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Interaction of 2-Keto-3-deoxyglucose with Thiol- and Amino-groups

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The chemical interaction of 2-keto-3-deoxyglucose with various thiol and amino compounds has been studied. The glucose-derivative was found to be much less reactive than the typical ketoaldehyde methylglyoxal. In contrast to the latter compound, 2-keto-3-deoxyglucose reacted slowly with thiols like cysteamine and glutathione; it was not bound to the insoluble polythiol SH-Sephadex, and it did not interact with bovine serum albumin. 2-Keto-3-deoxyglucose was, however, bound to amino-Sephadex, but at a lower rate than methylglyoxal.

2-Keto-3-deoxyglucose probably exists exclusively in a 1,5-pyranose ring form, explaining its low reactivity.

Recently Szent-Györgyi and co-workers isolated from various animal and vegetable tissues a compound, "retine", which strongly retarded the growth of tumor cells.[1] Chemical analyses suggested that retine was a glyoxal derivative containing an alpha-ketoaldehyde group. The antimitotic effect of retine was proposed to be due to interactions with thiol groups essential for cell division[2] and protein synthesis.[3] The biological role of ketoaldehydes is not yet known. It has been suggested, however, that glyoxal derivatives may be important regulators of cell division, and that cancer cells lack these inhibitors.[4]

The chemical structure of one of the physiologically occurring ketoaldehydes has now been deduced by Fodor, Sachetto, Szent-Györgyi and Egyud.[5] The compound, 2-keto-3-deoxyglucose (3-deoxy-D-*erythro*-hexosulose), whose function is completely unknown, appears to be present in most cells in a very high concentration, *viz.* approximately 0.05 M.[6]** The object of the present investigation has been to study the chemical interaction of 2-keto-3-deoxyglucose with thiol and amino groups. A typical ketoaldehyde, methylglyoxal, was included in the study for comparison. The results show that 2-keto-3-deoxyglucose is very much less reactive than methylglyoxal, probably because the former compound exists mainly or exclusively as a 1,5-pyranose ring form.[7]

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** However, see note added in proof.

MATERIALS AND METHODS

2-Keto-3-deoxyglucose was prepared from glucose and glycine essentially as described by Anet.[8] The concentration of this compound was determined as described by Neuberg and Strauss.[9] This involved precipitation of the 2,4-dinitrophenylhydrazone and subsequent treatment with alcoholic potassium hydroxide to yield a blue colour which was read at 560 mμ. Methylglyoxal was obtained as a 40 % aqueous solution (Koch-Light, Ltd., England). A dilute solution (0.01 M) was passed through a column containing Dowex-1-bicarbonate in order to remove acidic contaminants. The exact molarity of the purified methylglyoxal solution was determined by titration with alkali and hydrogen peroxide,[10] or with the osazone method used for 2-keto-3-deoxyglucose.

Amino-Sephadex was prepared by aminization of Sephadex G-25 (Pharmacia AB., Sweden) with 2-aminoethyl hydrogen sulphate (Koch-Light, Ltd., England) as previously described.[11] The product contained 0.20—0.25 mmoles NH_2 per g of Sephadex. Thiolated Sephadex was prepared by treating amino-Sephadex with *N*-acetylhomocysteine thiolactone (Koch-Light, Ltd., England) as described earlier.[11,12] The product contained 0.15—0.20 mmoles SH per g of Sephadex.

Cysteamine, dimethylcysteamine, reduced glutathione and cysteine were purchased from Fluka AG., Buchs, Switzerland. Bovine serum albumin was obtained from Sigma Chemical Company, USA. All other chemicals used were commercial products of high purity.

RESULTS

In order to test if methylglyoxal and 2-keto-3-deoxyglucose would react similarly with thiols of low molecular weight, the experiment described in Fig. 1 was performed. The thiol and the ketoaldehyde were mixed in equivalent amounts, and the reactions were followed by measuring the optical density at 295 mμ after different time intervals. The results show that cysteamine, dimethylcysteamine, and reduced glutathione all reacted very rapidly with methylglyoxal, resulting in an instantaneous increase in the optical density at 295 mμ. This increase most likely reflected formation of hemithioacetals, since it is well known that these addition products are formed immediately upon mixing ketoaldehydes and thiols.[13,14]

Fig. 1 clearly shows that 2-keto-3-deoxyglucose interacted with the same thiols at much lower rates. Thus, both dimethylcysteamine, cysteamine, and glutathione reacted in the same and slow manner, resulting in a gradual increase in the optical density. Fig. 1 therefore indicated that 2-keto-3-deoxyglucose was less reactive than methylglyoxal.

