Metabolism of Nitrilotriacetate by Cells of
*Pseudomonas* Species

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Received for publication 4 January 1973

A *Pseudomonas* species was isolated from soil which could degrade nitrilotriacetate (NTA) to CO₂, H₂O, NH₃, and cellular constituents without the accumulation of significant quantities of intermediates either in the presence or absence of several inhibitors. After extensive gas chromatography analysis, small quantities of aspartate, glycine, and aconitate were the only detectable compounds to accumulate during NTA degradation, and these compounds were not excreted from the cells. Manometric studies indicated that iminodiacetate, glycine, and glyoxylate are possible intermediates, whereas N-methyliminodiacetate, sarcosine, and acetate are not. The data are consistent with an oxidative cleavage of the C—N bond of NTA as the initial degradation step.

Nitrilotriacetic acid (NTA), N(CH₃COOH)₃, is a relatively simple, symmetrical molecule commercially produced from inexpensive raw materials. It forms water-soluble complexes with calcium and magnesium that are stable at pH 10 (washing pH) but not at pH 7, is substantially nontoxic (16, 19), and is biodegraded in sewage facilities (1, 15, 17, 20, 22), river water (23, 24), sea water (8), and soil (J. M. Tiedje and B. B. Mason, Bacteriol. Proc., p. 1, 1971). This combination of inexpensive starting materials and properties has prompted the commercial development of the trisodium salt of NTA as a partial replacement for phosphate in detergent formulations. NTA is now being used as a detergent builder in formulations marketed in Canada and Sweden; it was used in the United States until December 1970 at which time, at the request of the Surgeon General, it was voluntarily removed from all detergents. However, NTA continues to be marketed in the United States for a variety of industrial and agricultural uses.

As an ingredient of detergents, NTA becomes a component of waste water and, without degradation, would eventually find ubiquitous entry into our ecosystem. It is therefore essential that NTA biodegradation occur in a variety of environments without occurrence of intermediates that may be of environmental or public health concern. Epstein (7) has postulated that secondary amines, which could occur in the degradation of NTA, together with nitrite could form nitrosamines, some of which are known to be carcinogenic.

Bacteria have been isolated from fresh water (10) and sewage effluent (9) which could utilize NTA as the sole source of carbon and, in both cases, they were aerobic, gram-negative rods, presumably species of *Pseudomonas*. NTA was shown to be totally degraded to CO₂ and inorganic nitrogen by a sewage-derived bacterial culture (23) and to CO₂ and NH₃ by a bacterial pure culture (9). Because little effort has been devoted to verifying whether or not any intermediates occur, the present study was undertaken to provide information on intermediates as well as the possible pathway of degradation.

In the present study, we report on the isolation, growth, and metabolism of NTA by *Pseudomonas* sp. isolated from soil. Gas chromatography techniques were used to search for intermediates, and sequential induction studies were used to provide information on the pathway of NTA degradation. We did not find accumulation of any intermediates of apparent public health significance during or after NTA degradation.

MATERIALS AND METHODS

Medium. The mineral salts medium used throughout these experiments contained the following ingredients per liter: K₂HPO₄, 1.6 g; KH₂PO₄, 0.4 g; NH₄NO₃, 0.5 g (omitted when NTA was also used as the nitrogen source); MgSO₄·7H₂O, 0.2 g; 811

1 Published as Journal Article no. 6214 of the Michigan Agricultural Experiment Station.
CaCl₂, 2H₂O, 25 mg; FeCl₃, 6H₂O, 2.5 mg; and 0.1% (wt/vol) of carbonaceous substrate. The weights of NTA and inominocate (IDA), although added as the tri- and disodium salts, respectively, were calculated as the free acids. The final pH of the medium was 7.3.

**Culture.** The NTA-degrading bacterium was obtained by successive enrichment in a medium with NTA as the sole carbon source from a sandy soil surrounding a dry well that had received septic tank effluent. The organism was isolated by streaking on the same medium solidified with 1.8% agar. Purity of the isolate was established by streaking on nutrient agar and by examination by phase microscopy. The organism was maintained by periodic transfer on sterile agar slants and in liquid medium which contained NTA as the carbon source.

Characterization of the isolate was carried out by using standard physiological tests (21) and morphological observations. Flagellation was determined by electron microscopy.

Growth experiments were carried out in 250-ml Nephelo flasks to which a 5% NTA-grown inoculum was added. Turbidity was observed at 600 nm with a Spectronic 20 (Bausch & Lomb, Inc.) spectrophotometer. The flasks were incubated at room temperature (26 to 28 °C) on a rotary shaker.

