THE JOURNAL OF BIOLOGICAL CHEMISTRY

Vol. 265, No. 20, Issue of July 15, pp. 11576–11580, 1990
Printed in U.S.A.

Model Peptides Reveal Specificity of N^{α}-Acetyltransferase from *Saccharomyces cerevisiae**

(Received for publication, January 12, 1990)

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N^{α}-Acetylation is a major co-translational modification occurring at the α-NH_2 group of eukaryotic cytosolic proteins. In order to understand better the specificity of N^{α}-acetyltransferase, we used the purified enzyme from yeast (Lee, F.-J. S., Lin, L.-W., and Smith J. A. (1988) *J. Biol. Chem.* 263, 14948–14955) and synthetic peptides mimicking the NH_2 terminus of yeast and human proteins. Alcohol dehydrogenase I-(1–24) and 8 of the 19 synthetic analogues with substitutions at the NH_2-terminal residue were N^{α}-acetylated with varying efficiency. Penultimate amino acid substitutions, except for proline, had little influence on N^{α}-acetylation. Substitution of sequences from N^{α}-acetylated proteins into the yeast sequences which cannot be N^{α}-acetylated demonstrated that not only the first 3 NH_2-terminal residues but also more carboxyl-terminal residues were important for determining the specificity of N^{α}-acetyltransferase. Two other peptides mimicking yeast mitochondrial cytochrome *c* oxidase (subunit VI) and ATPase inhibitor, which are naturally non-acetylated, were efficiently acetylated. In addition, recombinant human alcohol dehydrogenase I and basic fibroblast growth factor, which are naturally N^{α}-acetylated, were not acetylated post-translationally.

EXPERIMENTAL PROCEDURES

Materials—Purified, recombinant proteins, human basic fibroblast growth factor (FGF), and human ADH I, expressed in yeast and *Escherichia coli*, respectively, were gifts from Dr. L. S. Cousens (Chiron Corporation) and from Professor Hans Jornvall (Karolinska Institute), respectively. The recombinant, human basic FGF consisted of a nearly equal mixture of the N^{α}-acetylated and non-acetylated forms of human basic FGF (Barr *et al.*, 1988). [^{3}H]Acetyl-CoA was from Amersham Corp., and unlabeled acetyl-CoA was from P-L Biochemicals. SP membrane was from Cuno Inc. Reagents for protein synthesis were from Applied Biosystems, except *t*-butoxycarbonyl amino acids were from Peninsula and solvents were from Anachem. Reagents and solvents for amino acid analysis and Ready-Solv EP scintillation mixture were from Beckman. Reagents for protein sequence analysis were from Applied Biosystems. All other chemicals were reagent grade or better.

Peptide Synthesis—Peptides were synthesized using the *t*-butoxycarbonyl chemistry and an Applied Biosystems 430A Peptide Synthesizer and characterized as described previously (Finnegan *et al.*, 1986). Protein sequence analyses of the ADH I-(1–24) peptide analogues were carried out using an Applied Biosystems 470A Protein Sequencer (Hewick *et al.*, 1981).

Purification of Yeast N^{α}-Acetyltransferase and N^{α}-Acetylation Assays—The purification of yeast N^{α}-acetyltransferase and the enzyme assays utilizing the various synthetic peptide and recombinant protein substrates were performed as described previously (Lee *et al.*, 1988). The data were reported as percent activity compared with the level of acetylation of a synthetic human adrenocortiotropic hormone peptide (ACTH), ACTH-(1–24), used previously as a control peptide for assaying for N^{α}-acetyltransferase (Woodford and Dixon, 1979).

TABLE I

Relative activity of yeast acetyltransferase for the N^{α}-acetylation of ADH I-(1–24) and substituted analogues

Substrate	Activity[a]
	%
ACTH (human) Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-Gly-Lys-Lys-Arg-Arg-Pro-Val-Lys-Val-Tyr-Pro	100 ± 5 (control)
Alcohol dehydrogenase I-(1–24) (yeast) Ser-Ile-Pro-Glu-Thr-Gln-Lys-Gly-Val-Ile-Phe-Tyr-Glu-Ser-His-Gly-Lys-Leu-Glu-Tyr-Lys-Asp-Ile-Pro	102 ± 5

