STUDIES ON THE NUTRITIONAL REQUIREMENTS OF BACTERIA VII. PYRIDINE-3-SULFONIC ACID AS A GROWTH FACTOR

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It has been well known that pyridine-3-sulfonic acid (PSA) is a growth inhibiting factor of some species of bacteria, reacting as an antagonist against niacin or its homologue.^(13,24) On the other hand, interesting informations have recently been accumulated indicating that the same substance can work as a growth promoting factor when given in addition to niacin or at sufficiently low concentration. Namely, McIlwain (1940)⁽¹³⁾ observed the growth stimulating effects of the combination of PSA and niacin on *Staphylococcus* and *Proteus*. Lwoff and Querido (1939)⁽⁷⁾ reported that PSA, when added to culture medium for *Proteus* at the concentration of 10^{-5} M, could substitute niacin, although the data hereby obtained could not provide unequivocal evidence for its capability of being growth factor.⁽⁵⁾

Studies have been made in our laboratory to clarify whether and how PSA can exert growth stimulating effects on *Staphylococcus*. The present paper will discuss the results obtained therefrom. Our first experiments revealed that PSA can substitute niacinamide when used at the concentration of 10^{-4} M. It appears unlikely, however, that PSA can be converted to niacin, exchanging its sulfonyl with carboxyl radical, during the period of bacterial growth. Therefore, it was presumed that this phenomenon may be due to the change caused by PSA to the balance between the velocity of synthesis and degradation of niacin.

At the same time, in view of the fact that niacin homologues are the precursors for DPN, actually acting form as growth factor, it was also considered probable that the activities of DPN destructive enzymes, DPN-nucleosidase^(9,16) and DPN-pyrophosphatase^(6,21), may be inhibited by the presence of PSA. The experimental results gave us suggestions verifying these presumptions.

EXPERIMENTAL

Strains: *Staphylococcus aureus* Terashima,
Shigella dysenteriae I (Ewing I)

Method:

The media used were Knight's semi-synthetic medium for *Staphylococcus* and synthetic medium for *Shigella dysenteriae* I.⁽¹²⁾ 10cc of bacterial suspension of *Staphylococcus* grown in Knight's medium for 24 hours at 37°C was made, washed three times with saline, suspended in the original amount of saline and then diluted decimally two times with saline. 0.1cc of this final dilution was inoculated into each 5cc of the test medium. In the case of *Shigella dysenteriae*, a loopful of the broth culture grown for 24 hours at 37°C was transferred to the synthetic medium (without niacinamide), from which three successive subcultures were repeated in the same medium, each time one loopful of the preceding culture being used as inoculum. 0.1cc of the final subculture was inoculated into each 5cc of the test medium, or, in case neutral red was contained in the test medium, the final subculture was washed three times with saline and re-suspended in one tenth of the original volume of saline, of which 0.2cc was inoculated into each 5cc of the test medium.

DPN, PSA and niacinamide:

DPN was extracted from brewer's yeast by the method of Williamson and Green⁽²³⁾ and its purity was 19% as determined by Colowick and

Kaplan's method⁽²⁾ and also by estimating the amount of niacin (BrCN-aniline method⁽⁴⁾) therein. Molar concentration of DPN was calculated on the basis of this purity. PSA was synthesized from pyridine and fuming sulfuric acid⁽⁸⁾ and used as sodium salt. Niacinamide was synthesized by our method⁽¹⁾.

Observation of growth:

Growth was observed by Colemann's Electrophotometer at the wave length of 500m μ , or, in case of the media with neutral red, 650m μ . The value of optical density is proportional to bacterial number. Therefore, its logarithm was calculated and plotted in the figures.

I. *PSA in Staphylococcus*

It was shown that PSA can substitute niacin when used at the concentration higher than 10^{-4} M and acts as a growth factor (Fig. 1). On the assumption that this is due to the inhibiting effects of PSA on the activity of DPN-nucleosidase, it was considered likely that the combined use of PSA and DPN might stimulate the growth and that, when given together with niacinamide, PSA might show no growth-stimulating effect, since niacinamide itself inhibits the action of DPN-nucleosidase of the brain⁽¹⁹⁾. Any way, PSA may act with DPN in different way from that with niacinamide. Actually, stimulating effects were observed in the next experiment whereby both PSA and DPN were added, as shown in Fig. 2. PSA together with 10^{-6} to 10^{-8} M of DPN remarkably accelerated the growth of *Staphylococcus*, whereas PSA together with 10^{-5} M of DPN had no effect. 10^{-2} M of PSA showed growth-stimulating effect in the later part of the growth period. PSA is not so much growth-inhibitory, so far as it is added at high concentrations as shown in Fig. 2.

Fig. 3 shows the growth observed in the medium containing PSA together with niacinamide. Growth-inhibitory effect of PSA could be observed after 24 hours, especially in case of the lower concentrations of niacinamide. It is interesting that, this time, contrary to the results of the preceding experiment with DPN, remarkable growth inhibitory, though not synergic, effects of the combination of PSA and niacinamide were observed.

II. *PSA in Shigella dysenteriae I*

So far, the investigation has been made on *Staphylococcus* which requires niacinamide as a growth factor. Then, what would be the effect

Fig. 1. Effective type of PSA and neutral red.

upon the growth of *Shigella dysenteriae* I which requires niacinamide as a growth-stimulating substance, being capable of synthesizing niacinamide to some extent but not sufficiently enough to support its own growth⁽¹¹⁾? The results are shown in Fig. 4. After 36 hours' incubation, PSA added together with niacinamide was found to be growth-inhibitory and PSA together with DPN had no influence on the growth.