Large quantities of cells were grown at 28 °C in either a 14-liter Microferm fermentor (New Brunswick Scientific Co.) or at room temperature in a 10-liter bottle equipped with a glass dispersion tube for aeration. The cells were harvested in log phase (optical density (OD) 0.3 to 0.45) and by centrifugation at 4 °C. The pellets were washed by suspension in cold, 0.02 M potassium phosphate buffer, pH 7.3, collected by centrifugation, and resuspended in fresh buffer so that an eightfold dilution of the suspension gave an OD of approximately 0.56. This suspension served as the washed cell preparation for the manometry and batch experiments.

**Manometry.** All studies were done by using a refrigerated Warburg manometer (Aminco) held at 30 °C. The vessels contained 0.2 ml of 20% KOH in the center well, 3.0 ml of washed cell suspension in the main reservoir, and 0.5 ml (10 μmol) of freshly prepared substrate in the side arm. The oxygen uptake values in Fig. 1 were obtained immediately prior to disassembly of each manometer. All data have thermobarometer and endogenous values subtracted. Certain oxygen uptake data are reported as theoretical percentages, i.e., the percentage of the total oxygen necessary to convert the substrate to CO₂, H₂O, and NH₃ (4.5 mol of O₂ per mol of NTA).

**Batch incubations.** Experiments to determine inorganic nitrogen products and organic intermediates from NTA degradation were carried out in 250-ml flasks incubated in a reciprocating water bath shaker maintained at 30 °C. The flasks contained a ratio of 1 mmol of NTA to 0.7 mg (dry weight) of washed cells. Samples were removed at the indicated time intervals for gas chromatography and inorganic nitrogen analyses. Within 0 to 5 min after adding the substrate to the cell suspension, a 3.5-ml sample was transferred to a Warburg manometer vessel; the vessel was thermally equilibrated in the Warburg bath for 5 min before being closed. Oxygen uptake determined in this manner was presumed to be indicative of the oxygen uptake occurring in the batch and thus was used to follow the progress of NTA metabolism. The values were extrapolated to zero time in order to estimate total oxygen uptake.

**Analyses.** Ammonia was determined by microdiffusion in Conway dishes and by titration (3). NTA was determined by the zinc-Zincon procedure (23), except that the ion exchange step was omitted because of the absence of interfering cations.

The fate of the NTA carbon was followed in a Warburg manometer experiment by using 14C-carboxyl-NTA (0.1 μCi/vessel) in which a manometer was disassembled every 15 min. The vessel was sealed with a serum stopper, the incubation was terminated, and 14CO₂ was driven off by the addition of 0.5 ml of 2% trichloroacetic acid. After 30 min the vessel was opened, and the contents of the center well were quantitatively transferred to a scintillation vial. Assimilated 14C was determined by assaying radioactivity associated with a 0.22-μm membrane filter (Millicore) which had retained cells from 0.5 ml of the suspension. The remaining cell suspension was centrifuged to remove cells prior to NTA analysis and to counting for soluble 14C compounds.

All radioactivity was assayed by liquid scintillation counting (Packard Tri-carb, model 307O) by using Bray's cocktail (2) plus 4% Cab-O-Sil. Counts were corrected for quenching by external standardization.

**Gas chromatography analysis.** Samples removed from batch experiments (or after the treatments described below) were immediately frozen in a dry ice-isopropanol bath and subsequently lyophilized to dryness. In experiment A (Table 1) the samples were divided into two portions with one portion sonically treated for 2 min (Biosonik III) to disrupt the cells. In experiment B (Table 1) the cells were divided into two portions; the cells were removed from one portion by filtration through a 0.45-μm Millipore membrane filter. In all other cases, the cells remained in the sample during the derivatization procedure.

The lyophilized samples (originally 1.0 ml) were stored in 2-dr vials or in 16- by 75-mm screw-capped test tubes fitted with Teflon-lined caps. A Teflon cap liner was inserted in the 2-dr vial cap prior to derivatization. The sample was treated with 1.0 ml of trifluoroacetic acid and rapidly stirred at room temperature (Vortex Genie) until it was either clear or translucent. The trifluoroacetic acid was stripped off with a stream of N₂, 100 μg of tetracosane was added as an internal standard, and the components of the sample were converted to trifluoracetyl, n-butyl ester derivatives by the procedure of Warren and Malec (25).

