Reports

Individuality of the Meromyosins

Abstract. Subtilisin, as well as trypsin and chymotrypsin, splits myosin into meromyosin-like components. Examination of the N- and C-terminal residues of the L-meromyosin-like products suggests that all three proteases produce identical L-meromyosins. It is proposed that the role of the enzymes is to break secondary bonds.

Gergely (*1*) and Perry (*2*) first showed that trypsin rapidly modified myosin to produce a water-soluble material with no loss of adenosine triphosphatase activity. Gergely (*3*) obtained two fractions, one soluble and the other insoluble at low ionic strength, most of the adenosine triphosphatase activity remaining in the soluble fraction. Mihalyi and Szent-Györgyi (*4*) showed that the reaction was monomolecular and that two distinct components were produced. Szent-Györgyi (*5*) separated and named the heavier component H-meromyosin (molecular weight, 230,000) and the lighter, L-meromyosin (molecular weight, 100,000) and also determined their physical properties. Gergely, Gouvea, and Karibian (*6*) obtained similar products, using chymotrypsin plus trypsin inhibitor, instead of trypsin. I found that a sample of subtilisin (*7*) also converted myosin into components resembling H- and L-meromyosin by means of a monomolecular reaction (*8*). The latter component could also be separated into ethanol-stable (L-meromyosin) and unstable fractions, as in the case of trypsin- and chymotrypsin-produced L-meromyosin (*9*). Electron micrographs of crystals of the subtilisin-produced L-meromyosin showed the typical banded structure with the bands 420 A apart. Recently Kominz has split myosin into two components by means of snake venom (*10*).

Laki (*11*) pointed out that the L-meromyosins should differ in detail, according to the specificity of the enzyme used, if their production is dependent upon the hydrolysis of certain peptide bonds in the original myosin. He quoted certain references to support this hypothesis (*10, 12*).

A detailed examination (*13*) by means of the Sanger technique (with fluorodinitrobenzene) of the N-terminal residues of the L-meromyosins produced by the action of trypsin, chymotrypsin (with trypsin inhibitor to inactivate any traces of trypsin) and subtilisin, for various times, has failed to reveal more than two equivalents per mole, and these are made up of traces of ten different N-terminal residues. However, it is possible to depolymerize the L-meromyosins under suitable conditions in 5*M* urea (*14*), and this results in approximately a tenfold increase in the N-terminal residues found. After 24 hours' treatment, with urea, fluorodinitrobenzene was added and stirred vigorously until it dissolved, and sodium bicarbonate was added to maintain *p*H > 8. After 4 hours the solution was thoroughly dialyzed and lyophilized. The results for the depolymerized L-meromyosins are given in Table 1 and are always the same, irrespective of the enzyme used. If the N-terminal residues are produced by hydrolysis, they would not *necessarily* differ with the enzyme used, unlike the C-terminal residues, but the probability of their remaining the same is small.

Treatment of the L-meromyosins with carboxypeptidase A in the presence of diisopropylfluorophosphate at 40°C (enzyme:substrate = 1:100; 0.6*M* KCl; 1-percent $NaHCO_3$; *p*H 8.3) for 15 minutes liberated aspartic acid, threonine, serine, alanine, glycine, valine, (iso)-leucine, phenylalanine, histidine, and tyrosine, the total being equivalent to 1 to 2 C-terminal residues per molecule of L-meromyosin. On depolymerization of L-meromyosin in urea solution, followed by carboxypeptidase treatment, approximately 20 C-terminal residues per molecule of L-meromyosin were obtained. That the acids listed above originated from the C-terminal residues was shown by the fact that similar results were obtained with the Akabori technique (*15*). Longer treatment of L-meromyosin with carboxypeptidase A resulted in a higher yield of the above-named acids. This was due to the fact that treatment at *p*H 8.3 alone was found to slowly dissociate L-meromyosin, and consequently the C-terminal residues of the subunits were eventually liberated by the carboxypeptidase A. Still further treatment liberated other non-C-terminal acids.