The secondary reaction which took place between cysteamine and methylglyoxal, and which was complete in the course of 10 min (Fig. 1), most likely reflected formation of a thiazolidine. Such compounds are usually formed from the hemithioacetals in the course of a few minutes.[13,15,16]

A different method for comparing the reactivity of 2-keto-3-deoxyglucose and methylglyoxal would be to measure the rate of binding of the ketoaldehydes to an insoluble macromolecule containing reactive thiol groups on its surface. Recently such a macromolecule was synthesised in our laboratory, *viz.* SH-Sephadex.[11] This material consists merely of Sephadex particles (cross-linked dextran) with a number of reactive thiol groups on its surface.[17] Fig. 2 shows that methylglyoxal rapidly was bound to the thiolated Sephadex. The binding was faster at pH 8.4 than at lower pH values, indicating that ionization of the thiol group plays a certain role. Addition of arsenite to the

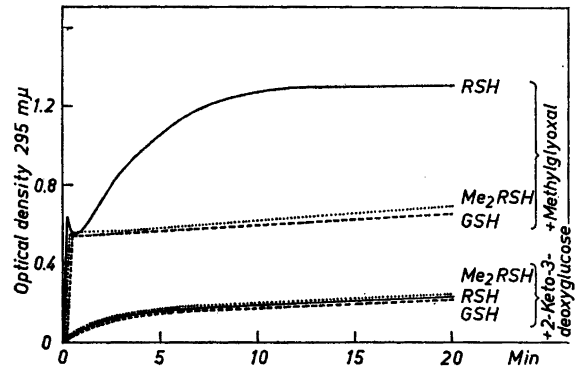

Fig. 1. Interaction of thiols with 2-keto-3-deoxyglucose and methylglyoxal at pH 7.4 and 20°. The thiol was either cysteamine (RSH), dimethylcysteamine (Me_2RSH), or reduced glutathione (GSH). Equimolecular amounts of the ketoaldehyde and the thiol were mixed. The optical density at 295 mμ was continuously recorded in a Zeiss RPQ 20A spectrophotometer. When 2-keto-3-deoxyglucose was tested the reaction mixture consisted of: 2-keto-3-deoxyglucose (0.015 M), the thiol (0.015 M), and phosphate buffer (0.1 M, pH 7.4). When methylglyoxal was tested the reaction mixture consisted of: methylglyoxal (0.005 M), the thiol (0.005 M), and phosphate buffer (0.1 M, pH 7.4).

"SH-Sephadex-ketoaldehydecomplex" immediately liberated the bound methylglyoxal. Also cysteine or cysteamine immediately eluted the ketoaldehyde. When thiols were used as eluting agents the methylglyoxal did not appear in the eluate in the free form, but as a thiazolidine.

Fig. 2 also clearly shows that 2-keto-3-deoxyglucose was not sufficiently reactive to become bound to the thiolated Sephadex.

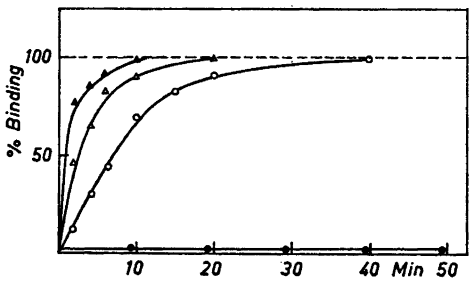

Fig. 2. Binding of 2-keto-3-deoxyglucose and methylglyoxal to SH-Sephadex at different pH. 2 g of dry, thiolated Sephadex was allowed to swell in 20 ml of 0.1 M phosphate buffers of different pH. 3 ml of 0.01 M ketoaldehyde was added to the SH-Sephadex suspension and mixed well using a magnetic stirrer. The stirring (at 20°) was interrupted at intervals, the SH-Sephadex particles allowed to settle, and aliquots (1 ml) of the supernatant fluid were withdrawn. The content of ketoaldehyde was subsequently determined as described in the text. The first aliquot, withdrawn immediately after mixing the ketoaldehyde and the SH-Sephadex, served as control. The difference between the control reading and the readings after different times of incubation expressed the amount of bound ketoaldehyde.

● 2-Keto-3-deoxyglucose at pH 4.8, 7.5, and 8.5, ○ methylglyoxal at pH 4.8, ▲ methylglyoxal at pH 7.5, △ methylglyoxal at pH 8.5.

