transoidal-type mechanism. The assignment of the proton NMR of A-2-C has been deduced by applying NOE difference experiments, Gd(III) line-broadening experiments and 2D-NOESY experiments of regio-and stereospecificially deuterated A-2-C's.

Glutamine Synthetase of some Fermentation Bacteria: Function and Application

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Metabolic activity of inorganic nitrogenous compounds affects not only microbial growth but also metabolite production in fermentation technology. We have worked on the enzymes participating in ammonia assimulation of some fermentation bacteria. This paper summarizes the results on glutamine synthetase and its application in practical field.

Glutamine synthetase (L-glutamate:ammonia ligase, EC. 6.3.1.2) catalyzes the formation of glutamine from glutamate and ammonia at the expense of cleavage of ATP and inorganic phosphate. The enzyme plays a dual role in nitrogen metabolism in bacteria; it is a key enzyme not only in the biosynthesis of various compounds through glutamine but also in the regulation of synthesis of some enzymes involved in the metabolism of nitrogenous compounds. The detailed works with the *Eschericia coli* and other enterobacterial enzymes revealed that glutamine synthetase is controlled by the following complex of mechanisms: (a) feedback inhibition by end products, (b) repression and derepression of enzyme synthesis, (c) modulation of enzyme activity in response to divalent cation and (d) covalent modification of enzyme protein by adenylylation and its cascade control. Comparative studies have also been made on the enzymes from other organisms.

1) Glutamine synthetase of *Gluconobacter suboxydans*

G. suboxydans, one of the type species of acetic acid bacteria, has a remarkable oxidizing-ability toward sugars and polyols, and has been utilized in fermentative production of various substances. The organism is also characterized by the lack of conventional TCA cycle. Investigators have therefore attempted to elucidate the biosynthesis of certain amino acids generally formed from intermediates of the cycle. For glutamic acid, was suggested a route involving some of the TCA cycle enzymes in conjunction with transaminations. However, the enzymatic mechanism of incorporation of ammonia into amino acid has not been elucidated because direct amination of α-ketoglutarate by glutamate dehydrogenase was not demonstrable in the organism. We confirmed that the organism assimilates ammonia as a nitrogen source and indicated glutamine synthetase/glutamate synthase pathway operates as the ammonia-assimilating system. Glutamine synthetase was crystallized and characterized together with glutamate synthase.

2) Glutamine synthetase of bifidobacteria.

Bifidobacteria, gram positive and strictly anaerobic, are one of the predominant organisms of mammalian intestinal flora. The organisms are supposed to take some part in reduction of putrefactive bacteria and toxic metabolites of the host. We investigated the enzymes involved in their ammonia assimilation, which might related with their detoxification activity in host intestine. Glutamine synthetase occurred in the organisms besides glutamate dehydrogenase, but glutamate synthase was not found in any strain. Glutamine synthetase was isolated from *Bifidobacterium bifidum* (human origin), *B. breve* (human origin) or *B. pesudolongum* (pig orgin). In addition,we found that bifidobacterial glutamine synthetase was inactivated by an enzymatic reaction, and characterized the inactivating enzyme. The results suggested that glutamine synthetase activity in the organism is controlled through adenylylation which appears similar to that found in the gram

negative bacteria.

3) Glutamine synthetase of glutamate producing bacteria.

The synthesis of glutamate from ammonia, the basis of the amino acid fermentation, is considered to be catalyzed by glutamate dehydrogenase, which is contained at high level in these bacteria. We found glutamate producers, *Micrococcus glutamicus (Corynebacterium glutamicum)* and *Brevibacterium flavum,* form a large amount of glutamine synthetase (2-3% of soluble protein) when grown on specific media containing glutamate as a nitrogen source. We elucidated that the enzyme participates in incorporation of ammonia to glutamate by coupling with glutamate synthase under the ammonia-limited conditions. The properties of the glutamine synthetase were discussed comparatively with those of the enzyme described above.

4) Glutamine production by coupling glutamine synthetase reaction with alcoholic fermentation of yeast.

Glutamine has been produced commercially by direct fermentation with certain bacteria, and is used in the treatment of gastric ulcers. In recent years, enzymatic synthesis has come to rival direct fermention as a means of producing amino acids. In case of glutamine, the need for a stoichiometric supply of ATP for the endergonic reaction of glutamine synthetase precludes the development of an economically viable method unless ATP can be regenerated and recycled.

We have established a process, "coupled fermentation with energy transfer," for production of various useful substances, in which catalytic amount of ATP is regenerated with the energy of sugar fermented by yeast. The enzyme(s) for the synthesis (energy-utilizing system) can involve those of yeast itself or of other organisms.

