THE AMINO TERMINAL SEQUENCE OF ATP-PHOSPHORIBOSYLTRANSFERASE, THE FIRST GENE PRODUCT OF THE HISTIDINE OPERON

Dennis Piszkiewicz[1], Tsafrira Rand-Meir[1], Ida Theodor[2], and Stanley M. Parsons[3]

[1]Department of Biological Chemistry, California College of Medicine, University of California, Irvine, California 92717

[2]Department of Pathology, California College of Medicine, University of California, Irvine, California 92717

[3]Department of Chemistry, University of California, Santa Barbara, California 93106

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SUMMARY: The amino terminal sequence of ATP-phosphoribosyltransferase of Salmonella typhimurium has been determined by automated Edman degradation. Since this protein is the first gene product of the histidine operon, comparison of its amino terminal sequence with the genetic code allows the deduction of a partial base sequence of its mRNA and the corresponding DNA. Five of the first nine residues of ATP-phosphoribosyltransferase align with the amino terminal sequence of histidinol dehydrogenase, the second gene product of the histidine operon.

INTRODUCTION

ATP-phosphoribosyltransferase (E.C. 2.4.2.17) is the first enzyme in the pathway of histidine biosynthesis in Salmonella typhimurium (1,2). It is subject to feedback regulation by histidine (3), and it is believed to be involved in regulation of expression of the histidine operon by direct interaction with DNA (4). Furthermore, this enzyme has been shown to interact with every regulatory signal known to affect expression of the histidine operon (5). The protein is composed of six identical polypeptide chains which contain about 310 amino acids each. Since ATP-phosphoribosyltransferase is the first gene product of the histidine operon (1,2), its amino acid sequence will aid in the elucidation and interpretation of the histidine operon DNA sequence. We have begun to determine the amino acid sequence of this protein and can now describe its amino terminal sequence.

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MATERIALS AND METHODS

ATP-phosphoribosyltransferase of Salmonella typhimurium was prepared by the method of Parsons and Koshland (6). Before sequence analysis it was carboxymethylated with [^{3}H]-iodoacetic acid, obtained from New England Nuclear.

The amino terminal sequence of the protein was determined by automated Edman degradation on a Beckman Model 890B sequencer. The DMBA (dimethylbenzylamine) program (7) and the DMAA (dimethylalylamine) program (8) were used in separate experiments. Identical results were obtained with both programs. PTH-derivatives were examined by gas chromatography on a Beckman GC-65 chromatograph, thin layer chromatography (9), and by identification of regenerated amino acids (10). Cysteines, which had been carboxymethylated with [^{3}H]-iodoacetic acid were detected by their radioactivity. Spot tests for tyrosine (11), histidine (11), and arginine (12) were also performed.

Tryptic peptides were produced by the method described earlier (13); and all manual sequencing methods used were those of Landon et al. (10).

RESULTS

ATP-phosphoribosyltransferase has been subjected to automated amino-terminal end group analysis. Identical results were obtained with the DMBA (7) and DMAA programs (8); for simplicity only results obtained with the former program are given here. Those residues identified, the method of identification, and the yield of PTH-derivative as measured by gas chromatography are given in Table I.

The amino-terminal sequence of ATP-phosphoribosyltransferase has been confirmed in part by the sequences of tryptic peptides isolated from this region (T. Rand-Meir and D. Piszkiewicz, unpublished results). The peptide corresponding to residues 1 through 6 has not yet been isolated; however, the peptide corresponding to residues 7 and 8 has been isolated: Leu, 0.97 (1); Arg, 1.02 (1), yield 61%. The peptide corresponding to residues 9 through 13 has also been isolated: Ile, 1.93 (2); Ala, 1.02 (1); Glx, 1.02 (1); Lys, 1.00 (1); yield, 58%. Its sequence was determined by four steps of the manual Edman degradation; PTH-derivatives were identified by both gas chromatography and thin layer chromatography.

