, after temperature equilibration at 30°, 0.5 ml. of *Nitrobacter* suspension (containing 0.25–0.5 mg. dry wt. of cells) was added from the side arm. In the experiment with arsenite, the initial nitrite concentration was 17 mM and in all other experiments 33 mM. Results are expressed as a percentage of the control value in the absence of inhibitor.

		Oxygen in gas phase (%)						
	Concentration	1.25	2.5	5.0	7.5	10	15	20
Compound	(mM)	Percentage of control value						
Nitrate	33	200	233	109	68	66	—	—
Arsenite	8	—	167	191	—	107	82	73
Cyanate	0.3	214	104	74	60	36	—	—
	0.2		130					50
	0.3		145					34
	0.5		118					24
	0.7		97					18
	1.0		54					8

Fig. 1. Relationship between nitrite concentration and the rate of nitrite oxidation by *Nitrobacter* at different oxygen concentrations. In each vessel there was 2.5 ml. of 0.03 M-phosphate buffer, pH 7.5, containing the nitrite. In the side arm there was 0.5 ml. of washed *Nitrobacter* suspension containing 0.25–0.5 mg. dry wt. of cells. The gas phase in each vessel was changed by flushing with the appropriate mixture of oxygen and nitrogen before temperature equilibration at 30°. Oxygen concentrations used were: ○, 20%; □, 10%; △, 2.5%.

arsenite or cyanate in place of nitrate (Table 1); chloride and sulphate had little effect at any oxygen concentration tested. The effect on nitrite oxidation of varying cyanate concentrations at low and normal oxygen concentrations is also shown in Table 1.

As Meyerhof (1917) showed, the rate of nitrite oxidation by *Nitrobacter* first of all increases as the nitrite concentration is increased, reaches a maximum and then begins to diminish as the nitrite concentration is further increased; typical curves for the variation of the rate of nitrite oxidation with nitrite concentration at various oxygen concentrations are shown in Fig. 1. This 'humped' type of curve is characteristic of enzyme systems in which there is inhibition by excess of substrate. Fig. 1 also shows how the optimum nitrite concentration for nitrite oxidation varies with oxygen concentration.

DISCUSSION

The site of the inhibition by excess of nitrite may be either the enzyme (e.g. by formation of

abnormal enzyme-substrate complexes) or a component of the electron-transport system involved in nitrite oxidation. The fall in optimum nitrite concentration as the oxygen concentration is lowered (Fig. 1) may be explained if it is assumed that a transport system carries nitrite into the cell. The type of mechanism suggested is:

At moderate nitrite concentrations (up to about 20 mM) and normal oxygen concentrations (20% oxygen in the gas phase) the rate at which nitrite is transported into the cell and the rate at which it is oxidized via the carrier-cytochrome system are assumed to be roughly equal, so that there is no appreciable accumulation of nitrite within the cell. When the oxygen concentration is lowered, however, nitrite should accumulate at the enzyme surface and inhibit nitrite oxidation. At lower oxygen concentrations therefore the optimum oxidation rate should occur at a lower nitrite concentration in the medium.

Nitrate, arsenite and cyanate (being somewhat similar in chemical structure to nitrite) may interfere with the nitrite-transport system. By lowering the rate of transport of nitrite, these ions decrease the rate of nitrite oxidation at normal oxygen concentrations. At lower oxygen concentrations, however, they prevent accumulation of nitrite within the cell and thus stimulate nitrite oxidation.

We have, of course, no direct proof that these explanations are correct. They can be proved only when it becomes possible to separate the ion-transport mechanisms of *Nitrobacter* (if they exist) from the nitrite-oxidizing system. Since Aleem & Alexander (1958) claim to have obtained from *Nitrobacter* a cell-free system capable of oxidizing nitrite, proof or disproof of the validity of our explanations may be obtained soon.

SUMMARY

1. A study has been made of the influence of nitrite, and of certain other ions, on nitrite oxidation by *Nitrobacter* at normal and lowered oxygen concentrations.

2. Ions similar in structure to nitrite (nitrate, arsenite and cyanate) inhibited nitrite oxidation at normal oxygen concentrations and stimulated nitrite oxidation at decreased oxygen concentrations. Other ions (chloride and sulphate) had little effect at any oxygen concentration.

3. It was confirmed that nitrite inhibited its own oxidation. The nitrite concentration at which

a maximum rate of oxidation occurred decreased as the oxygen concentration was decreased.

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The Pyridine Nucleotide Metabolism of Mitochondria Incubated with and without Added Substrates and Metabolic Inhibitors

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The experiments described previously (Birt & Bartley, 1960*a, b*) have shown that the amounts and state of reduction of the endogenous pyridine nucleotides of rat-liver mitochondria are controlled by the components of the incubating medium. This paper is concerned with a general study of the effects of the temperature of incubation, the addition of various substrates, 2,4-dinitrophenol and amytal on the behaviour of the pyridine nucleotides in these particles; in particular, factors altering the dynamic steady-state levels of reduced nucleotide (by changing rates of oxidation or reduction of the coenzymes, or both) have been investigated.

MATERIALS AND METHODS

The chemicals used in the preparation of media for the isolation and incubation of rat-liver mitochondria and the details of the preparative procedure have been described previously (Birt & Bartley, 1960*a*). The mitochondria were isolated in a medium containing sucrose (0.25M) plus nicotinamide (25 mM) and incubated in a 'saline' medium containing KCl (0.1M), 2-amino-2-hydroxymethylpropane-1:3-diol (tris)-HCl buffer (pH 7.4, 50 mM), MgSO₄ (5 mM), sodium ethylenediaminetetra-acetate (EDTA, 1 mM), adenosine triphosphate (ATP, 1 mM) and nicotinamide (25 mM).

The methods of incubation and estimation of O₂ uptake and pyridine nucleotide analysis were as described by Birt & Bartley (1960*b*) and Bassham, Birt, Hems & Loening (1959).

RESULTS

Changes in pyridine nucleotides during incubation without added substrates at different temperatures

A number of mitochondrial suspensions without added substrate were incubated at temperatures in the range 0–30° (Table 1). During 1 hr. at both 0° and 10°, there was no destruction of coenzyme and no decline in reduced pyridine nucleotide; indeed, at both temperatures there was a tendency for the amount of reduced diphosphopyridine nucleotide (DPNH) to increase slowly, though the level of reduced triphosphopyridine nucleotide (TPNH) did not change significantly. At and above 15°, however, the total coenzyme level declined by approximately 20% in 1 hr.; di- and tri-phosphopyridine nucleotides (DPN⁺ and TPN⁺) disappeared at similar rates. There was also a net loss of reduced coenzyme during 1 hr. The DPNH level usually fell, in 15–30 min., to a value below which little further decline occurred, but the oxidation of TPNH commonly continued throughout the hour of incubation, especially at 20° (similar data were obtained with mitochondria incubated at 25° without substrate). The last two columns of Table 1 indicate the relative changes occurring in the proportions of the di- and tri-phosphopyridine nucleotides present in the reduced form; values greater than 1.0 were the result of a decrease below the initial proportion of reduced coenzyme; values less than 1.0 of an increase over the initial proportion of reduced coenzyme. At 15° and above, the proportional change after incubation for 5 min. was always greater in the triphosphopyridine

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