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Site specificity of protein glycation

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Summary. The rate of nonenzymatic glycation of a protein amino group is dependent upon a number of factors, such as the accessibility to glucose molecules in solution, and local acid-base catalysis of the rearrangement of the Schiff base that is formed initially. This is illustrated by a study of the site specificity of liver alcohol dehydrogenase, in which an attempt has been made to interpret the data in terms of the three-dimensional structure of the enzyme molecule.

Keywords: Amino acids – Protein – Enzyme – Glycation – Glycosylation

Causes of site specificity

Many of the factors that account for the site specificity of glycation of amino groups of proteins have been summarised by Baynes et al. [1].

The first stage of glycation (see Fig. 1) is the formation of the Schiff base, II, which is in equilibrium with the more stable glycosylamine, I. An important factor to be considered here is the accessibility of the amino group to glucose in solution. A second factor is the pK_A value. For example, if a terminal α-amino group and a lysyl ε-amino group have equal access to glucose, the former will react faster because it has a lower pK_A. Hence, compared with the ε-amino group, the α-amino group will give higher concentrations of I and II, and this will affect the rate of subsequent reactions.

Site specificity is governed mainly by factors that affect the second stage of glycation, which involves the conversion of the Schiff base (II) into the enol (III) by an Amadori rearrangement (see Fig. 1). In this rate-controlling step, a base catalyst is required to remove the proton from C-2 of II. Other tautomerization steps are subject to acid or base catalysis. It is therefore assumed that the overall rate of glycation of a protein amino group to form a 1-deoxyfructosyl derivative (IV and V; mainly the latter) depends upon its proximity to a group, B/BH^+, that behaves as an acid-base catalyst. Baynes et al. [1] have summarised reports from several laboratories indicating that lysine residues that are significantly glycated are adjacent to aspartate, glutamate, lysine or histidine in the primary sequence.

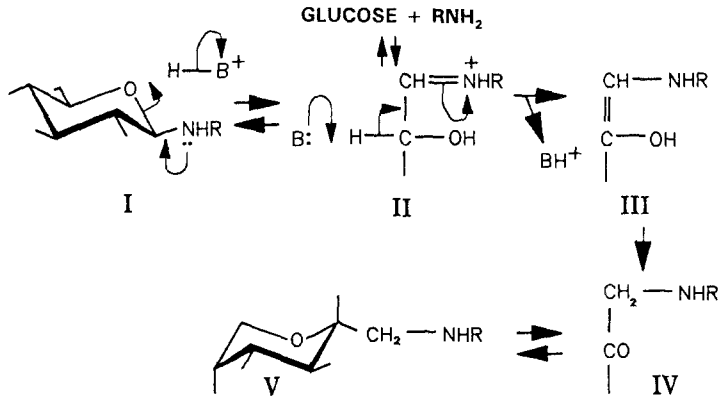

Fig. 1. Mechanism of glycation. *R* protein; *B* basic group

It has also been suggested [1] that phosphate or bicarbonate ions, or ligands such as diphosphoglycerate, bound to a specific area of a protein, accelerate glycation of nearby amino groups by acting as local acid-base catalysts.

In this talk I wish to discuss our work on the site specificity of glycation of liver alcohol dehydrogenase (LADH). It has proved to be a good model for examining some of the principles mentioned above, since the three-dimensional structure of the horse enzyme is known [2] and the human enzyme is structurally similar [3].

Site of in vivo glycation of human LADH

Class I isozymes of human LADH, which are characterised by a high affinity for ethanol, possess approximately 0.1 mol of lysine-attached hexose/mol of enzyme, as a result of in vivo glycation [4]. In order to identify the glycation sites, we treated LADH with borotritide to label the attached hexose, and then digested it with trypsin. Each of the six glycated peptides obtained gave a blank result at an Edman sequencing cycle corresponding to a lysyl residue. It was assumed that a hexitol-lysine residue occurred at this position in the sequence, and that it did not give an identifiable PTH derivative. Judging from the sequencing results [4] and the relative sizes of the RP-HPLC peaks in Fig. 2A, approximately 75% of in vivo glycation had occurred at Lys-231. 10% of it had occurred at Lys-10, and only 5% at each of lysines 39, 248 and 325. Glycation is therefore relatively specific to Lys-231. Human LADH resembles several other proteins, such as haemoglobin [5], serum albumin [6], Cu-Zn-superoxide dismutase [7] and ribonuclease A [8], in that the amino groups exhibit differential reactivity towards glucose.

Sites of in vivo glycation of horse LADH

Class I horse LADH (EE isozyme) was then studied. In this case the tryptic peptide HPLC profile (Fig. 2B) was similar to that of the human one (Fig. 2A), and 70–75% of the tritium label proved to be associated with Lys-231; less than