The Akabori technique was also used to determine the presence of C-terminal lysine residues. After a correction of 53 percent for decomposition during the hydrazinolysis (*16*) had been made, less than 0.1 equivalent of C-terminal lysine was found in a mole of trypsin-produced L-meromyosin and considerably less in chymotrypsin-produced L-meromyosin. There was no increase in these values on depolymerizing the L-meromyosins. This small amount of lysine from the trypsin-produced L-meromyosin was probably due to specific hydrolysis of the myosin by the trypsin and resultant adsorption of the C-terminal lysyl peptide material on the L-meromyosin.

Thus, although it is true that certain amino acids, which would be expected as a result of the enzyme specificity, appear in traces as C-terminal residues, it should be pointed out that, with the exception of lysine, they are present in all the L-meromyosins produced by different enzymes and only appear in amounts equivalent to one or more C-terminal residues when the L-meromyosins are depolymerized. It is suggested, therefore, that the proteases first modify the myosin by breaking secondary bonds, which are stable with respect to urea treatment, to produce the meromyosins, through activity somewhat similar to the "denaturase" action postulated by Linderstrøm-Lang (*17*). Only the final degradation phase is due to specific hydrolysis. This does not entirely rule out the possibility that a "trigger" mechanism is "fired" by the hydrolysis of

Table 1. N-terminal residues of depolymerized L-meromyosins. Average chain weight, 5000.

N-terminal residue	Equivalents/ 100,000 g (± 0.2 equivalents)
Aspartic acid	2
Glutamic acid	2
Threonine	1
Serine	2
Alanine	2
Glycine	1 or 2?
Valine	2
Isoleucine	2
Lysine	4
Arginine	2

Instructions for preparing reports. Begin the report with an abstract of from 45 to 55 words. The abstract should *not* repeat phrases employed in the title. It should work with the title to give the reader a summary of the results presented in the report proper.

Type manuscripts double-spaced and submit one ribbon copy and one carbon copy.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each.

For further details see "Suggestions to Contributors" [*Science* 125, 16 (1957)].

one or more peptide bonds, resulting in the formation of the meromyosins containing latent N- and C-terminal residues which can be liberated by depolymerization in 5*M* urea solution. However, this is unlikely, as the operation of the "trigger" mechanism would vary according to the specificity of the protease used, and these details would probably be detected on comparison of the C-terminal residues.

Laki (*11*) concludes "that the meromyosins are the proteolytic split products of myosin and as such should not be considered as pre-existing subunits of myosin." However, he agrees that "since tracer studies show that the two fragments of myosin have different turnover rates [(*18*)], at least two subunits of some kind pre-existing in the muscle can be postulated." I would, therefore, like to draw attention to the work of Marshall and Holtzer (*19*), who used an immunological staining technique with the antibodies of myosin, L- and H-meromyosin. The areas of the sarcomere, stained by the antibodies of L- and H-meromyosin, were more than the length of a myosin molecule apart, suggesting that myosin is either dissociated into L- and H-meromyosin in the muscle fibrils or that the molecule is greatly extended (*20*).

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References and Notes

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20. This study was supported by research grant H-2905 from the National Heart Institute, U.S. Public Health Service; by the American Heart Association; and by the Muscular Dystrophy Associations of America.

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Specific Action of Adenine as a Feedback Inhibitor of Purine Biosynthesis

Abstract. Purines can prevent the formation of aminoimidazole precursors which are accumulated by bacterial mutants genetically blocked in purine biosynthesis. If the block does not interfere with interconversions among adenine, guanine, hypoxanthine and xanthine, then any of the purines can act as a feedback inhibitor. If conversion of the other purines to adenine is prevented, then adenine becomes a specific requirement for inhibition; this indicates that feedback control operates at a level involving adenine or one of its congeners.

