Vol. 119, No. 2, 1984 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
March 15, 1984 Pages 556-560

FORMATION OF PYRROLIDINE BY ANAEROBIC POLYAMINE DEGRADATION

H. Kneifel and S.M. Schoberth

Institute for Biotechnology, KFA Juelich,
D-5170 Juelich , West Germany

Received December 24, 1983

SUMMARY. High-resolution mass spectrometry was used to identify p y r r o l i d i n e as a product of anaerobic polyamine degradation. Due to the widespread occurrence of polyamines in the living world this process could contribute considerably to the presence of pyrrolidine in the environment.

Polyamines occur widely in nature. Their involvement in processes related to growth and cell development has stimulated research on polyamine biochemistry (1).

Previous experiments have shown that the polyamines putrescine ($H_2N-(CH_2)_4-NH_2$), spermidine ($H_2N-(CH_2)_3-NH-(CH_2)_4-NH_2$) and spermine ($H_2N-(CH_2)_3-NH-(CH_2)_4-NH-(CH_2)_3-NH_2$) can be degraded anaerobically to methane (2). Disappearance of these polyamines was accompanied by the occurrence of a new amino compound which remained stable until the end of the experiments.

We now present results obtained with high-performance liquid chromatography (HPLC) and mass spectrometry which have led to the identification of pyrrolidine as a product of anaerobic polyamine degradation.

MATERIALS AND METHODS

Chemicals - All chemicals were the purest grade available and purchased from Merck, Darmstadt, with the exception of dansyl chloride and pyrrolidine which were obtained from Fluka,Neu-Ulm.

Polyamine degradation - Strict anaerobic conditions according to the Hungate-technique (3) were used. Digesting sludge from a local sewage plant was inoculated into the following medium which was modified from Schoberth (4):
distilled water, 1 l; resazurine, 1 mg; 1 M potassium phosphate buffer pH 7.0, 4 ml; $FeSO_4x7H_2O$, 20 mg; NH_4Cl, 400 mg; $MgSO_4x7H_2O$, 200 mg; trace minerals and vitamins (5), 10 ml each; sodium thioglycolate, 500 mg; sodium dithionite, 50 mg. The gas phase was 80% N_2 - 20% CO_2 (v/v). The pH was adjusted to 6.8 with $KHCO_3$.

0006-291X/84 $1.50

Putrescine, spermidine and spermine were added as indicated. The incubation temperature was 30°C. Methane formation was monitored using syringe techniques and gas chromatography. Degradation experiments were carried out in duplicate using screw-capped plasma bottles (B. Braun Melsungen AG) closed by butyl rubber stoppers no. 4 (Bellco). Each bottle contained 100 ml digesting sludge and 100 ml medium in a total volume of 603 ml. Samples of 2 ml each were used to quantitate polyamines.

Polyamine analysis - Analysis was performed by dansylation of the amines in aqueous solution and subsequent HPLC with fluorescence detection as previously described (6). HPLC fractions containing the unknown dansyl derivative were pooled, dried in a desiccator under vacuum and subjected to mass spectrometry.

Mass spectrometry - A Kratos MS 25 mass spectrometer with a direct inlet system and data processing by the data system DS 55 was used with the electron energy 70 eV, trap current 100 uA, and ion source temperature 250°C.

The mass spectrum of the dansylated unknown compound was in good agreement with that of dansyl pyrrolidine (7) and contained the following major mass peaks: m/e = 304 (32%, m^+); 171 (100%), 170 (24%), 172 (14%), 128 (11%), 154 (10%).

Atomic composition of the dansylated unknown compound was established by high resolution mass spectrometry:

exact mass measured for M : 304.1243
exact mass for $C_{16}H_{20}N_2O_2S$: 304.1240.

RESULTS

In Fig. 1 a typical HPLC chromatogram of the dansylated reaction mixture is shown. With the exception of peak x all major peaks of the chromatogram could be identified by comparison with retention times of standards. After semipreparative isolation of x by collection of the corresponding HPLC peak, evaporation and analysis by mass spectrometry, x was shown to be pyrrolidine.

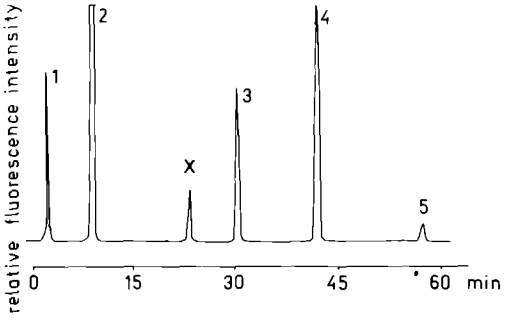

Fig. 1 HPLC chromatogram of the dansylated reaction product of spermine degradation. Peaks represent dansyl derivatives of the following compounds: 1: H_2O; 2: ammonia; x: pyrrolidine; 3: 1,3-diaminopropane; 4: 1,8-diaminooctane (internal standard); 5: spermine.

