The Oxidation of Aminoacetone by a Species of *Arthrobacter*

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1. A micro-organism similar to *Arthrobacter globiformis* has been isolated from sewage by elective growth on a medium containing L-threonine as sole source of carbon and nitrogen. 2. Washed cell suspensions of the organism catalyse the complete disappearance of aminoacetone from the medium and its almost complete oxidation. 3. In the presence of iodoacetate, aminoacetone disappearance is accompanied by the accumulation of methylglyoxal, about 70% of the aminoacetone removed being accounted for in this way. 4. It is suggested that the conversion of aminoacetone into methylglyoxal is catalysed by an amine oxidase.

Aminoacetone was first isolated from a biological source by Elliott (1959, 1960c), who obtained it from the medium in which *Staphylococcus aureus* cells had been incubated aerobically with threonine. It was subsequently shown to be formed from threonine (Neuberger & Tait, 1960, 1962; Green & Elliott, 1964; Turner, 1966a), from glycine plus a source of acetyl-CoA (Elliott, 1958, 1960c; Neuberger & Tait, 1962; Turner, 1966a), from 1-aminopropan-2-ol (Turner, 1966a,b) and from 4-amino-3-hydroxybutyrate (Turner, 1966a) by a variety of micro-organisms. The formation of aminoacetone from threonine (Urata & Granick, 1961, 1963; Green & Elliott, 1964) and from glycine plus acetyl-CoA (Urata & Granick, 1961, 1963) by preparations of certain animal tissues was also demonstrated.

On the basis of his early work, Elliott (1959, 1960c) suggested that the above reactions could be integrated with already known ones into a metabolic cycle, which might provide a route for the conversion of threonine and glycine into acetyl-CoA, with the intermediary formation of aminoacetone, methylglyoxal, D-lactate and pyruvate. Of the postulated reactions of this cycle, all except the conversion of aminoacetone into methylglyoxal are well documented. This reaction has previously only been shown to be catalysed by a fraction of ox plasma (Elliott, 1960a). However, it has been demonstrated that the further metabolism of aminoacetone is catalysed by plasmas of sheep, goat and horse (H. Blaschko, personal communication), guinea-pig liver mitochondria and homogenates of various guinea-pig tissues (Urata & Granick, 1961, 1963), preparations of *Streptococcus faecalis* and *Corynebacterium erythrogenes* (Green &

Elliott, 1964) and cell suspensions and fragments of *Pseudomonas* spp. (Higgins & Turner, 1966); but in none of these cases have any products been identified.

The present paper is concerned with the metabolism of aminoacetone by a species of *Arthrobacter*. Cell suspensions of this organism have been shown to catalyse the almost complete oxidation of aminoacetone. By inhibiting this process after the postulated first step, it has been demonstrated that methylglyoxal is an intermediate in the pathway of metabolism of at least the bulk of the added aminoacetone.

MATERIALS

The toluene-*p*-sulphonic acid salt of aminoacetone was prepared as described by Elliott (1960b). Methylglyoxal was obtained as a 30% (w/v) solution in water from K & K Laboratories Inc. (Plainview, N.Y., U.S.A.). Methylglyoxal 2,4-dinitrophenylhydrazone was prepared as described by Elliott (1960a). Iodoacetic acid, a product of British Drug Houses Ltd. (Poole, Dorset), was recrystallized from light petroleum, and solutions, prepared immediately before use, were neutralized with NaOH.

Basal salts medium contained KH_2PO_4 (0.27g.), $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ (1.08g.), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (0.26g.), $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (3mg.), $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ (2mg.) and $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ (2mg.) in 1l. of water, adjusted to pH 7.0 with 2N-HCl.

The bacterial species used in these investigations was isolated from sewage and identified as a species closely related to *Arthrobacter globiformis*, as described below.

METHODS

Growth of bacteria and preparation of cell suspensions. The organism was maintained in slope culture on the growth medium described below containing 1% of agar.

