Inhibition of Ribonuclease I in *Escherichia coli* by Polymyxin B

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The "latent" RNase I of *E. coli* ribosomes is avtivated by polymyxins in media containing enough magnesium (10^{-4} to 10^{-3} M) to prevent RNase I-induced autolysis of ribosomal RNA under normal conditions [1, 2]. On the other side RNase I-induced RNA depolymerization in membrane injured cells of *E. coli* is inhibited by polymyxin B in EDTA containing buffers [3]. The following communication presents some more detailed data concerning this inhibitory action of polymyxin B.

Material and Methods

E. coli was a wild type derived from the collection of the Max v. Pettenkofer-Institut München. Toluenized cells, ribosomes and acid-soluble ribosomal RNase were prepared from stationary cells as described previously [3]. All experiments were performed in 0.05 M Tris-HCl-0.001 M EDTA buffer p_H 7.5 at 37 °C. RNase I activity was determined by measuring the appearance of acid-soluble UV-absorbing material (260 mμ) after 15 min (ribosomes, toluenized cells) and 60 min (acid-soluble RNase) of incubation. The data were corrected for the absorption of polymyxin B at 260 mμ. The RNase I-induced autolysis of ribosomal RNA was carried out with 2×10^9 cells per ml and with 150 μg ribosomes per ml. For the determination of the RNase I activity of polymyxin-precipitated ribosomes and of the acid-soluble RNase preparation 0.1 ml ribosome suspension (150 μg/ml) or 0.1 ml enzyme preparation were added to 2 ml of 0.01% polyadenylic acid (poly-A). The specific activity of the used enzyme preparation (per ml) corresponded to the activity of about 10 μg ribosomes. Polymyxin B sulfate was purchased from Pfizer G.m.b.H. Karlsruhe (bactericidal activity 7661 units per mg).

Results and Discussion

Both autolysis of ribosomal RNA in isolated ribosomes and toluenized cells and depolymerization of poly-A by polymyxin-precipitated ribosomes and acid-soluble RNase were inhibited in a similar manner (Fig. 1). This process was characterized by a reduced rate of RNA degradation, but total cleavage of the available RNA into acid-soluble material could be obtained by prolonged incubation. Direct and irreversible

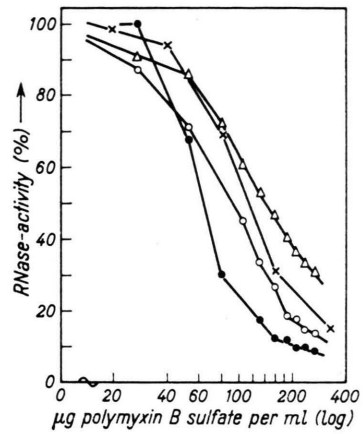

Fig. 1. Inhibition of RNase I activity of various fractions of *E. coli* by polymyxin B.

denaturation of RNase I by polymyxin B was excluded since ribosomes (150 μg/ml) treated with excess polymyxin B (600 μg/ml) had the original RNase I activity against external poly-A when the free polymyxin B had been removed by several wash procedures. In all examined fractions of *E. coli* the polymyxin B-exhibited RNase I inhibition was not affected by the length of the polymyxin B treatment. The possibility that RNase I activity was masked by the observed formation of 70 S sedimenting particles in the presence of polymyxin B [4], may be ruled out because isolated acid-soluble RNase which is most probably identical with RNase I [5], was inhibited by polymyxin B too.

If intact cells of *E. coli* were incubated in the presence of increasing amounts of polymyxin B (50 to 500 μg/ml), no remarkable RNA depolymerization was detectable within 15 min of incubation in spite of a totally destroyed permeability barrier of the cells. But if the free polymyxin B was washed off, the resulting RNA degradation reached 50% of the normal rate in toluenized cells or isolated ribosomes independently on the used concentration of polymyxin B.

