STUDIES OF THE REACTION BETWEEN PROTEINS AND REDUCING SUGARS IN THE "DRY" STATE

V. THE REACTIONS OF D-GALACTOSE, 2-DEOXY-D-GALACTOSE, D-GLUCOSAMINE AND N-ACETYL-D-GLUCOSAMINE WITH CASEIN

by

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INTRODUCTION

Since theories to explain the rearrangement and ultimate decomposition with browning of the first formed products (presumably N-glycosides) of the interaction of the free amino groups of proteins or amino acids with aldoses usually involve changes at carbon 2 of the carbohydrate, it seemed of interest to compare the reactions with casein of sugars modified at carbon 2 with those of the unmodified sugars. 2-deoxy-D-galactose has therefore been compared with galactose, and D-glucosamine (2-amino-D-glucose) and N-acetyl-D-glucosamine with glucose: the reaction of glucosamine with acetylated casein has also been investigated. Browning in some of these systems was found to be extremely rapid.

The reactions of the glucosamines with protein have an added interest because of the natural occurrence together of these substances in mucoids and glycoproteins.

METHODS

As in previous work[1,2,3,4] a 2% solution of casein at pH 6.3 containing 1.5 equivalents of the carbohydrate (on the casein amino-N basis) was freeze-dried, adjusted to a suitable moisture content by equilibration with an atmosphere of 70% relative humidity (R.H.), and stored at 37° C. The course of the reaction was followed by the loss of free amino-N, using the VAN SLYKE method with a reaction time of 30 minutes at 20° C[2], and by the increase in brown colour of the solid, using the LOVIBOND-SCHOFIELD Tintometer[1].

RESULTS

Reaction of D-galactose and 2-deoxy-D-galactose with casein

Fig. 1A shows that whereas galactose reacted with the free amino groups of casein at a rate very similar to that previously observed with glucose[2], 2-deoxy-galactose reacted with the amino groups considerably more slowly. The development of a brown discoloration however was very much more *rapid* with the modified than with the normal

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sugar (Fig. 1B), the marked lag or induction period which precedes the onset of browning in the galactose system (similar to that previously observed for glucose[2] and for lactose[5]) being absent (Fig. 2).

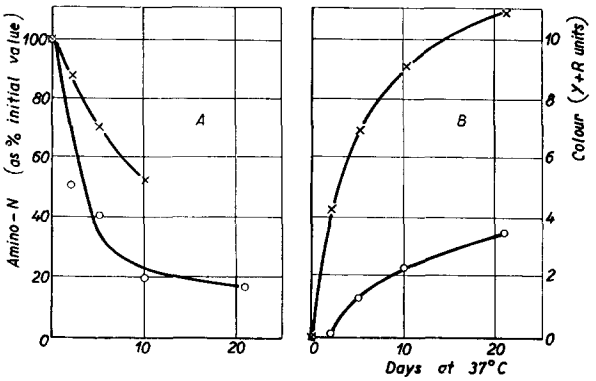

Fig. 1. Relative rates of reaction of galactose (O) and 2-deoxy-galactose (X) with casein at pH 6.3, 37° C and 72% relative humidity.

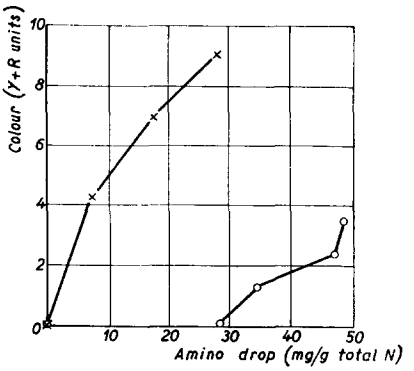

Fig. 2. Relationship between disappearance of amino-N and production of brown discoloration in the reactions of galactose (O) and 2-deoxy-galactose (X) with casein.

Reaction of D-glucosamine with casein

Amino-N. The results for glucosamine (Fig. 3A) showed that free amino-N disappeared from the glucosamine-casein system much more rapidly than from a glucose-casein system under similar conditions, and with approximately the same high temperature coefficient. The free amino-N content of the glucosamine-casein mixture continued to fall, particularly at 37° C, after the level corresponding to its glucosamine content had been reached, but ceased to fall before reaching the level corresponding to the protein amino-N content of the mixture. These observations suggested that the glucosamine amino group was taking part in some reaction, perhaps with the protein, but that the protein amino groups were *not* reacting with the reducing groups of the glucosamine.