We utilized the glutamine synthetase in the process for glutamine production, and this was the first application of "coupled fermentation with energy transfer" to production of nonphosphorylated compounds. After preliminary demonstration of the fermentation principle with the *Gluconobacter* enzyme, we produced glutamine in high concentration by use of the enzymes from the glutamate producers (50-60 g/L). By analyzing characteristics and efficiency of the glutamine production, the following factors were found to be important for the satisfactory production: (1) the ratio of the activities of yeast fermentation of sugar and glutamine synthetase, (2) the effect of contaminating enzyme(s) in the glutamine synthetase preparation, which interferes yeast fermentation or energy coupling, (3) the function of glutamine synthetase in the fermentation mixture, and (4) the Harden-Young effect of alcoholic fermentation by yeast.

The glutamine synthetase is active toward methylamine or ethylamine replaceably with ammonia, and forms corresponding γ-glutamyl derivatives (flavor enhancing materials). When methylamine was added into the fermentation mixture, 17g/L of γ-glutamylmethylamide was produced in 4 hr.

5) Rapid and sensitive determination of ammonia by glutamine synthetase

Recently, significance of ammonia determination is increasing in the field of environmental ecology or clinical chemistry because the substance is an index of pollution in water and for ammonemia or some hepatic diseases in serum. Furthermore, the procedure is valuable for detection of diagnostically important ammonia-forming enzymes. Among the methods for ammonia determination so far known, that with glutamate dehydrogenase has been recognized to be simple and convenient for coupling with the other enzyme reactions. However, glutamate dehydrogenase has high Km value for ammonia generally, and this suggested the enzyme might be unsuitable for rapid determination of ammonia at low concentration. Considering the "scavenging" role of glutamine synthetase under physiological conditions with low ammonia concentration, we designed a system by use of the enzyme, where ADP formation is determined spectrophotometrically at 340 nm with pyruvate kinase and lactate dehydrogenase. The analysis of 10 nmoles of ammonia (0.14 ug NH^4-N) by 1 unit of glutamine synthetase was finished in 5 min whereas in more than 20-30 min by several

glutamate dehydrogenases. The new method could be modified by substituting pyruvate oxidase, peroxidase and appropriate chromgens for lactate dehydrogenase and NADH. Thus, the sensitivity was increased about 2 times at visible reions, and the procedures were available for simultaneous determination of the activity of such enzymes as adenosine deaminase, guanase, urease and so on.

에이 : 오. 티 - 이소옥탄 역미셀계에서의 리파제의 특성과 이 효소에 의한 유지의 가수분해

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초 록 : 이온성 계면활성제 존재하에서 유기용매에 물을 첨가하면 물이 계면활성제에 약해 둘러싸이면서 유기용매에 용해되어 의사 이상계가 형성된다. 이러한 계를 역미셀 또는 W/O microemulsion이라고 하며 계면활성제로 둘러 쌓인 물분자 집합체를 water pool 이라고 한다. 그런데, 10여년 전 water pool에 bipolymer를 용해시킬 수 있다는 사실이 밝혀짐에 따라 이러한 체계를 생체막을 단순화시킨 모형막으로서 막을 통해 일어나는 여러가지 현상의 규명에 이용하거나 물에 불용성인 기질의 효소 촉매반응의 반응계로 이용하는 연구가 꾸준히 이루어져 있다. 본 강연은 역미셀계에 리파제를 용해시켜 유지의 가수분해를 유도함으로써 지방산을 생산하는 방법에 관한 연구이다. 역미셀계에서 리파제의 특성은 에멀전계와 비교했을 때 큰 차이가 없었으며 물과 계면활성제의 몰 비율(R값)은 효소의 초기반응 속도에 커다란 영향을 끼치는 인자로 나타났다. 올리브유 농도가 5% (v/v), AOT 농도가 0.1M, 초기 물 농도가 1.0M의 조건에서 유지의 회분식 가수분해 실험을 행한 결과 이 기질은 거의 완전히 가수분해 되었으며, 이 반응계에서 R값과 초기 물 농도는 반응의 평형에 커다란 영향을 끼치는 것으로 나타났는데 초기 물 농도가 증가할수록 평형 가수분해율은 증가하였다. 이러한 결과를 반응속도론 측면에서 분석한 결과 역미셀계에서 리파제 반응은 에멀전계에서와는 달리 2차 반응을 따르는 것으로 나타났다. 물 농도가 평형 가수분해율과 속도 변수에 끼치는 영향을 수학적으로 표시하기 위하여 2차 가역적 반응 속도론에 근거하여 가수분해율, 평형상수, 속도상수 등을 나타내는 식을 유도하였고 이를 바탕으로 여러가지 실험 조건하에서 리파제 반응의 반응 시간에 따른 가수분해율을 예측한 결과는 실제 실험 결과와 잘 일치하였다(편차는 5%). 또한 속도상수와 R값과의 관계식 및 유도한 방정식을 이용하여 추정한 초기속도와 평형 가수분해율을 최대화하는 R값은 각각 10.4와 11.4였다.