Derivatized samples were stored in 0.5 ml of Freon 113 (1,1,2-trichlorotrifluoroethane). Portions of a sample (1 to 3 uliters) were chromatographed on a Vario-Aerograph 2100 dual-column gas chromatograph equipped with flame ionization detectors and operating in the dual-differential mode. The signal from the gas chromatograph was fed to an Infotronics CRS-104 digital integrator and then to both channels
of a Varian Aerograph, model 30, dual-pen recorder. For the gas chromatogram presented in this paper, the range of the low-sensitivity pen was 0 to 20 mV and that of the high-sensitivity pen was 0 to 1 mV.

Glass U-shaped columns (1.9 m long with 2 mm inside diameters; Applied Science, State College, Pa.) packed with ethylene glycol adipate (Analabs Inc., stabilized grade; 0.65%, wt/wt) on acid-washed Chromosorb W (80-100 mesh) were used. Column preparation details and operating parameters have been presented previously (25).

The identity of NTA, tri-n-butyl ester; N-trifluoroacetyliminodiacetic acid, di-n-butyl ester; N-trifluoroacetetyl-glycine, n-butyl ester; and N-trifluoroacetyl-aspartic, n-butyl ester have been confirmed by gas chromatography mass spectrometric analysis (Varian Aerograph 1700 gas chromatograph and Varian CH-7 mass spectrometer). The identity of aconitic and isocitric acids was confirmed by peak enhancement experiments. The relative standard deviations of the values presented in Tables 1 and 3 are ±15%. The procedure is capable of detecting 10 to 20 × 10⁻⁸ g of NTA, IDA, glycine, sarcosine, and N-methyliminodiacetic acid.

Chemicals. N-Oxalyliminodiacetic acid (N-oxalyl-IDA) and N-nitrosoiminodiacetic acid (N-nitroso-IDA) were synthesized by previously described procedures (25). High-purity NTA and NTA-amine oxide were supplied by Monsanto Industrial Chemicals Co. ¹⁴C-Monocarboxyl-labeled NTA (3.35 mCi/mmol) was a gift from Procter and Gamble Co. Sodium glyoxylate and nitrolotrpropionic acid were purchased from Sigma Chemical Co.

RESULTS

Characterization and growth of isolate. The NTA-degrading isolate was a small, gram-negative, aerobic, rod which did not hydrolyze starch, produce indole, or grow on lactose. No acid or gas was produced during growth on glucose. The bacterium was motile during early log phase by means of a single, polar flagellum. On the basis of these morphological and physio-

Table 1. Products produced during the metabolism of NTA by washed cells of Pseudomonas sp.

| Expt                  | Time (min) | Chemicals found (µg) |
|-----------------------|------------|----------------------|
|                       |            | NTA*     | IDA    | Aspartate | Glycine | Aconitate | Isocitrate |
| (A) Buffer and sonicated cells | 0         | 210      | 3.4    | 0.1       | 0       | 9.0       | 3.3        |
|                        | 10        | 161      | 3.4    | 2.6       | 0       | 6.6       | 1.6        |
|                        | 20        | 98       | 3.4    | 3.6       | 0       | 8.2       | 1.0        |
|                        | 30        | 30       | 3.4    | 3.1       | 0       | 7.9       | 0.4        |
|                        | 50        | 0        | 2.7    | 0.3       | 0       | 12.5      | 1.0        |
|                        | 75        | 0        | 1.9    | 0.5       | 0       | 11.0      | 1.0        |
|                        | 120       | 0        | 1.2    | 0         | 0       | 11.0      | 1.0        |
| (B) Buffer, cells removed | 0         | 176      | 8.8    | 0         | 0       |          |            |
|                        | 10        | 146      | 6.7    | 0         | 0       |          |            |
|                        | 30        | 1        | 1.6    | 0         | 0       |          |            |
|                        | 100       | 1        | 0      | 0         | 0       |          |            |
| Buffer and whole cells | 0         | 192      | 7.0    | 0         | 0.5     |          |            |
|                        | 10        | 130      | 4.6    | 2.3       | 1.7     |          |            |
|                        | 20        | 66       | 4.0    | 3.7       | 1.2     |          |            |
|                        | 30        | 0        | 0.8    | 1.4       | 0.4     |          |            |
|                        | 40        | 0        | 1.1    | 0         | 0       |          |            |

* NTA added prior to incubation (192 µg).
logical characteristics, the organism was identified as *Pseudomonas* sp.