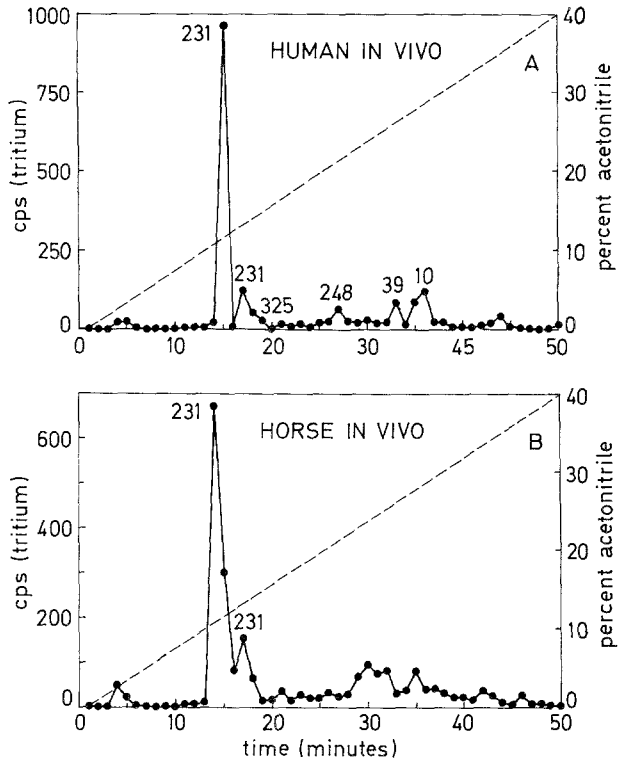

Fig. 2. Reverse-phase HPLC of tryptic peptides from (**A**) human and (**B**) horse LADH, glycated in vivo. Each peptide contained a single hexitol-lysine residue. The number refers to the position of the corresponding glycated lysine residue in the original LADH subunit

10% of it was associated with any one of the other glycated sites. Therefore the in vivo site specificity was very similar to that observed for human LADH.

Proposed catalytic role of histidine imidazole groups

Inspection of a computer image (Fig. 3) of the crystal structure of horse LADH revealed that Lys-231 possesses the only ε-amino group of the subunit that is within 9 Å of an imidazole group (His-348). Computer images of two other protein molecules were then examined. It was thus established that four of the main glycation sites of haemoglobin [5], βVal-1, αLys-16, αLys-66 and αLys-61, are close to imidazole groups. Also the principle glycation sites of ribonuclease A [8]. Lys-1 (α-amino group), Lys-7 and Lys-41, are close to imidazole groups (not illustrated here). We therefore propose that a nearby imidazole group accelerates glycation of an amino group by acid-base catalysis of the Amadori rearrangement of the Schiff base. This hypothesis is supported by the observation that small peptides containing a histidine residue are glycated rapidly [10]. The effectiveness of catalysis by protein imidazoles may be increased by nearby carboxylates of acidic residues.

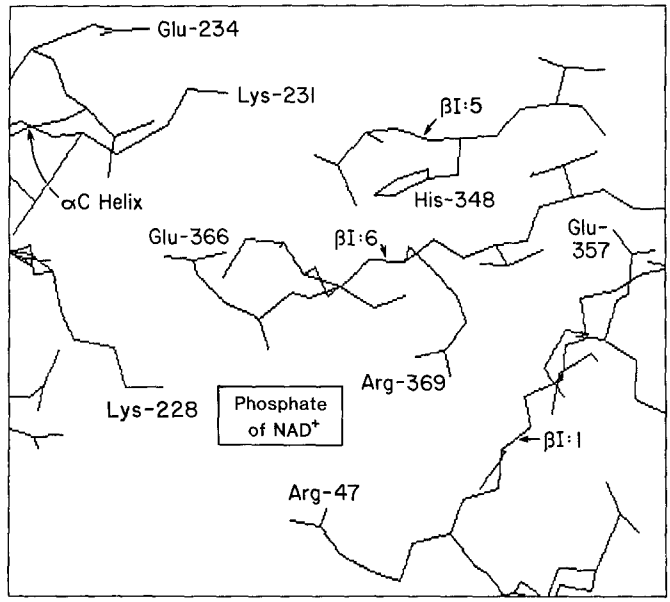

Fig. 3. Location of lysines 228 and 231 in horse ADH subunit. The computer image was generated from coordinates in [9]

Sites of glycation of horse LADH in vitro

On glycation with [^{14}C]glucose in phosphate buffer, followed by borohydride treatment and tryptic hydrolysis, the main radioactive peptide represented glycation at Lys-231. An additional peptide appeared in the HPLC profile, representing glycation of Lys-228. The presence of a large number of less abundant peptides in the HPLC profile showed that low levels of glycation had occurred at other sites. NAD^+ prevented glycation of Lys-228, but did not affect the reactivity of other groups. When phosphate in the medium was replaced by sulphate, the HPLC profile resembled that obtained from in vivo glycated enzyme (Fig. 2B), i.e. glycation had occurred at Lys-231, but not at Lys-228.

Conclusions

These results can be rationalised as follows. Lys-231 is glycated under all of the conditions employed, because its amino group is always accessible to glucose, and is close to the imidazole group of His-348, which acts as a catalyst. Glycation of Lys-228 in the absence of NAD^+ depends upon the presence of the phosphate ion. The latter birds to the cluster of three positive groups which form the binding site for the pyrophosphate group of NAD^+ (11) (see Figure 3); here it performs acid-base catalysis of glycation of Lys-228. When the same anion binding site is occupied by sulphate, the latter does not promote glycation at Lys-228, since it is completely ionised, and is not an acid-base catalyst. When NAD^+ is bound to LADH, it blocks access of Lys-228 to glucose molecules, accounting for the lack of glycation of Lys-228 in NAD^+-containing solutions in vitro, and in the liver in vivo. A relatively low level of glycation of other groups is promoted by phosphate ions that are less strongly bound to the enzyme.

We hope that we have convinced you that, in studying site specificity of glycation, it is useful to examine proteins with known three-dimensional structures. Work on LADH has allowed us to consider several factors, such as the accessibility of amino groups to glucose (which can be affected by ligand binding), and catalysis by (a) nearby groups that are part of the protein molecule, and (b) immobilised anions.

Acknowledgments

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