Fig. 2. Relationship between DPN and PSA in *Staphylococcus aureus*.

Fig. 3. Relationship between niacinamide and PSA in *Staphylococcus aureus*.

Fig. 4. Relationship between PSA and either DPN or niacinamide in *Shigella dysenteriae*.

Fig. 5. Neutral red as a growth-accessory substance in *Shigella dysenteriae*

III. *Growth-stimulating effect of neutral red on Shigella dysenteriae I*

Neutral red is a typical inhibitor of DPN-nucleosidase in case it is an animal brain tissue preparation⁽¹⁹⁾. It was thought that, if the activity of PSA as a growth factor is due to its inhibitory-effect against DPN-nucleosidase, neutral red might also be a growth factor for *Staphylococcus* of a growth-stimulating substance for *Shigella dysenteriae* I. As shown in Fig. 5, the growth curve of *Shigella dysenteriae* I indicated that neutral red has remarkable growth-stimulating effect at the concentration of 10^{-3}M . Contrary to the case of PSA, neutral red at 10^{-2}M completely inhibits the growth. Similar experiments on *Staphylococcus* failed to demonstrate the similar effect of neutral red, presumably due to strong growth-inhibitory effect of neutral red on this bacteria, even in the presence of methyl-naphthoquinone⁽¹⁴⁾.

DISCUSSION

According to the considerations made by Fildes' school⁽³⁾, the growth factor can be considered as "essential metabolite" which can not be synthesized by bacteria. If the velocity of synthesis by bacteria of essential metabolite is negligibly slow, this essential metabolite works as a growth factor. If the velocity is limited (there might be varied steps) and slower than usual, this essential metabolite can become growth accessory substance. The quantitative order of growth factor during metabolism is usually within $\text{m}\mu\text{M}/\text{mg}/\text{hr}$ ⁽¹⁵⁾.

The vitamin-activity of PSA appears to be slightly different from that of ordinary vitamins in the point that the quantitative order of PSA needed during growth period may probably be within $\mu\text{M}/\text{mg}/\text{hr}$. It means that PSA is not a real growth factor, and that sulfonyl group of PSA can not be exchanged with carboxyl group during the growth period. The above findings show that PSA inhibits the activity of DPN-nucleosidase, thus exerting growth factor-like effects. Growth-inhibitory effects of PSA, when added together with niacinamide, may probably be due to the inhibition of the assimilation of niacinamide. It is comparable to the fact that pantoyletaurine can inhibit the assimilation as well as inactivation of pantothenic acid⁽¹⁵⁾. In this meaning, the vitamin-activity of PSA can be understood to have the following mode. In the growth of bacteria which can synthesize DPN by themselves the DPN-synthesis is faster than its degradation, while in the growth of bacteria which are unable to synthesize

DPN and require niacinamide as a growth factor the DPN synthesis is slower than its degradation. PSA is a substance which can control the balance between the synthesis and degradation of DPN. When it acts as an inhibitor of DPN-degradation (PSA with DPN), it apparently becomes a growth factor or a growth accessory substance, and when it acts as an inhibitor of niacinamide assimilation (PSA with niacinamide), it apparently becomes a growth-inhibitory substance instead. PSA, any may, can be a vitamin-like substance.

The findings described above should not be regarded as those restricted to the problem of DPN only. It can be suggested that other inhibitors of vitamin-destructive enzyme may act as vitamins in wider sense, since they protect the active form of vitamin against the vitamin-destructive enzyme; for example, pantoyletaurine against coenzyme-A destructive enzyme, sulfanilic acid against folic acid destructive enzyme and so on.

It has been known that DPN is less effective than niacinamide in term of their growth promoting effect at equimolecular concentration⁽²²⁾. For the interpretation of this phenomenon, there have been several considerations about factors accounting for it; for example, cell permeability⁽¹⁰⁾, other substance than DPN and so on⁽²⁰⁾. As confirmed in our experiments, DPN and niacinamide are equally effective at 10^{-5}M , whereby PSA has no effect. DPN is less effective than niacinamide at 10^{-6} , 10^{-7} and 10^{-8}M , whereby PSA remarkably promote the growth-stimulating effect of DPN. The bacterial growth in the presence of DPN at 10^{-6} to 10^{-8}M together with PSA at 10^{-3}M is equal to that of niacinamide alone at 10^{-6} to 10^{-8}M . Thus, the fact that DPN is less effective than niacinamide can be explained by the action of DPN-nucleosidase. As well known, *Lactobacillus* which has negligible DPN-nucleosidase can grow equally well in medium containing either DPN or niacinamide^(17,22).

SUMMARY

(1) PSA can substitute niacinamide at the concentrations of 10^{-4} , 10^{-3} , 10^{-2}M for the growth of *Staphylococcus aureus* Terashima.

(2) PSA is a growth accessory substance for *Shigella dysenteriae* I at the concentrations of 10^{-3} , 10^{-2}M .

(3) These two phenomena are thought to be due to the fact that PSA inhibits DPN-nucleosidase and hence acts as a vitamin. PSA added to the medium together with DPN accelerates to great extent the growth of

Staphylococcus and *Shigella dysenteriae* I. PSA added to the medium together with niacinamide, on the contrary, inhibits the growth of both species of bacteria.

(4) Neutral red, an inhibitor of DPN-nucleosidase, can also become a growth-accessory substance for *Shigella dysenteriae* I.

(5) It is presumable from the data obtained above that inhibitor of vitamin-destructive enzymes may act as vitamins.

(6) Discussions were given on the problem that DPN is less effective than niacinamide even at equimolecular concentration.

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