The isolate grew equally well (maximum OD of approximately 0.5) when NTA was the sole carbon or sole carbon and nitrogen source. Resting cells of the isolate grown on NTA degraded all of the added NTA and converted 70% of the carboxyl label to $^{14}$CO$_2$ within 1 h as shown in Fig. 1. Twenty minutes later, the initial phase of oxygen uptake was completed with 53% of the theoretically possible quantity of oxygen having been consumed. The $^{14}$C assimilated was maximum in the 60 to 120 min period, but only accounted for 7% of the label. Essentially no $^{14}$C-labeled soluble materials other than NTA were found in solution. An additional 5% of the original label was retained in the Warburg vessel side arm and did not reach the cells.

The only inorganic nitrogen product detected during NTA degradation was ammonia (Table 2), which accounted for all of the added nitrogen. In controls, in which cells were incubated without substrate and with acetate, 2.63 and 0.00 μmol, respectively, of ammonia were produced after 90 min. It is assumed that, during NTA metabolism, the available carbon skeleton will result in some assimilation of nitrogen, as occurs during acetate metabolism, thus limiting the quantity of endogenous ammonia produced. Ammonia production occurred at a constant rate, which extended 30 min beyond the inflection point for oxygen uptake before leveling off.

**Search for NTA intermediates.** The gas chromatography analytical procedure was effective in separating the naturally occurring amino and organic acids as well as the imino and nitroso acids. Figure 2 shows representative chromatograms for the 0-, 10-, and 20-min samples described in Table 1, experiment B. Note the enhancement of peaks corresponding in retention time to aspartic acid and glycine as well as the disappearance of the NTA and IDA peaks. The analysis was sensitive enough to detect an intermediate present (if present) in quantities equivalent to 0.1% by weight of the originally added NTA. However, in experiments where cells were extracted, the background produced by the extracted cellular components (Fig. 2) was such that the possibility that small quantities of NTA intermediates might have been masked could not be ruled out.

Six experiments were conducted in which intermediary products in NTA metabolism by washed cells were searched for by the chromatography procedure. Representative data from three of these experiments are summarized in Tables 1 and 3. All experiments confirmed that NTA was totally degraded within 1 h. IDA was found in all experiments. The majority of IDA,

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**Table 2. Inorganic nitrogen products formed during NTA metabolism by washed cells**

| Time (min) | Nitrogen product formed (μmol)* | Ammonia | Nitrite* | Nitrate* |
|------------|--------------------------------|---------|---------|---------|
| 0          | 1.65                           | 0       | 0       | 0.03    |
| 30         | 3.56                           | 0       | 0       | 0       |
| 60         | 5.43                           | 0       | 0       | 0       |
| 90         | 7.89                           | 0       | 0       | 0       |
| 120        | 8.65                           | 0       | 0       | 0       |

* Maximum possible from NTA (8.60 μmol).
* Detection limit (0.005 μmol).
* Detection limit (0.02 μmol).
TABLE 3. Effect of 4 × 10⁻⁴ M arsenate on metabolism of NTA by washed cells

| Time (min) | O₂ uptake (% of theoretical) | Chemicals found (µg) | NTA* | IDA | Glycine |
|-----------|-------------------------------|----------------------|------|-----|---------|
| 0         | 0                             | 162                  | 5.5  | 0.0 |         |
| 30        | 14 (20)*                      | 118                  | 10.2 | 0.6 |         |
| 60        | 21 (42)                       | 46                   | 3.1  | 0.5 |         |
| 90        | 28 (53)                       | 34                   | 1.9  | 0.4 |         |
| 150       | 42                            | 6                    | 2.1  | 0.3 |         |

* NTA added prior to incubation (192 µg).
* O₂ uptake values for cells from the same preparation but not inhibited with arsenite.

However, appeared to be chemically formed from NTA during the derivatization procedure, because a sample of highly purified NTA subjected to derivatization yielded 1 to 5% IDA. The presence of this chemically formed IDA made it impossible to determine whether small quantities of IDA accumulated during the biodegradation of NTA. The only indication of IDA as an intermediate was that in two experiments (Table 1), peaks with the same retention time as IDA persisted after NTA had disappeared. However, the identity and biological origin of these small peaks could not be confirmed.

The accumulation of aspartic acid and, to a lesser extent, glycine (Table 1) suggests that they are either intermediates or shunt metabolites from NTA dissimilation. It is also apparent that these amino acids are not excreted from the cell (Table 1). The presence of IDA in the buffer surrounding the cell supports the above argument that IDA is of chemical rather than biological origin, because other amino acids did not leak from the cells.