Auxotrophic mutants of bacteria that accumulate the substrates of their blocked reactions have been extremely useful for studying feedback control of biosynthetic processes. The formation of the precursor serves as an index of the potential capacity of the bacteria for *de novo* synthesis of the metabolite in question. In the case of purine biosynthesis, feedback inhibition has been studied at the level of several aminoimidazole intermediates accumulated by purine-requiring mutants. The formation of the ribotides of both 5-aminoimidazole and 5-amino-4-imidazolecarboxamide (AICA) (excreted as their respective ribosides) is prevented by those purines which can support the growth of the mutants (1, 2).

Nonproliferating suspensions of strain B-96/1, a mutant of *Escherichia coli* B, accumulate AICA because of a mutational impairment in transformylase activity. An additional, genetically unrelated, requirement for tryptophan allows the nonproliferating condition to be maintained when growth-promoting purines are added in the absence of tryptophan. Under these conditions, all purines which can serve as growth factors (adenine, hypoxanthine, xanthine, guanine, and isoguanine) cause a direct and immediate cessation of AICA formation (2). Half-maximal inhibition is obtained with as little as 0.02 to 0.04 μmole of any purine per milliliter. Since interconversions between the purines can proceed unhampered beyond the transformylase block in strain B-96/1, it was not known whether each of the various purines exerted a separate inhibition or whether there was only one inhibitory form to which the others could be converted.

In order to resolve this question, a system was required which contained an early block to allow for accumulation of precursors as well as an additional late block beyond the pivotal position of inosinic acid to prevent interconversions of the exogenously supplied purines. In addition, an unrelated deficiency in amino acid formation would be desirable to permit analysis under nonproliferating conditions. The chance isolation of strain B-94, another mutant of *Escherichia coli* B, provided these requirements. This mutant is lacking in adenylosuccinase, a bifunctional deacylase which is required for two separate functions in the biosynthesis of adenylic acid (*3*). One reaction involves the desuccinylation of SAICAR (*4*), the succinyl derivative of AICA-ribotide; the other involves a similar splitting of adenylosuccinic acid to yield adenylic acid. Consequently, loss of this enzyme results in (i) the accumulation of SAICAR (excreted as both riboside and ribotide in the proportion 85:15) and (ii) a block in the process by which inosinic acid is aminated to adenylic acid so that interconversions which lead to adenylic acid are prevented and a specific requirement for adenine is manifested. Strain B-94 also exhibits a growth requirement for arginine which is unrelated and genetically distinct from the adenylosuccinase deficiency.

Table 1. Comparison of the inhibitory action of purines on the formation of AICAR by strain B-96/1 and on SAICAR by strain B-94.

Purine	Amount required for 50% inhibition (μmole/ml)	
	AICAR (B-96/1)	SAICAR (B-94)
Adenine	0.02	0.03
Hypoxanthine	0.02	0.24
Guanine	0.04	7.20
Xanthine	0.03	> 10.00

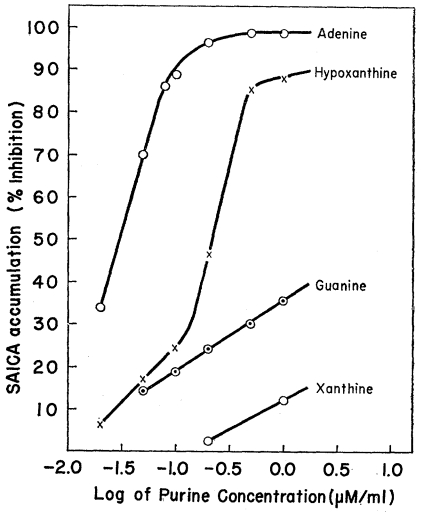

Fig. 1. Dose-response curves of the inhibitory action of various purines on the formation of SAICAR by strain B-94. The yield of SAICAR was determined after an incubation period of 2 hours at 37°C.