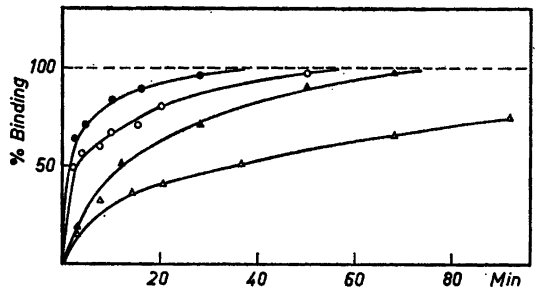

Fig. 3. Binding of 2-keto-3-deoxyglucose and methylglyoxal to NH_2-Sephadex at different pH. The experimental conditions were as described in Fig. 2, except that amino-Sephadex was used instead of thiolated Sephadex. At pH 4.5 no binding of either methylglyoxal or 2-keto-3-deoxyglucose occurred (not shown).
○ Methylglyoxal at pH 7.5, ● methylglyoxal at pH 8.9, △ 2-keto-3-deoxyglucose at pH 7.5, ▲ 2-keto-3-deoxyglucose at pH 8.9.

In Fig. 3 the ability of methylglyoxal and 2-keto-3-deoxyglucose to react with aminogroups was compared. Use was made of the insoluble macromolecule amino-Sephadex. This material, which is a precursor of SH-Sephadex, consists of Sephadex molecules in which some of the hydroxyl groups have been replaced with ethylamino groups. Fig. 3 demonstrates that both methyl-

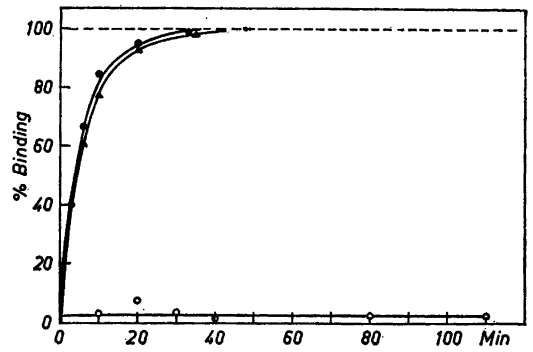

Fig. 4. Binding of 2-keto-3-deoxyglucose and methylglyoxal to albumin at pH 7.8 and 37°. The reaction mixture consisted of: Bovine serum albumin (5 ml of a 10 % solution in 0.2 M phosphate buffer) and 0.75 ml of 0.01 M ketoaldehyde. The final pH was 7.8 and the incubation was carried out at 37°. Aliquots (0.5 ml) of the reaction mixture were withdrawn at intervals and added to 2 ml of 10 % trichloroacetic acid. The first aliquot, withdrawn immediately after mixing the ketoaldehyde and the protein, served as control. The precipitated protein was removed by centrifugation. To an aliquot of the filtrate (1.5 ml) was added 0.1 ml of 2,4-dinitrophenylhydrazine reagent. The resulting osazone was washed and dissolved in ethanol-KOH as described in the text. The blue color was read at 560 mμ. The difference between the control reading and the readings obtained after different times of incubation, expressed the amount of protein-bound ketoaldehyde.
○ Albumin+2-keto-3-deoxyglucose, ● albumin+methylglyoxal, ▲ albumin+*p*-CMS (10^{-3} M)+methylglyoxal.

glyoxal and 2-keto-3-deoxyglucose interacted with amino-Sephadex, and that the reactions were faster at higher pH. At pH 4.0 no reaction occurred. Further, it is evident from the figure that methylglyoxal reacted more rapidly with amino-Sephadex than did 2-keto-3-deoxyglucose.

Concomitant with the binding of methylglyoxal to amino-Sephadex, the latter material gradually turned yellow as the reaction proceeded. This was probably due to formation of double bonds (Schiff bases) between the amino groups of the insoluble material and the ketoaldehyde. A similar color change was not observed in the case of 2-keto-3-deoxyglucose.

Displacement of the bound ketoaldehydes from the amino-Sephadex was extremely difficult to accomplish. Neither arsenite, cysteamine, dilute acids, dilute bases, or neutral buffers of high ionic strength had any eluting effect. Strong solutions (1—2 M) of hydrochloric acid and sodium hydroxide, however, slowly liberated and decomposed the ketoaldehyde.

It was thought desirable to investigate the interaction of 2-keto-3-deoxyglucose and methylglyoxal not only with an insoluble, but also with a soluble macromolecule containing amino groups and thiol groups. Bovine serum albumin was chosen for this purpose. Figs. 4 and 5 demonstrate that 2-keto-3-deoxyglucose was not bound to this protein at any pH. Methylglyoxal, on the other hand, was rapidly bound to albumin at pH above 7. Increasing the pH caused an increase in the rate of binding. Moreover, this reaction proceeded at the same rate whether or not the thiol group of the albumin was blocked by *p*-chloromercuri-phenylsulphonate, indicating that thiol groups were not involved. The reaction between methylglyoxal and albumin was accompanied by an increase in the optical density at 330 mμ. A pale yellow color therefore appeared similar to that observed when methylglyoxal reacted with amino-Sephadex. This suggested that methylglyoxal was bound to albumin *via* amino groups.