**Effect of inhibitors.** Malonate, arsenite, and 2,4-dinitrophenol (DNP) were used as inhibitors in an attempt to accumulate intermediates of NTA degradation. Malonate at the level of 3 × 10⁻³ M was not inhibitory to oxygen uptake. Arsenite at levels of 1 × 10⁻⁴ to 4 × 10⁻⁴ M caused a 10 to 50% inhibition in the rate of oxygen uptake. Table 3 shows data from one of four experiments in which arsenite was used as an inhibitor. No intermediates were detected by gas chromatography in three of the four experiments. In one experiment, small quantities of glycine were detected during NTA degradation. Substitution of 0.02 M tris(hydroxymethyl)aminomethane buffer, pH 7.3, for phosphate buffer in an arsenite-inhibition experiment also did not cause accumulation of any intermediates nor did it significantly alter the rates of oxygen uptake.

When DNP was used as an inhibitor there was little decrease in rate of oxygen uptake (Fig. 3); however, the total quantity of oxygen consumed was 13% greater than without DNP. No NTA intermediates were found by gas chromatography analysis in any of the DNP-inhibited samples.

**Manometry.** The pattern of oxygen uptake by washed cells grown on NTA or IDA and incubated with several of the postulated NTA intermediates is illustrated in Fig. 4. The two graphs show similar patterns, except for IDA which was degraded more rapidly by IDA- than by NTA-grown cells. Glycine was also metabolized without a lag. Acetate was metabolized after a 20-min lag and sarcosine after a lag of approximately 1 h.

The ability of the *Pseudomonas* isolate to grow on and oxidize a variety of substrates is summarized in Table 4. Acetate and NTA supported the most rapid growth. The glyoxylate and glycine mixture supported more rapid growth than when each was supplied independently. NTA-grown cells were able to oxidize all substrates on which they grew, although acetate, glycolate, and sarcosine were oxidized only after a lag. IDA-grown cells could metabolize NTA and acetate as well as, glycine slightly better than, and IDA much better than NTA-grown cells. Acetate-grown cells could not metabolize NTA and only slowly metabolized IDA; they metabolized glycine after a lag of 1 h.

**DISCUSSION**

Our results provide evidence that NTA degra-
The classical pattern of oxygen uptake suggested that NTA was rapidly oxidized during the initial phase of oxygen uptake and that accumulated intermediates were more slowly oxidized during the second phase. The inflection point in the oxidation curve occurred between 50 to 55% of theoretical oxygen uptake.

**Table 4. Growth on and oxidation of several potential NTA intermediates by the Pseudomonas isolate**

| Substrate        | Generation time (h) | Oxidation, QO₂ (μliter per mg x hr) |
|------------------|---------------------|--------------------------------------|
|                  | NTA                 | IDA                                  | Acetate  |
| NTA              | 5.9                 | 87                                   | 88       | 0        |
| IDA              | 8.5                 | 20                                   | 82       | 9        |
| Glycine          | 9.9                 | 27                                   | 37       | 28*      |
| Acetate          | 4.4                 | 46*                                  | 45       | 87       |
| Glyoxylate       | 13.5                | 6.4                                  |          |          |
| Glyoxylate-glycine (2:1) | 32.1 | 12                                   |          |          |
| Glycerate        |                     | 19                                   |          |          |
| Serine           |                     | 21*                                  |          |          |
| Glycolate        |                     | 8.9                                  | 27*      |          |
| Sarcosine        |                     |                                      |          |          |
| Methylamine      |                     | 8.2                                  |          |          |
| N-Methyl-IDA     |                     | No growth                            | 0        |          |
| N-Oxalyl-IDA     |                     | No growth                            | 0        |          |
| NTP*             |                     | No growth                            | 0        |          |
| N-Nitroso-IDA    |                     | No growth                            | 0        |          |
| NTA-amine oxide  |                     |                                      | 0        |          |

*Oxygen uptake occurred after a distinct lag period.
* NTP, Nitritotripropionic acid.
in all experiments. This relatively low value, together with the total disappearance of NTA prior to this point, suggested that intermediates could have accumulated. Furthermore, the initial rate of ammonia production continued well beyond the inflection point in oxygen uptake, which indicated the presence of nitrogen-containing intermediates. The high values of $^{14}$CO$_2$ produced and the low amount of $^{14}$C assimilated do not necessarily disprove this hypothesis, because 50% of the carboxyl label could have been lost in the initial stages of degradation via pathways involving condensation of two C$_3$ compounds to form a C$_4$ compound and CO$_2$. However, an extensive search for metabolites by gas-liquid chromatography yielded no intermediates which occurred in any significant amount. The probable explanation is that the unoxidized NTA intermediates were assimilated into cellular polymers and thus were not recoverable as intermediates. This hypothesis is supported by the finding that total oxygen uptake was greater in the presence of DNP, which is known to reduce assimilation (4).