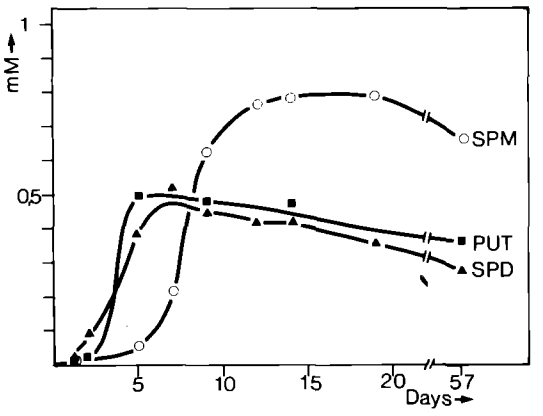

Fig. 2 Pyrrolidine concentrations during polyamine degradation. Concentrations of polyamines (PUT = putrescine, SPD = spermidine, SPM = spermine) at the start of the experiments: 25 mM.

Spermidine and putrescine yielded pyrrolidine only when higher polyamine concentrations were used (c = 25 mM), pyrrolidine formation from spermine already started at c = 10 mM. Fig. 2 shows the time course of the formation of pyrrolidine from the polyamines investigated. It can be seen that the pyrrolidine level remained relatively stable from the point of its occurrence until the end of the experiment (57 days). The polyamines investigated had already completely disappeared after 5 (putrescine), 9 (spermidine) and 20 days (spermine) (2).

DISCUSSION

Degradation of polyamines has been studied intensively in mammalian systems (serum, seminal plasma, brain) (8,9) and in plants (10). Fewer examples are known from bacteria (8). These degradation processes are generally oxidation reactions followed by cyclization of the intermediates formed. The oxidation of spermidine by Serratia marcescens may serve as a typical example (s. Fig. 3). Formation of pyrrolidine from polyamines under anaerobic conditions could be explained by assuming a reduction step resulting in hydrogenation of pyrroline.

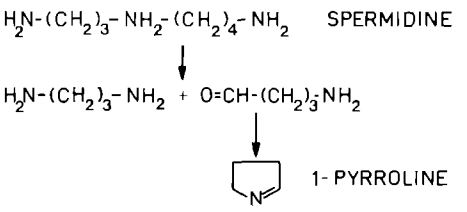

Fig. 3 Oxidation pathway of spermidine in Serratia marcescens (8).

Since they are precursors of carcinogenic nitrosamines, the occurrence of secondary amines in the environment has attracted attention. Next to dimethylamine and diethylamine, pyrrolidine was always reported among the most prevalent secondary amines. Pyrrolidine concentrations were usually in the order of 1 - 80 µg/kg in sediments (11) and 0.2 - 2 µg/l in surface waters (12). In pig manure, where anaerobic conditions prevail during storage in tanks, 3 mg pyrrolidine/l was found (Kneifel, unpubl.). It should be mentioned that many alkaloids (e.g. nicotine) are pyrrolidine derivatives. Values of 0.1 - 5 mg pyrrolidine/kg were reported for vegetables (11).

The origin of pyrrolidine in the environment was thought to be the result of proline decarboxylation (11), but its formation from polyamines by heating (e.g. cooking) has also been considered (13). Polyamines not only occur widely in nature but their concentrations can also be quite high. Putrescine concentrations in algae (14) and bacteria (6), for example, can reach more than 0.3% of the dry material. Polyamine degradation under anaerobic conditions, therefore, must be assumed to be a major source of pyrrolidine in the environment.

ACKOWLEDGMENTS

We thank H. Paal (Kratos GmbH) for performing the mass spectrometric investigations, H. Gross and G. Neumann for measuring the pyrrolidine concentrations and E. Schunck for performing the degradation experiments.

REFERENCES

1. Bachrach, U., Kaye, A., and Chayen, R. (eds.) (1983) Advances in Polyamine Research, Vol. 4, Raven Press, New York. See also previous volumes.
2. Schoberth, S.M. and Kneifel, H. (1982) XIII Intern.Congr. Microbiol. Abstr. Paps. p. 14:11, Am. Soc. Microbiol.
3. Hungate, R.E. (1969) Methods in Microbiol., Vol. 3B, pp. 117-132, Academic Press, London.
4. Schoberth, S. (1973) Arch. Mikrobiol. 92, 365-368.
5. Wolin, E.A., Wolin, M.J., and Wolfe, R.S. (1963) J. Biol. Chem. 238, 2882-2886.
6. Scherer, P. and Kneifel, H. (1983) J. Bacteriol. 154, 1315-1322.
7. Seiler, N., Schneider, H., and Sonnenberg, K.-D. (1970) Z. Anal. Chem. 252, 127-136.
8. Bachrach, U. (1981) Polyamines in Biology and Medicine (D.R. Morris and L.J. Marton eds.), pp. 151-168, Marcel Decker, New York.
9. Seiler, N., Bolkenius, F.N., and Rennert, O.M. (1981) Med. Biol. 59, 334-346.
10. Smith, T.A. (1981) Polyamines in Biology and Medicine (D.R. Morris and L.J. Marton eds.), pp. 77-82, Marcel Decker, New York.
11. Duré, G. (1978) Hydrochem. hydrogeol. Mitt. 3, 91-112.
12. Neurath, G.B., Dünger, M., Pein, F.G., Ambrosius, D., and Schreiber, O. (1977) Fd. Cosmet. Toxicol. 15, 275-282.
13. Lijinsky, W. and Epstein, S.S. (1970) Nature 225, 21-23.
14. Kneifel, H. (1979) Marine Algae in Pharmaceutical Science (H.A. Hoppe, T. Levring and Y. Tanaka eds.), pp. 365-401, Walter de Gruyter, Berlin.