The growth medium consisted of 0.1% L-threonine in basal salts medium. Cells were grown in 4l. batches at 30°

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with forced aeration. When almost fully grown they were harvested on a Sharples centrifuge, washed once by resuspension in basal salts medium and centrifugation, and then resuspended in basal salts medium to the required cell density. This cell suspension was stored at 0° and used within 4 days.

Weight of cellular material. The actual dry weight of the cell suspension could not be determined because the cells sedimented incompletely from a water suspension. Therefore a measure of the amount of cellular material present, referred to as the 'dry weight', was obtained by the following procedure. Trichloroacetic acid was added to a sample of cell suspension to a final concentration of 5% (w/v) in a tared tube. After 15 min. this was centrifuged and the supernatant discarded. The sediment was washed twice by resuspension in acetone and centrifugation, and then dried at 80° overnight.

Standard enzymic assay. The reaction mixture contained, in a total volume of 3.0 ml., 1.5 ml. of basal salts medium, 2.0 μ moles of aminoacetone toluene-*p*-sulphonate and cell suspension (4.5–6.0 mg. dry wt.). Incubations were carried out in 6 cm. x 1 cm. tubes shaken aerobically at 25° for 30 min. The reaction was stopped by the addition of 1.0 ml. of 40% (w/v) trichloroacetic acid. For inhibited incubations iodoacetate was present at a concentration of 0.1–0.2 M, the actual concentration being determined with each batch of cell suspension as that required to inhibit completely the disappearance of 2.0 μ moles of methylglyoxal from the medium under standard assay conditions. In this case the incubation mixture, with aminoacetone omitted, was preincubated for 20 min. at 25° before the reaction was started by the addition of aminoacetone.

Manometry. Uptake of O₂ and evolution of CO₂ were determined manometrically in a Warburg apparatus at 25° with double-side-armed flasks (Umbreit, Burris & Stauffer, 1964). The main compartment of each flask contained 1.5 ml. of basal salts medium and cell suspension (approx. 6.0 mg. dry wt.) in a total volume of 2.7 ml. When O₂ uptake only was to be determined, 0.2 ml. of 20% (w/v) KOH was added to the centre well. The reaction was started by the addition of 2.0 μ moles (0.3 ml.) of aminoacetone toluene-*p*-sulphonate from the first side arm and was followed to completion. Then, when O₂ uptake and CO₂ evolution were both to be determined, CO₂ was released from solution by the addition of 0.2 ml. of 40% (w/v) trichloroacetic acid from the second side arm, and the reaction was followed to completion. The contents of the flask were then removed and analysed to verify that the aminoacetone had completely disappeared. Uptake of O₂ was calculated from the results obtained in the presence of KOH in the centre well. The results obtained in the absence of KOH in the centre well were corrected for this O₂ uptake, and the CO₂ evolution was calculated (Umbreit *et al.* 1964). Both O₂ uptake and CO₂ evolution were corrected for endogenous values obtained in the absence of added substrate.

Chemical determinations. Before determination of substrates or products in an acidified enzymic reaction mixture, the precipitated material was removed by centrifugation. Aminoacetone was determined by the method of Mauzerall & Granick (1956), the sample being previously brought to pH 2–4 with NaOH. Methylglyoxal was determined by a method based on that of Tonhazy, White & Umbreit (1950) for pyruvate. The sample was brought to

1.0 ml. with water and 1.0 ml. of 0.1% 2,4-dinitrophenylhydrazine in 2N-HCl was added. This was kept for 10 min. at room temperature, and then 5.0 ml. of 2.5N-NaOH was added. The extinction at 540 m μ was determined within 1 hr.

Thin-layer chromatography. This was carried out as described by Bachelard (1965), with MN-cellulose 300G plates and butan-1-ol-ethanol-0.5N-NH₃ (7:1:2, by vol.) as ascending solvent. After drying, the plates were sprayed with 2% (w/v) KOH in 95% ethanol.

Infrared spectra. These were determined on mulls in Nujol.

RESULTS

Isolation and identification of the bacterial species. The organism was isolated from sewage by elective growth on the medium described, which contained L-threonine as sole source of carbon and nitrogen, followed by plating out on agar medium of the same composition.

