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ENZYMIC POLYPEPTIDE DEGRADATION

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ABSTRACT OF THE DISCLOSURE

Disclosed herein is a process for degrading heterodetic polypeptides by utilizing microorganisms of the genus Actinoplanes.

This invention relates to the degradation of heterodetic polypeptides. More particularly, this invention relates to the enzymic degradation of heterodetic polypeptide antibiotics, employing growing cultures of a microorganism of the genus Actinoplanes.

During the course of antibiotic treatment, it is often desirable to eliminate any antibiotic activity present in blood and other body fluid samples in order that hitherto unsuspected bacteria can be detected or that further biochemical tests can be performed.

Such inactivation previously required comparatively drastic treatment, such as acid hydrolysis, in order to achieve the desired degree of antibiotic degradation, whereas, by the present invention, a method is provided whereby such antibiotics can be safely and thoroughly removed from blood or other body fluid samples without the necessity of subjecting said samples to the drastic conditions heretofore required.

Further, the method of the present invention may be employed to carry out a partial degradation of polypeptide antibiotics. Specifically, opening of the ester bonds in the lactone ring of such polypeptides as, for instance, dactinomycin and vernamycin B's may be achieved. Such degradates may then be chemically modified, for instance, by attaching another amino acid residue and rejoining the ring, thus providing an analog of the original antibiotic. The present invention thus provides the laboratory worker and researchist with a new and valuable diagnostic and investigative tool not heretofore available in the art.

In one embodiment of the invention, a microorganism of the genus Actinoplanes is grown under aerobic conditions in a culture medium containing assimilable carbon, nitrogen, and minerals. To the culture thus obtained is added a quantity of antibiotic (or sample containing antibiotic), such as dactinomycin, and the incubation is continued. The presence of the antibiotic in the growing culture stimulates the production of an enzyme which, in turn, causes the degradation of the polypeptide antibiotic. The degradation so achieved is readily determined by paper ionophoresis and ion exchange chromatographic behavior, as well as by degradation by chemical processes.

The above degradation processes is generally applicable to heterodetic polypeptide (that is, any polypeptide containing a lactone ring in its structure). Examples of heterodetic polypeptides which may be degraded in the process of this invention include, but are not limited thereto, dactinomycin, cactinomycin, actinomycin, etamycin, staphylomycin S, pristinamycin I, mikamycin B, vernamycin B and echinomycin (and the related triostin complex).

The degradations take place readily and effectively under the conditions satisfactory for the growth of the Actinoplanes; that is, a temperature between about 20 and 50° C., preferably about 20 to 40° C., and a pH between about 6 and 9, preferably between about 6.5 and 8, with agitation and aeration. Optimally, the degradations take place at a temperature of about 30° C. and a pH of about

7. The culture medium should contain an assimilable source of carbon such as sucrose, glucose, glycerol or the like, a nitrogen source such as peptone, urea, ammonium sulfate or the like, and several inorganic salts found generally to be effective to promote the growth of microorganisms. Applicant has found the use of the commercially available culture medium designated Staley's Special Nutrient 4–S, employed in combination with glycerol, to be particularly suitable although any standard culture medium known in the art meeting the above requirements may be utilized.

The Actinoplanes genus microorganism are operable as a group in the process of the present invention. Examples of species of this genus are *Actinoplanes missouriensis* (ATCC 14,538), *Actinoplanes utahensis* (ATCC 14,539), *Actinoplanes philippinensis* and Actinoplanes species IMRU F3–15. The use of Actinoplanes species IMRU F3–15 is preferred in the present process.

The invention will be more clearly understood from the following operating examples, which are intended to be illustrative only, and not as limitations on the scope of the invention.

