PRELIMINARY NOTES

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The effect of a competitive inhibitor on the acetylation of tyrosyl and lysyl residues of staphylococcal nuclease

About five of the seven tyrosine residues of staphylococcal nuclease appear to be accessible to solvent, as judged by studies of ultraviolet absorption[1]. The binding of deoxythymidine 3',5'-diphosphate (pdTp) causes a large red shift in tyrosine absorption[1], displacement of the tyrosine titration curve[2], decreased tyrosyl exposure to neutral solvents[1] and quenching of tyrosyl fluorescence[3].

This report describes the reactivity of the tyrosyl residues of nuclease toward chemical modification by acetylimidazole, a reagent reported to be relatively specific for tyrosine acetylation[4–6]. Five tyrosyl and 9–10 lysyl residues are acetylated in the native enzyme with almost complete loss of deoxyribonuclease and ribonuclease activities; there is complete return of deoxyribonuclease and partial return of ribonuclease activities on O-deacetylation. Only 2 tyrosyl and 8–9 lysyl residues are acetylated in the presence of pdTp and Ca^{2+} and there is no loss of deoxyribonuclease activity; a small fall in ribonuclease activity occurs which is reversed on O-deacetylation.

Acetylation is performed by adding aliquots of 0.5 M acetylimidazole freshly dissolved in 10 mM Tris buffer (pH 7.5) to nuclease (2 mg/ml) dissolved in the same

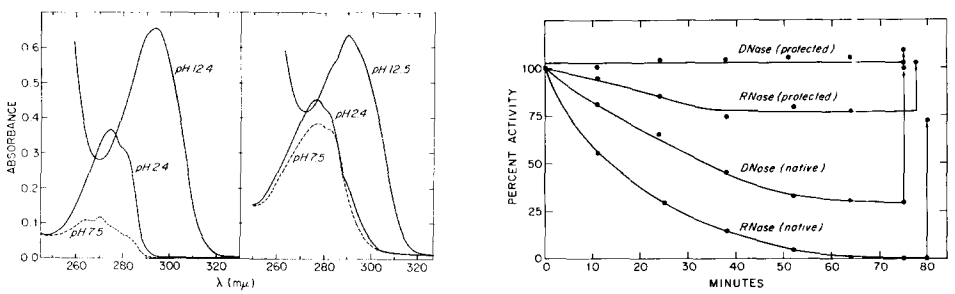

Fig. 1. Ultraviolet absorption spectra used to estimate the content of *O*-acetyl tyrosine. Right: A 1-ml sample of nuclease, acetylated with a 65-fold molar excess of acetylimidazole, in 5 mM Tris buffer (pH 7.5) was O-deacetylated by addition of 50 μl of 5 M NaOH (pH 12.5) and then acidified with 50 μl of 6 M HCl (pH 2.4). The protein concentration ($2.9 \cdot 10^{-5}$ M) was obtained from the spectrum in acid, which is identical to that of native nuclease. Left: 0.27 mM *N*-*O*-diacetyl tyrosine subjected to the same procedures as nuclease.

Fig. 2. Effect of acetylation with 50-fold molar excess of acetylimidazole on deoxyribonuclease (DNase) and ribonuclease (RNase) activities in the presence and absence of pdTp and $CaCl_2$. Arrows (at right) show change in activity occurring after alkaline deacetylation.

buffer. After 1 h (24°) the mixture is passed through Sephadex G-25 with 5 mM Tris buffer (pH 7.5). The *O*-acetyl tyrosine content is determined as described in Fig. 1. The $\Delta\varepsilon_M$ at 275 mμ for O-deacetylation of *N*-*O*-diacetyl tyrosine was 1150.

Five tyrosyl residues are readily acetylated in the native enzyme with con-

TABLE I

EFFECT OF NUCLEOTIDES AND Ca^{2+} ON THE ACETYLATION OF TYROSINE RESIDUES OF NUCLEASE

Nuclease (0.10 mM) was reacted with a 70-fold excess of acetylimidazole. Ca^{2+} when present was 10 mM.