The organism isolated showed the following characteristics in common with *Arthrobacter globiformis* (Breed, Murray & Smith, 1957): it was Gram-negative; it occurred in young cultures as rods about 3 μ x 0.6 μ , often curved or bent, but in older cultures as very short rods or cocci (approx. 0.6 μ diam.); it was non-motile; it formed cream waxy colonies (approx. 1 mm. diam.) on plain agar and asparagine-agar plates; it turned nutrient broth turbid, with the production of slight sediment but no surface growth; it grew abundantly on potato as a soft pale-brown layer; it turned milk alkaline; it produced a little acid but no gas from glucose, and neither acid nor gas from lactose; it hydrolysed starch; it grew on NH₄⁺ and, to a slight extent, NO₃⁻ as nitrogen source, and citrate as carbon source; it was catalase-positive. Its characteristics differed from those listed for *A. globiformis* in the following ways: gelatin was not liquefied, although growth on gelatin plates and stabs was observed; acid production from sucrose or mannitol was not observed; the formation of NO₂⁻ from NO₃⁻ was not detected. In addition, the organism was oxidase-positive, a characteristic not referred to in the published list.

Oxidation of aminoacetone. In preliminary work it was found that the organism, grown aerobically on L-threonine as sole carbon and nitrogen source, catalysed the very rapid disappearance of aminoacetone. The rate of aminoacetone disappearance decreased if the cells were not aerated during growth or if threonine was replaced by other carbon and nitrogen sources.

Under the conditions of the standard assay aminoacetone disappearance was totally dependent on the presence of unboiled cells. The disappearance of aminoacetone from the reaction mixture reached completion after about 50 min. incubation, the amount disappearing/unit time increasing at

the end of the reaction to about double that at the start. The rate of reaction over the whole range from zero to complete disappearance of aminoacetone was 8.4 μmoles of aminoacetone disappeared/min./mg. dry wt. The rate over the first 30 min. of the reaction (standard assay conditions) was 6.8 μmoles of aminoacetone disappeared/min./mg. dry wt. The amount of aminoacetone disappearing/mg. dry wt./30 min. increased slightly with the amount of cell suspension added over the range 1.5–7.5 mg. dry wt./3 ml. Omission of shaking during the incubation decreased the amount of aminoacetone disappearing by 80%, and the addition of 20 μmoles of 2-oxoglutarate decreased it by 90%. The gas exchanges during the oxidation of aminoacetone, determined by Warburg manometry and averaged over two separate experiments, were 2.62 μmoles of oxygen absorbed and 2.97 μmoles of carbon dioxide evolved/ μmole of aminoacetone disappeared.

Formation of methylglyoxal from aminoacetone

Accumulation of a carbonyl compound in the presence of iodoacetate. Iodoacetate inhibited the complete oxidation of aminoacetone, causing the accumulation of a compound that reacted with 2,4-dinitrophenylhydrazine to form a product giving a blue-violet colour in alkali. This compound was subsequently identified as methylglyoxal. The amount accumulated during the reaction period increased with iodoacetate concentration to reach a maximum at about 0.2 M-iodoacetate. Over the same range the amount of added methylglyoxal disappearing fell from a high value to zero and that of aminoacetone fell by about 10% (Fig. 1).

Identification of the reaction product as methylglyoxal. Three lines of evidence support the view that the carbonyl compound formed from aminoacetone is methylglyoxal. First, the absorption spectra in alkali of the 2,4-dinitrophenylhydrazones of the reaction product and of authentic methylglyoxal were identical. Secondly, thin-layer chromatography of each dinitrophenylhydrazone, and a mixture of the two, gave rise to one main spot, R_F 0.87, with an additional spot remaining at the origin. All the spots turned blue-violet on being sprayed with ethanolic potassium hydroxide. Thirdly, the 2,4-dinitrophenylhydrazone of the reaction product was purified by recrystallization from anisole. This derivative, authentic methylglyoxal 2,4-dinitrophenylhydrazone and a mixture of the two all had the same melting point (299–301°, decomp., uncorr.). The derivatives of the reaction product and authentic methylglyoxal gave i.r. spectra identical in all respects.