Example 1

A culture of Actinoplanes species IMRU F3–15 is grown in a submerged culture in a medium containing 30 grams per liter of Staley's Special Nutrient 4–S and 20 grams per liter of glycerol. The culture is grown under moderate aeration (100 ml. medium in a 250 ml. Erlenmeyer flask placed on a rotary shaker at 280 r.p.m. and 1 inch displacement) at a temperature between 23 and 37° C. After about four days incubation, 5 ml. of vegetative growth is used to inoculate a series of flasks (100 ml. of medium per each 500 ml. Erlenmeyer flask) and the second series of flasks are placed on the shaker. After about three days incubation, 0.2 ml. of a 50% aqueous ethanol solution containing 5 mg. of dactinomycin per ml. are added to each flask and the fermentation continued for 12 to 18 hours. At the end of the incubation period, the contents of the flasks are collected by centrifugation and resuspended in 0.1 to 0.2 M phosphate buffer (pH 7.0). The solids are again collected by centrifugation and resuspended in the phosphate buffer. This cell suspension, containing the enzyme, is used to degrade the antibiotics directly by placing the suspension in aerated beakers, adding dactinomycin to give a concentration of 10 to 1,000 mcg. per ml. and aerating the mixture for 4 to 10 hours. At the end of this period essentially all of the antibacterial activity has disappeared (as measured by bioassay using *Staphylococcus aureus* 209P). The degradation products are recovered by either solvent extraction or ion exchange chromatography, and include dactinomycinic acid and dactinomycin monolactone.

Example 2

Actinoplanes species IMRU F3–15 is grown in shaken flasks (100 ml. per 250 ml. Erlenmeyer flask) placed on a rotary shaker (180 r.p.m., 1 inch displacement), located in a room maintained at 28–30° C. The nutrient medium contained Staley's Special Nutrient 4–S, 30 grams, glycerol, 20 grams, and distilled water q.s. 1 liter. The flasks are inoculated with 5% transfer of vegetative growth of a 4-day old culture grown in this medium. After three days incubation, 1 mg. of actinomycin complex is added to one flask and incubation continued for 18 hours. The contents of the flasks are then collected by centrifugation and the solids resuspended in 100 ml. of phosphate buffer (0.02 M, pH 7.0). Ten ml. of this suspension is placed in a 50 ml. Erlenmeyer flask and 1 mg. of antibiotic added to the suspension. A zero-time sample is taken and extracted with $CHCl_3$ (equal volume). After three hours incubation at 30° C., a second sample is taken (usually 5 ml.)

and similarly extracted. The antibiotic content of the extracts is determined by bioassay using *Staphylococcus aureus* 209P (agar diffusion assay). More than 90% of the antibiotic activity of the treated actinomycin has disappeared during the incubation.

Example 3

The process of Example 2 is followed except that vernamycin B is substituted for the actinomycin complex. In this case, also, antibiotic activity decreases by over 90%.

Example 4

Following the procedure of Example 2, but substituting echinomycin for the actinomycin complex, substantially the same result is achieved. The antibiotic activity is diminished by over 90% during the incubation.

Example 5

Etamycin is substituted for the actinomycin complex of Example 2, with the result that more than 90% of the antibiotic activity of the treated etamycin has disappeared during incubation.

What is claimed is:

1. A process for degrading heterodetic polypeptides selected from the group consisting of dactinomycin, cactinomycin, acitnomycin, etamycin, staphylomycin S, pristinamycin I, mikamycin B, vernamycin B, and echinomycin which comprises exposing said polypeptide to the action of a microorganism of the genus Actinoplanes for a time sufficient to achieve substantially degradation of said polypeptide.

2. A process in accordance with claim 1 wherein said exposure takes place at a temperature between about 20 and 50° C. and at a pH between about 6 and 9.

3. A process in accordance with claim 1 wherein the microorganism is Actinoplanes species IMRU F3–15.

4. A process in accordance with claim 1 wherein the heterodetic polypeptide is dactinomycin and the microorganism is Actinoplanes species IMRU F3–15.

References Cited

Katz et al., Science, vol. 126, 1957, pp. 402–403, copy in Scientific Library.

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