After extensive studies our inability to find accumulated intermediates, particularly secondary amines, in the presence and absence of several inhibitors suggests that it is unlikely that significant quantities of intermediates would accumulate during or after NTA degradation in nature. And, even if some intermediates accumulated, present evidence suggests that they would not be likely to be excreted from the cell. The absence of accumulation of both secondary amines and nitrite appears to limit the potential of this isolate to contribute to the formation of nitrosamines.

Although the rate of IDA oxidation was much slower than the rate of NTA oxidation, IDA did not accumulate in quantities expected when IDA preceded a rate-limiting degradation step. Quantities of this magnitude could easily have been detected; however, it was impossible to determine whether or not trace quantities of IDA were present because of the small but irregular quantities of IDA formed from NTA during the derivatization. This slower rate of IDA oxidation, which is contrary to a previous report (9), appears to be due to a limited rate of IDA uptake. This explanation is supported by the above finding that IDA did not accumulate in NTA-metabolizing cells and that IDA-grown cells (which presumably would have the appropriate transporting permeases) could degrade IDA more rapidly than they could degrade NTA (which was as rapidly as NTA-grown cells could degrade NTA). On the other hand acetate-grown cells (which like NTA-grown cells may have lacked IDA permeases) oxidized IDA only very slowly. Thus, the difference in rate of IDA oxidation in acetate- and NTA-grown cells is consistent with the explanation that the former lacked IDA-degrading enzymes, whereas the latter contained the enzymes which were induced by growth on NTA. The finding that IDA oxidation is induced by growth on NTA supports the suggestion that IDA is an NTA degradation intermediate. Recent data from studies with cell-free extracts provide direct evidence that IDA is an intermediate (J. M. Tiedje, M. K. Firestone, and B. B. Mason, unpublished data).

There appear to be three possibilities for the initial step in NTA degradation: decarboxylation, nonoxidative cleavage, and oxidative cleavage of the C—N bond. The absence of growth on and oxidation of N-methyl-IDA and the presence of a lag period prior to sarcosine oxidation appear to eliminate decarboxylation as the initial degradation step. Previous findings also suggested that N-methyl-IDA was not an intermediate in pure culture (9) or in river water (24). The nonoxidative cleavage does not appear to be the primary degradation step, because a definite lag phase was present prior to acetate and glycolate oxidation.

The oxidative cleavage hypothesis is supported by the fact that glyoxylate, as well as IDA, was oxidized without a lag. A similar type of oxidative cleavage of C—N bonds has been shown in which secondary and tertiary amines are cleaved to form amine and aldehyde products (5, 6, 13). In this case, the analogous products would be IDA and glyoxylate. Glyoxylate can be subsequently metabolized via the glycerate pathway (11), the β-hydroxyaspartate pathway (12) or via amination to glycine followed by condensation to serine (18). In this study, the oxidation of glycerate and serine without a lag is consistent with glyoxylate being an intermediate. IDA could be expected to be degraded by a similar oxidative cleavage forming glycine and glyoxylate. Both of these compounds were oxidized without a lag by IDA-grown cells, as they were by NTA-grown cells. In addition, in three experiments glycine was found to accumulate during NTA degradation. The absence of oxidation of N-oxalyl-IDA is not inconsistent with the oxidative cleavage hypothesis, for a less oxidized intermediate, for example an α-hydroxy derivative, would be a more likely precursor of an aldehyde product. NTA-amine oxide does not appear to be the intermediate because it was not oxidized by NTA-grown cells.

The NTA- and IDA-degrading enzymes appear to have a relatively high degree of substrate specificity, since N-methyl-IDA, N-
oxalyl-IDA, nitrotripropionic acid, sarcosine, NTA-amine oxide, and N-nitroso-IDA were not attacked.

Our finding that NTA is readily oxidized to inorganic products and that no intermediates accumulated under a variety of conditions indicates that environmental concern for hazardous intermediates in NTA degradation seems unwarranted.

ACKNOWLEDGMENTS

The technical assistance of Mary K. Firestone is gratefully acknowledged. The research was supported in part by a grant from the Procter and Gamble Co.

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