Polymyxins are very potent reagents to precipitate ribosomes from *E. coli* [4, 6]. This precipitation is most probably caused by formation of electrostatic bonds between the negative charged phosphate groups of ribosomal RNA and the five free positive charged amino groups of the polymyxin B molecule [4]. These observations along with the results reported in the present communication suggest that RNase I-induced depolymerization of RNA or poly-A in EDTA containing media is inhibited by polymyxin B because the phosphodiester linkages in the polynucleotide are protected by the bound polymyxin. In the presence of polymyxin *and* magnesium, however, autolysis of ribosomes seems to be a more complicated process. On the one hand

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RNase I is activated, on the other side a simultaneous inhibition can be observed [1, 2]. The experimental data thus far available permit no real understanding mainly because until now very little is known about the mechanism of the activation of the "latent" RNase I in ribosomes.

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Zur Frage der Identität und des Vorkommens von Neurohormon D in verschiedenen Bereichen des Zentralnervensystems von *Periplaneta americana*

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Neue und neueste Befunde lassen immer deutlicher erkennen, daß mit mehreren Neurohormonen aus dem Bereich des Zentralnervensystems der Insekten zu rechnen ist. Aus dem Gehirn sind bisher außer dem Aktivationshormon der Häutung hormonale Faktoren für den Wasserhaushalt und die Exkretion, für den Farbwechsel, den sog. „Tanning-Effekt", für verschiedene Stoffwechselprozesse wie Regulation des Blutzuckers und für den Eiweißstoffwechsel festgestellt worden. Ebenso kennt man bei verschiedenen Insektenarten wirksame Faktoren aus einzelnen Ganglien des Bauchmarks.

Andererseits fragt es sich, inwieweit *ein* bestimmtes Neurohormon in *verschiedenen* Ganglien produziert wird. Dies scheint nach früheren Befunden für Neurohormon D der Fall zu sein [1, 2], das aus dem Zentralnervensystem isoliert und als Peptid mit einem Mol.-Gew. von etwa 2000 charakterisiert werden konnte [3].

Zur genauen Klärung dieser Frage wurden getrennt voneinander Gehirn und Bauchmark von 5000 *Periplaneta americana* verarbeitet. Die dann nacheinander durchgeführte dünnschicht- und papierchromatographische Auftrennung des mit einem Propanol-Wasser-Gemisches erhaltenen Extraktes nach einem bereits beschriebenen Schema [3] ergab von Aminosäuren freies Neurohormon D. Zumindest liegen jene möglichen Mengen unter der Nachweisgrenze mit Ninhydrin.

Es zeigte sich, daß sich der biologisch aktive Bereich in beiden Aufarbeitungen sowohl hinsichtlich seiner physiologischen Aktivität als auch seines chemischen Verhaltens gegenüber verschiedenen Peptidreagenzien völlig gleichartig verhielt.

Noch klarer läßt sich die Identität beider Proben aus dem IR-Spektrogramm des jeweils aus dem Bauchmark und dem Gehirn aufgearbeiteten Faktors erkennen (s. Abb.).

Die auf verschiedene Weise erhobenen Befunde zeigen, daß Neurohormon D sowohl im Cerebralganglion als auch in den Ventralganglien produziert wird. Ungeklärt bleibt jedoch, ob das Neurohormon in allen Ganglien des Bauchmarks gebildet werden kann.

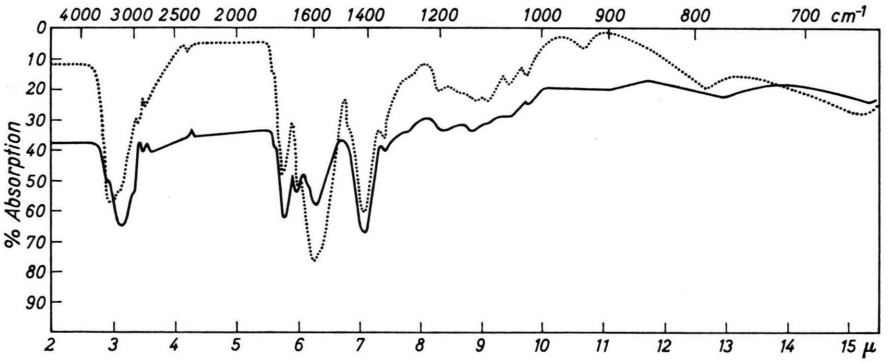

Abb. 1. IR-Spektrogramm von Neurohormon B aus dem Bauchmark aufgearbeitet · · · · , aus dem Gehirn aufgearbeitet ——.

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