Support for this hypothesis was provided by dialysis of the 28 hour glucosamine-casein reaction product, when a protein residue of approximately the same free amino-N content as the original casein was obtained. By reaction with fluorodinitrobenzene[6] followed by acid hydrolysis and chromatographic separation of the products this protein was shown to have most if not

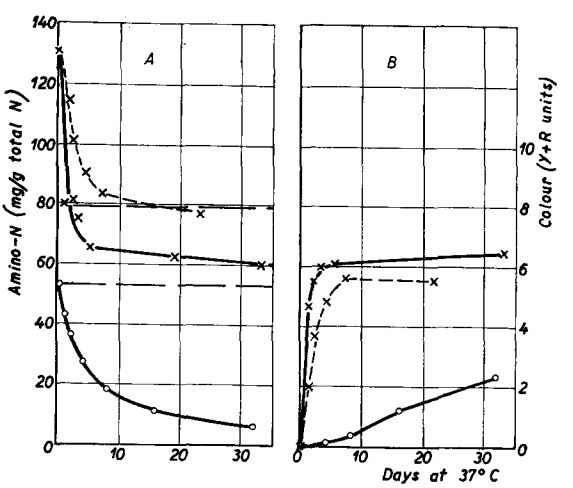

Fig. 3. Relative rates of reaction of glucose (O) and glucosamine (X) with casein at pH 6.3 and 70% relative humidity. Temperature 37° C. (—) or 25° C (---). The broken lines in Fig. 3A indicate the contributions of the casein (52) and glucosamine (79) to the initial amino-N content of the mixture (131).

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all of its lysine ε-amino groups still free, and to contain no recoverable glucosamine bound to the protein with its amino group free.

Colour. The results for colour production were also striking (Fig. 3B). They showed an immediate and extremely rapid development of a brown discoloration at both 37 and 25° C, which however ceased or became very slow as the amino-N reaction approached completion. This is in marked contrast to the glucose reaction under the same conditions, where browning only develops after a lag period (during which the drop in free amino-N is quite rapid) and continues to increase, although at a falling rate, after the amino-N reaction has virtually ceased.

Reaction of D-glucosamine with acetylated casein

Since the above results suggested that the free amino groups of casein do *not* react with the reducing group of glucosamine as they do with that of the unsubstituted sugars, the reaction between glucosamine and acetylated casein (in which practically all the free amino groups had been "blocked" by acetylation with very little other change in the protein molecule[4]) was studied.

The results (Fig. 4) showed that the rate of loss of free amino groups in the acetylated casein-glucosamine system was even greater than in the casein-glucosamine system: the rate of colour production was approximately the same, although the level reached was a little higher in the acetylated casein system.

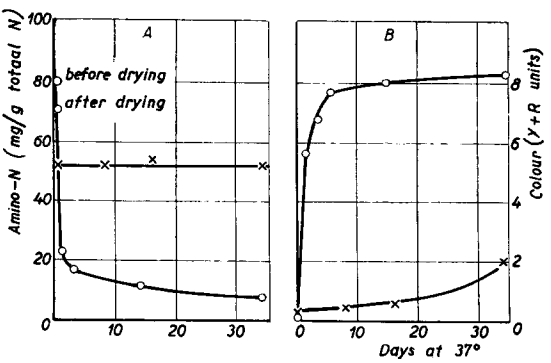

Fig. 4. Reaction at pH 6.3, 37° C and 70% relative humidity of glucosamine with acetylated casein (O), and of N-acetyl-glucosamine with casein (X).

These observations gave further support to the view that in the glucosamine-casein system the amino group of the glucosamine was reacting, but that the free amino groups of the protein were not.

Reaction of N-acetyl-D-glucosamine with casein

While the experiments with glucosamine and casein or acetylated casein can be considerated to have established the reactivity of the amino group and the probable inactivity of the reducing group of glucosamine towards casein, further confirmation of the absence of the normal aldose-protein amino group reaction was sought by a study of the *N*-acetyl glucosamine-casein system.

