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Application of mass spectrometry to enzyme inhibition studies

Identification of the amino-acid residue alkylated in an enzyme by an irreversible inhibitor is usually accomplished by comparison of the molar ratios of the amino-acids found in the native and inhibited enzyme. Reduction in the content of a specific amino-acid in the modified enzyme is evidence for alkylation of that residue (Lawson & Schramm, 1962; Schoellmann & Shaw, 1963; Gundlach, Stein & Moore, 1959). The alkylated residue behaves differently from the parent amino-acid on the ion-exchange column used in the analysis and can on occasions be detected on the scan and subsequently identified using a synthetic sample (Lawson & Schramm, 1962; Gundlach & others, 1959).

Amino-acid analysis is satisfactory for the detection of changes in the titres of histidine and methionine since these titres are usually low in proteins and loss of one residue in the alkylation is readily discernable. The predominance of serine and, in certain enzymes, lysine, limits the use of the method since the corresponding changes in these titres are within the limits of accuracy of the analysis. Furthermore, amino-acid analysis cannot be used with confidence to detect the site of alkylation where impure enzyme preparations are used, especially if a separation technique is used for removal of excess inhibitor since native and inhibited enzyme preparations may then have variable compositions (Al Shabibi, 1972).

Mass spectrometry is a technique which may be unambiguously applied to the identification of a minor constituent in a mixture of components by precise mass measurement of either its parent ion, M^+, or main fragmentation ion. We have shown using bovine ribonuclease and the irreversible inhibitor iodoacetate that mass spectrometry could be a useful new tool for the rapid identification of an alkylated amino-acid in a protein hydrolysate. We consider that this new method would be particularly useful for impure or degraded modified enzyme.

Ribonuclease is alkylated by iodoacetate at the active site, reaction occurring with histidine (His-119) at pH 5·5–6·0 and with lysine at pH 8·5–10·0 (Gundlach & others, 1959). Reaction between iodoacetate and ribonuclease at pH 8·5 was followed by

acid hydrolysis of the inhibited enzyme and conversion of the constitutent amino-acids to their ethyl esters. Mass spectrometry of the esterified mixture at 70 eV gave a scan which showed ions corresponding to all mass numbers up to mass 300, an indication that low molecular weight peptides were present in the mixture. Precise mass measurements of the ion at mass 187 gave a mass of 187·1444 ± 0·0009 for this ion which corresponded to the expected fragment ion (Biemann, Seibl & Gapp, 1961) $\overset{+}{H_2N} = CH\cdot(CH_2)_4\cdot NH\cdot CH_2\cdot CO_2Et$, (187·1446) derived from a ϵ-carboxy-methyl lysine residue. The product from the reaction conducted at pH 5·6 when treated in a similar manner showed an ion at mass 196 which at high resolution was resolved into two ions, mass 196·0974 ± 0·0010 ($C_{10}H_{14}NO_3$) and 196·1082 ± 0·0010 ($C_9H_{14}N_3O_2$, 196·1086). The latter ion corresponded to the expected ion, $EtO_2C\cdot CH_2C_3H_2N_2-CH_2\cdot CH=\overset{+}{N}H_2$, derived from N-carboxymethylhistidine.

A control sample of ribonuclease, which had not been reacted with iodoacetate, when treated in a similar manner showed the presence of only traces of the relevant ions.

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Chemical antagonism by iodine of the pharmacological activity of some sympathomimetic amines

In a series of experiments concerning the dependence of β-adrenoceptor-mediated effects on cellular metabolism, it was found that inhibition of pendular movements of the rabbit isolated intestine, produced by isoprenaline, was prevented by iodoacetate (Hall, 1971). When pyruvate was added to the organ bath in the continued presence of iodoacetate, the inhibitory response to isoprenaline was restored. These experiments were interpreted in support of the concept that β-adrenoceptor-mediated effects are dependent on the integrity of cellular glycolytic pathways. In a more recent series of experiments attempts were made to repeat the observation using a sample of iodoacetate from a different commercial source. The new sample was without effect on isoprenaline responses. Fresh samples of iodoacetate from both suppliers were then compared. Those from the one supplier always blocked responses to isoprenaline, while those from the other did not. Chemical analysis showed that the active samples of iodoacetate contained about 0·6% free iodine. The activity of the active iodoacetate solutions now appears to have been due to their content of free iodine.

Experiments were made on segments of rabbit small intestine and on the rat portal vein. All tissues were suspended in Krebs solution at 37°, and gassed with 5% CO_2