Addition	*Nucleotide concn. (mM)*	*Deoxyribonuclease activity (%)*[7]		*Tyrosines acetylated*
		Acetylated	*Deacetylated*	
None		20	96	5.5
pdTp	0.70	40	100	4.2
Ca^{2+}		18	97	5.7
pdTp + Ca^{2+}	0.19	115	110	2.4
pdTp + Ca^{2+}	0.70	111	113	2.1
Urea (8 M)		6	80	7.3
pdTp + Ca^{2+} + urea (8 M)	0.70	10	81	7.1
CMP + Ca^{2+}	0.36	29	75	5.6

comitant loss of 80% of the deoxyribonuclease activity (Table I). If Ca^{2+} is present, pdTp prevents this loss of activity and protects 3 tyrosines from reacting. In 8 M urea all 7 tyrosyls are acetylated, and there is no protection by the nucleotide. A non-binding (CMP) nucleotide has no effect. Removal of the *O*-acetyl groups by alkaline treatment restores to normal the deoxyribonuclease activity (Table I), indicating that tyrosyl residues may be essential for catalytic integrity.

The fall in ribonuclease activity is more rapid and extensive than the fall in deoxyribonuclease activity (Fig. 2). Ribonuclease activity in the presence of pdTp falls by 25% whereas deoxyribonuclease activity is unchanged. Since this activity is restored on O-deacetylation, it may be that one or both of the two tyrosyls acetylated is involved in RNA hydrolysis. The ribonuclease activity of the enzyme, acetylated without nucleotide, is only partially restored on deacetylation, suggesting that a residue forming an alkaline-stable derivative is involved in RNA but not DNA hydrolysis.

TABLE II

ACETYL CONTENT OF NUCLEASE TREATED WITH ACETYLIMIDAZOLE

pdTp (0.60 mM) and $CaCl_2$ (10 mM) were added to the solution of native enzyme (0.17 mM).

Enzyme	*Molar excess of reagent*	*Moles/mole enzyme*						
		Before alkaline treatment			*After alkaline treatment*			
		Acetyl tyrosine	*[^{14}C]-Acetyl groups*	*% Deoxy-ribo-nuclease activity*	*Acetyl tyrosine*	*[^{14}C]-Acetyl groups*	*Lysine content**	*% Deoxy-ribo-nuclease activity*
Native	10	0.9	2.5	88	0	1.6		100
Protected	10	0.2	1.5	100	0	1.1		100
Native	50	3.0	9.8	48	0	7.3		100
Native	100	5.2	14.4	7	0	9.6	10.2	94
Protected	100	2.3	10.1	94	0	7.9	9.0	100

* Determined by deamination with nitrous acid, followed by acid hydrolysis and amino acid analysis.

Studies using [^{14}C]acetylimidazole reveal that the number of alkaline-labile acetyl groups is equal to the number of spectrally determined *O*-acetyl tyrosines (Table II). This excludes aliphatic hydroxyl acetylation. However, large amounts of alkaline-stable [^{14}C]acetyl groups are present. Acetyl histidine is probably not present since no loss of radioactivity occurs after 4 h at pH 2.0. It is likely that lysine accounts for the large content of [^{14}C]acetyl as suggested by studies of nitrous acid deamination (Table II).

The inhibitor, pdTp, appears to protect at least one lysine residue from acetylation (Table II). Further support for this is obtained from peptide maps of trypsin digests of nuclease treated with 10-, 20-, and 100-fold excess of [^{14}C]acetylimidazole, which show one much less intense radioactive peptide in the maps of samples acetylated in the presence of pdTp. Amino acid analysis, after elution of the peptide, suggests that the lysine residue at position 84 (ref. 10) is protected from acetylation.

It is of interest that nuclease has full deoxyribonuclease activity in spite of having 8–10 of its amino groups blocked. Moreover, such a modified nuclease does not differ significantly from the native enzyme in many catalytic properties, *i.e.*, pH optimum, optimum Ca^{2+} concentration, K_m and v_{max} for DNA, and inhibition by pdTp.

Laboratory of Chemical Biology,
National Institute of Arthritis
and Metabolic Diseases,
Bethesda, Md. 20014 (U.S.A.)

PEDRO CUATRECASAS
SARA FUCHS*
CHRISTIAN B. ANFINSEN

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* Visiting Scientist ,on leave from the Weizmann Institute of Science, Rehovoth, Israel.