Time-course and stoichiometry. Aminoacetone

Fig. 1. Effect of iodoacetate concentration on the rate of aminoacetone disappearance, methylglyoxal accumulation and methylglyoxal disappearance. The conditions were those of the standard assay (see the Methods section) in the presence of iodoacetate, the iodoacetate concentration being varied as indicated. ○, Aminoacetone disappeared; Δ, methylglyoxal accumulated; □, methylglyoxal disappeared.

Fig. 2. Time-course of aminoacetone disappearance and methylglyoxal accumulation in the presence of iodoacetate. The conditions were those of the standard assay (see the Methods section) in the presence of iodoacetate, except that the incubation time was varied as indicated; the iodoacetate concentration was 0.2 M. ○, Aminoacetone; Δ, methylglyoxal.

disappeared from the reaction mixture at a constant rate until almost all had been metabolized. At the same time methylglyoxal accumulated (Fig. 2). However, the two rates were not equal, only 70% of the aminoacetone metabolized being accounted for as methylglyoxal, although sufficient iodoacetate to inhibit completely the metabolism of added methylglyoxal was present in the reaction mixture.

Oxygen uptake and carbon dioxide production. The amounts of oxygen absorbed and carbon dioxide evolved during the conversion of aminoacetone into methylglyoxal were determined by Warburg manometry. The values obtained, the average of two separate experiments, were 0.52 μ -mole of oxygen absorbed and 0.00 μ mole of carbon dioxide evolved/ μ mole of aminoacetone disappeared.

DISCUSSION

It has been found that a bacterial strain closely related to *A. globiformis*, grown on L-threonine as sole carbon and nitrogen source, catalyses the oxidation of aminoacetone at a rapid rate. From the amounts of oxygen absorbed and carbon dioxide produced during the reaction, it would appear that oxidation is 80–90% complete. The theoretical equation for complete oxidation is:

Iodoacetate inhibits the complete oxidation of aminoacetone, but aminoacetone disappearance continues at only a slightly decreased rate and most of it could be accounted for as methylglyoxal, which accumulated. It seems probable that iodoacetate, which effectively inhibits the oxidation of added methylglyoxal, acts primarily by inhibiting the glyoxalase reaction, which requires glutathione as cofactor (Lohmann, 1932). If this is the case, however, the question arises as to why the amount of methylglyoxal accumulating was less than the amount of aminoacetone disappearing although sufficient iodoacetate was present to block completely the disappearance of added methylglyoxal. Possible explanations would seem to be either that iodoacetate blocks methylglyoxal uptake by the cells slightly more effectively than methylglyoxal oxidation, or that only part of the aminoacetone metabolism proceeds by a pathway involving methylglyoxal. However, the results presented do indicate that at least 70% of added aminoacetone is metabolized by a pathway involving methylglyoxal.

From determinations of oxygen uptake and carbon dioxide formation during the conversion of aminoacetone into methylglyoxal and from the fact

that 2-oxoglutarate inhibits aminoacetone oxidation, it seems likely that this reaction is catalysed by an amine oxidase:

The hydrogen peroxide formed would then be removed by catalase, which has been shown to be present in cells of the organism used.

The above results are similar to those of Elliott (1960a), which indicated that the conversion of aminoacetone into methylglyoxal is catalysed by an amine oxidase in ox plasma. However, other workers have obtained indications that this conversion can also proceed by a transamination mechanism; Urata & Granick (1963) found that 2-oxoglutarate stimulated aminoacetone disappearance in guinea-pig liver mitochondria, and J. M. Turner (personal communication) obtained similar results with extracts of *Bacillus subtilis* and *Pseudomonas* spp.

The work described provides support for the postulated aminoacetone cycle (Elliott, 1959, 1960c). All the reactions of this cycle have now been shown to occur in both micro-organisms and animal tissues (for references see Green & Elliott, 1964), although evidence for the actual operation of the pathway is lacking.

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