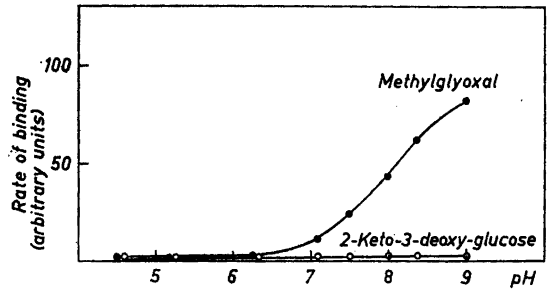

Fig. 5. Rate of binding of 2-keto-3-deoxyglucose and methylglyoxal to albumin at different pH. Bovine serum albumin was dissolved in phosphate buffers (0.2 M) of different pH. To 0.5 ml of the resulting 10 % albumin solutions was added 0.1 ml of 0.01 M ketoaldehyde. The pH of the final reaction mixture was determined in a pH-meter. After incubation at 20° for a definite period (60 min in the case of 2-keto-3-deoxyglucose, and 15 min in the case of methylglyoxal), 2 ml of 10 % trichloroacetic acid was added to the solutions. The control incubations consisted of the ketoaldehyde, trichloroacetic acid, and 10 % albumin solution added in the order stated and thoroughly mixed. The amount of protein-bound ketoaldehyde was then determined as described in Fig. 4.

DISCUSSION

Several authors have studied the interactions of ketoaldehydes with thiols. They have shown that a number of reactions may take place, depending upon the experimental conditions and the thiol in use.[13,14,18–22] For example, it is known that the ketoaldehyde methylglyoxal readily reacts with thiols like cysteine and cysteamine first forming hemithioacetals.[13,14] These thiols contain a primary amino group, and ring formation may therefore occur as a secondary reaction yielding a thiazolidine.[13,15,16] This compound is considerably more stable than the hemithioacetal.[13] Both the thiazolidine and the hemithioacetal, however, exist in equilibrium with the starting materials, and will consequently react more or less as free thiols towards typical SH-reagents.[13]

A ketoaldehyde may also react directly with NH_2 groups yielding simple addition compounds and Schiff bases.[23] Schiff bases may also arise due to rearrangement of certain thiazolidines (K. F. Nakken, personal communication).

From the results presented it is evident that 2-keto-3-deoxyglucose was much less reactive than the typical ketoaldehyde methylglyoxal. Thus, interaction of 2-keto-3-deoxyglucose with soluble thiols of low molecular weight was a slow process in comparison to the instantaneous reaction shown by methylglyoxal. Binding to the insoluble polythiol SH-Sephadex did not occur, in contrast to methylglyoxal which was bound in the course of a few minutes. On the other hand, 2-keto-3-deoxyglucose showed a definite affinity for the aminogroups present on the surface of amino-Sephadex. However, also in this case methylglyoxal reacted faster. The higher reactivity of the latter compound was also evident when the interaction with a typical protein was studied. Thus, methylglyoxal was rapidly bound to albumin, most likely *via* protein-amino groups, whereas 2-keto-3-deoxyglucose showed no reaction towards this protein. Both ketoaldehydes formed 2,4-dinitrophenylhydrazones in the cold. Also this simple reaction proceeded markedly slower with 2-keto-3-deoxyglucose than was the case with methylglyoxal.

As suggested by Anet [7] 2-keto-3-deoxyglucose probably exists exclusively in a 1,5-pyranose ring form, *i.e.* 2-keto-3-deoxyglucose will in its reactions behave more like a ketosugar than as an open chain ketoaldehyde. This probably explains why methylglyoxal is considerably more reactive than the glucose derivative. In view of the low reactivity of 2-keto-3-deoxyglucose it is also understandable that this compound apparently has little ability to prevent bacterial cell-division.[24] This contrasts methylglyoxal and other typical ketoaldehydes, which are known to inhibit the mitosis of *E. coli* in low concentrations.[25] The latter, highly reactive ketoaldehydes will probably react with a number of different protein-bound amino groups and thiol groups,[23] and will most likely lead to cell depletion of the amino acid cysteine thus preventing protein synthesis.[16] One might anticipate, on the other hand, that 2-keto-3-deoxyglucose because of its lower reactivity, most likely will react only with quite specific proteins, polypeptides, or other cellular constituents.

The present investigation demonstrating a comparatively low reactivity of 2-keto-3-deoxyglucose makes it unlikely that this compound in the free form is a regulator of mitotic activity. However, intracellular reactions leading to a complete opening of the 1,5-pyranose ring or displacement of the equilibrium in favour of the open chain form, *i.e.* in favour of a free ketoaldehyde group, would immediately make the molecule much more reactive.

Note added in proof. A recent paper by Otsuka and Egyud [26] indicates that 2-keto-3-deoxyglucose is not a physiologically occurring compound.

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