First amino acid substitution		Second amino acid substitution	
Substrate	Activity	Substrate	Activity
	%		%
Ala - - - - - - -	0	- Ala - - - - - - -	148 ± 10
Arg - - - - - - -	0	- Arg - - - - - - -	102 ± 5
Asn - - - - - - -	0	- Asn - - - - - - -	116 ± 5
Asp - - - - - - -	0	- Asp - - - - - - -	150 ± 11
Cys - - - - - - -	0	- Cys - - - - - - -	123 ± 7
Gln - - - - - - -	0	- Gln - - - - - - -	125 ± 7
Glu - - - - - - -	0	- Glu - - - - - - -	111 ± 6
Gly - - - - - - -	23 ± 4	- Gly - - - - - - -	84 ± 5
His - - - - - - -	19 ± 3	- His - - - - - - -	119 ± 6
Ile - - - - - - -	0	- Leu - - - - - - -	117 ± 7
Leu - - - - - - -	0	- Lys - - - - - - -	113 ± 8
Lys - - - - - - -	0	- Met - - - - - - -	120 ± 7
Met - - - - - - -	15 ± 3	- Phe - - - - - - -	110 ± 6
Phe - - - - - - -	9 ± 3	- Pro - - - - - - -	0
Pro - - - - - - -	70 ± 5	- Ser - - - - - - -	122 ± 7
Thr - - - - - - -	103 ± 6	- Thr - - - - - - -	128 ± 8
Trp - - - - - - -	0	- Trp - - - - - - -	81 ± 5
Tyr - - - - - - -	20 ± 3	- Tyr - - - - - - -	129 ± 8
Val - - - - - - -	69 ± 4	- Val - - - - - - -	110 ± 7

[a] Data reported as mean activity ± S.D. (n = 3–5).

TABLE II

Relative activity of yeast acetyltransferase for the N^{α}-acetylation of superoxide dismutase, enolase, and various chimeric synthetic peptides

Substrate	Activity[a]
	%
ACTH-(1–24) Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-Gly-Lys-Lys-Arg-Arg-Pro-Val-Lys-Val-Tyr-Pro	100 ± 5 (control)
Superoxide dismutase-(1–24) (human) Ala-Thr-Lys-Ala-Val-Cys-Val-Leu-Lys-Gly-Asp-Gly-Pro-Val-Gln-Gly-Ser-Ile-Asn-Phe-Glu-Gln-Lys-Glu	86 ± 6
Superoxide dismutase-(1–24) (yeast) Val-Gln-Ala-Val-Ala-Val-Leu-Lys-Gly-Asp-Ala-Gly-Val-Ser-Gly-Val-Val-Lys-Phe-Glu-Gln-Ala-Ser-Glu	0
[Ala^{-1},Thr^{1}]Superoxide dismutase-(1–24) (yeast) Ala-Thr-Gln-Ala-Val-Ala-Val-Leu-Lys-Gly-Asp-Ala-Gly-Val-Ser-Gly-Val-Val-Lys-Phe-Glu-Gln-Ala-Ser	14 ± 4
[Ala^{-1},Thr^{1},Lys^{2}]Superoxide dismutase-(1–24) (yeast) Ala-Thr-Lys-Ala-Val-Ala-Val-Leu-Lys-Gly-Asp-Ala-Gly-Val-Ser-Gly-Val-Val-Lys-Phe-Glu-Gln-Ala-Ser	54 ± 5
[Ala^{-1},Thr^{1},Lys^{2},Ala^{3},Val^{4},Cys^{5}]Superoxide dismutase-(1–24) (yeast) Ala-Thr-Lys-Ala-Val-Cys-Val-Leu-Lys-Gly-Asp-Ala-Gly-Val-Ser-Gly-Val-Val-Lys-Phe-Glu-Gln-Ala-Ser	80 ± 5
Enolase-(1–24) (human) Ser-Ile-Leu-Lys-Ile-His-Ala-Arg-Glu-Ile-Phe-Asp-Ser-Arg-Gly-Asn-Pro-Thr-Val-Glu-Val-Asp-Leu-Phe	54 ± 5
Enolase-(1–24) (yeast) Ala-Val-Ser-Lys-Val-Tyr-Ala-Arg-Ser-Val-Tyr-Asp-Ser-Arg-Gly-Asn-Pro-Thr-Val-Glu-Val-Glu-Leu-Thr	0
[Ser^{1},Ile^{2}]Enolase-(1–24) (yeast) Ser-Ile-Ser-Lys-Val-Tyr-Ala-Arg-Ser-Val-Tyr-Asp-Ser-Arg-Gly-Asn-Pro-Thr-Val-Glu-Val-Glu-Leu-Thr	60 ± 7
[Thr^{2},Lys^{3}]Enolase-(1–24) Ala-Thr-Lys-Lys-Val-Tyr-Ala-Arg-Ser-Val-Tyr-Asp-Ser-Arg-Gly-Asn-Pro-Thr-Val-Glu-Val-Glu-Leu-Thr	20 ± 4
[Tyr^{2}]Enolase-(1–24) (yeast) Ala-Tyr-Ser-Lys-Val-Tyr-Ala-Arg-Ser-Val-Tyr-Asp-Ser-Arg-Gly-Asn-Pro-Thr-Val-Glu-Val-Glu-Leu-Thr	0
[Ala^{3}]Enolase-(1–24) (yeast) Ala-Val-Ala-Lys-Val-Tyr-Ala-Arg-Ser-Val-Tyr-Asp-Ser-Arg-Gly-Asn-Pro-Thr-Val-Glu-Val-Glu-Leu-Thr	0

[a] Data reported as mean activity ± S.D. (n = 3–5).