DISCUSSION

ATP-phosphoribosyltransferase is the first gene product of the histidine operon of Salmonella typhimurium. It is believed to be an auto-regulatory protein in that it regulates its own biosynthesis, probably by

Table 1

Amino Terminal Sequence of ATP-phosphoribosyltransferase Determined by Automated Edman Degradation

Step	Amino Acid	Methods of Identification [a]	Yield (n moles) [b]
1	Met	GC, TLC, Reg	120
2	Leu	GC, TLC, Reg	139
3	Asp	GC, TLC, Reg	49
4	Asn	GC, TLC, Reg	
5	Thr	GC, TLC, Reg	
6	Arg	phenanthrenequinone	
7	Leu	GC, TLC, Reg	84
8	Arg	phenanthrenequinone	
9	Ile	GC, TLC, Reg	
10	Ala	GC, TLC, Reg	80
11	Ile	GC, TLC, Reg	
12	GLn	GC, Reg	
13	Lys	Reg	
14	---		
15	Gly	GC, Reg	
16	---		
17	Leu	GC, Reg	

[a] PTH-derivatives were identified by the following methods: gas chromatography, GC; thin layer chromatography, TLC (9); regeneration of amino acid by acid hydrolysis (10) and identification by paper electrophoresis at pH 1.9 or analysis, Reg; phenanthrenequinone spot test for arginine (12).

[b] The repetitive yield was calculated as 94%.

direct interaction with histidine operator DNA (4). The amino acid sequence of this protein is essential for determining where in the nucleic acid structure regulatory functions end and amino acid coding begins. By comparison of the amino-terminal sequence of ATP-phosphoribosyltransferase with the genetic code (14) it is possible to deduce a partial base sequence of its mRNA and the corresponding DNA (Table 2). Degeneracy of the genetic

Table 2

Amino Terminal Sequence of ATP-Phosphoribosyltransferase and Possible Nucleotide Sequences of its mRNA[a]

	1				5					10					15		
Amino Acid	MET-		LEU-		ASP-		ASN-		THR-	ARG-		LEU-	ARG-		ILE-	ALA-	
Base	AUG		UUU		GAU		AAU		ACU	CGU		UUU	CGU		AUU	GCU	
			C	C		C		C		C	A	C	C	C	A	C	
				A					A	A		A	A			A	
				G					G	G		G	G			G	

	ILE-	GLN-	LYS-		-GLY-		-LEU										
	AUU	CAA	AAA		GGU		UUU										
	C				C		C	C									
	A				A		A										
		G	G		G		G										

[a]Bases shown are present in one or more of the codons for the amino acids indicated; however, not all combinations of these bases are allowed by the genetic code to give the correct amino acid.

Table 3

Comparison of Amino Terminal Sequences of ATP-Phosphoribosyltransferase and Histidinol Dehydrogenase[a]

	1				5					10					15		
ATP-Phosphoribosyltransferase	Met-		Leu-		Asp-		Asn-		Thr-	Arg-		Leu-	Arg-		Ile-	Ala-	
	1						5								10		
Histidinol dehydrogenase	(Met)		Ser-		Phe-		Asn-		Thr	-		Leu	-		Ile-	Asp-	

	Ile-	Gln-	Lys-	X	-Gly-	X	-Leu										
							15										
	Asn-	Ser-	Cys-	Ser-	Pro-	Glu-	Gln-	Gln									

[a] N-formylmethionine is assumed to be at the amino terminus of histidinol dehydrogenase. Homologous amino acid residues are enclosed in rectangles.

code prevents determination of the complete base sequence. However, the identity of 23 of the first 39 bases (corresponding to 13 amino acids) and 26 of the first 51 bases (corresponding to 17 amino acids) can be deduced unambiguously.

One intriguing result of this project is the discovery that the amino-terminal sequence of ATP-phosphoribosyltransferase is homologous with the amino-terminal sequence of histidinol dehydrogenase (15) (Table 3). Histidinol dehydrogenase is the second gene product of the histidine operon and the terminal enzyme of the histidine biosynthetic pathway (1,2). Allowing for two insertions, five of the first nine residues of ATP-phosphoribosyltransferase align with the dehydrogenase (Table 3). We could find no other obvious homologies of the amino-terminal sequence of ATP-phosphoribosyltransferase with that of any other protein sequenced to date (16), nor could we find any obvious homologies in structure of tryptic peptides of ATP-phosphoribosyltransferase (T. Rand-Meir and D. Piszkiewicz, unpublished results) and histidinol dehydrogenase (15). It is possible that these homologous amino acid sequences reflect a common structure of the nucleic acids which code for them; however, the role of this common feature remains to be determined.

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