Glucosamine was converted to the mono-*N*-acetyl derivative by the method of ZUCKERKANDE AND MESSINER-KLEBERMASS[7], and freeze-dried with casein and stored at pH 6.3, 37° C and 70% R.H. as in previous experiments.

Neither the free amino-N content nor the colour of the mixture showed any appreciable change in periods considerably longer than those sufficient for practically complete reaction in systems containing free glucosamine (Fig. 4). After a prolonged lag period however the free amino-N values began to fall, resulting in a loss of about 10% after 110 days, and brown colour increased, reaching a figure of 5.3 yellow plus red units in the same period.

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DISCUSSION

The experiments reported in this paper show that both the hexose sugars modified by substitution of the hydroxyl group at carbon 2 which have been tested, namely 2-deoxy-galactose and 2-amino-glucose (glucosamine), produce browning and associated deleterious changes in the "dry" protein-sugar system much more rapidly than do the parent sugars galactose and glucose themselves. This observation appears to be in contrast to that of HURD AND KELSO[8], who found that whereas 3,4-dideoxyaldopentose (tetrahydropyran-2,3-diol) gave rise to a strong brown discoloration in its reaction with glycine in aqueous solution, 2,3,4-trideoxyaldopentose (tetrahydropyran-2-ol) did not, "thereby demonstrating the importance of the α-hydroxyaldehyde function in this reaction".

Since in the glucosamine reaction the destruction of amino groups and discoloration both develop to the same extent and even more rapidly when the casein amino groups have been blocked by acetylation, it is obvious that the amino groups involved are essentially those of the glucosamine. The high residual free amino-N and unimpaired lysine content of the casein recovered after reaction with glucosamine, and the comparative stability of the N-acetylated glucosamine-casein system show that the more usual type of reaction between the protein free amino groups and the reducing group of the carbohydrate is either absent or very slow in the casein-glucosamine system, presumably owing to some inhibiting action of the adjacent amino or acetyl-amino group.

The interaction between glucosamine amino groups and the protein appears to be even more effective than the usual protein amino group glucose reaction in causing browning, and insolubility of the protein also develops, but the chemical nature of the changes involved and the extent to which glucosamine becomes attached to the protein have not yet been determined.

ACKNOWLEDGEMENT

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SUMMARY

1. 2-deoxy-galactose and 2-amino-glucose (glucosamine) both produce much more rapid browning with casein at pH 6.3, 37° C and a water content corresponding to a relative humidity of 70% than do galactose or glucose.

2. The reaction of glucosamine with casein differs fundamentally from that of glucose. The glucosamine amino groups disappear rapidly and discoloration and insolubilityof the protein develop, but the protein amino groups appear not to be involved in the primary reacion.

RÉSUMÉ

1. Le 2-déoxy-galactose et le 2-amino-glucose (glucosamine) produisent, l'un et l'autre, un brunissement beaucoup plus rapide avec la caséine à pH 6.3, à 37° C, et à un contenu aqueux correspondant à 70% d'humidité relative, que ne le font le galactose ou le glucose.

2. La réaction de la glucosamine avec la caséine diffère essentiellement de celle du glucose. Les groupes aminés de la glucosamine disparaissent rapidement et il y a développement de décoloration et d'insolubilité de la protéine, mais les groupes aminés de la protéine, semble-t-il, ne sont pas impliqués dans la réaction primaire.

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ZUSAMMENFASSUNG

1. Sowohl 2-Deoxy-Galaktose als auch 2-Amino-Glukose (Glukosamin) verursacht mit Kasein, bei 37° C, pH 6.3 und einem Wassergehalt entsprechend 70% relativer Feuchtigkeit viel raschere Bräunung als Galaktose oder Glukose.

2. Die Reaktion des Glukosamins mit Kasein ist wesentlich anders als jene der Glukose. Die Aminogruppen des Glukosamins verschwinden rasch, und Verfärbung und Unlöslichkeit des Proteins entwickeln sich, aber die Aminogruppen des Proteins scheinen von der Primärreaktion nicht betroffen.

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