TABLE III

Relative activity of yeast acetyltransferase for the N^{α}-acetylation of synthetic peptides mimicking mitochondrial proteins

Substrate	Activity[a]
	%
ACTH-(1–24) Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-Gly-Lys-Lys-Arg-Arg-Pro-Val-Lys-Val-Tyr-Pro	100 ± 5 (control)
Cytochrome *c* oxidase-(1–24) (yeast, mitochondrial, subunit VI) Ser-Asp-Ala-His-Asp-Glu-Glu-Thr-Phe-Glu-Glu-Phe-Thr-Ala-Arg-Tyr-Glu-Lys-Glu-Phe-Asp-Glu-Ala-Tyr	60 ± 5
ATPase inhibitor (1–24) (yeast, mitochondrial) Ser-Glu-Gly-Ser-Thr-Gly-Thr-Pro-Arg-Gly-Ser-Gly-Ser-Glu-Asp-Ser-Phe-Val-Lys-Arg-Glu-Arg-Ala-Thr	76 ± 6

[a] Data reported as mean activity ± S.D. (n = 3–5).

N^{α}-Acetylation of synthetic peptides, mutagenized model proteins, and naturally occurring proteins

Peptide/protein substrates for N^{α}-acetyltransferases	N^{α}-Acetylated amino acid residue																				Reference
	Ala	Arg	Asn	Asp	Cys	Gln	Glu	Gly	His	Ile	Leu	Lys	Met	Phe	Pro	Ser	Thr	Trp	Tyr	Val	
Yeast alcohol dehydrogenase I-(1–24) and its 19 NH_2-terminally substituted peptide analogues	(−)[a]	−	−	−	−	−	−	+	+	−	−	−	+	+	+	+[b]	+	−	+	+	This work in Table I
Recombinant plant thaumatin and its 19 variants with NH_2-terminal substitution	+[c]	−	−	−	+/−	−	−	+	−	−	−	−	+	−	−	+	+	−	−	−	Huang *et al.* (1987)
Human hemoglobin β-chain variants	+	−	−	−	+	−	−	+	−	−	−	−	+	−	−	+	+	−	−	+/−	Boissel *et al.* (1988)
Various N^{α}-acetylated proteins (118)	+	ND[d]	+	+	ND	ND	+	+	ND	ND	ND	ND	+	ND	ND	+	+	ND	+	+	Driessen *et al.* (1985)
Various cytosolic, N^{α}-acetylated eukaryotic proteins (71)	+	ND	ND	+	ND	ND	+	+	ND	ND	ND	ND	+	ND	+	+	+	ND	ND	+	Flinta *et al.* (1986)
Side-chain type[e]	Alk	+	NH_2	−	SH	NH_2	−		+		Alk	+	SCH_3	Ar		OH	OH	Ar	Ar/OH	Alk	
Maximum side-chain length (Å)	1.51	7.40	3.68	3.74	2.83	4.93	4.97	−	4.64	3.91	3.90	6.37	5.46	5.10	2.40	2.41	2.54	6.64	6.43	2.55	Hirel *et al.* (1989)
Radius of gyration (Å)	0.77	2.38	1.45	1.43	1.22	1.75	1.77	−	1.78	1.56	1.54	2.08	1.80	1.90	1.25	1.08	1.24	2.21	2.13	1.29	Levitt (1976)
Mean solvent-accessible surface area (Å^2)	31.5	93.8	62.2	60.9	13.9	74.0	72.3	25.2	46.7	23.0	29.0	110	30.5	28.7	53.7	44.2	46.0	41.7	59.1	23.5	Rose *et al.* (1985a)
N-end rule category[f]	S^{I}	S^{I}	S^{III}	S^{II}	S^{II}	S^{III}	S^{II}	DS	S^{I}	DS	S^{I}	S^{I}	DS	S^{I}	DS	S^{I}	S^{I}	S^{I}	S^{I}	DS	Gonda *et al.* (1989)

[a] [Ala^1]ADH I-(1–24) was not acetylated by yeast N^{α}-acetyltransferase, although the purified enzyme is capable of N^{α}-acetylating the NH_2-terminal alanyl residue of human superoxide dismutase-(1–24) (see Table II) and of recombinant human superoxide dismutase protein (Hallewell *et al.*, 1987).

[b] NH_2-terminal residue of yeast alcohol dehydrogenase I.

[c] NH_2-terminal residue of recombinant thaumatin expressed in yeast.

[d] ND, N^{α}-acetylated residue not detected in any protein of this data base.

[e] Abbreviations: +, positively charged; −, negatively charged; Alk, alkyl; Ar, aromatic; OH, hydroxyl; SH, sulfhydryl; NH_2, amide; SCH_3, methylated thiol.

[f] Abbreviations: S^{x}, stabilizing residues (x = I (primary), II (secondary), III (tertiary)); DS, destabilizing residues.