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Preliminary communication

3,7-Anhydro-2-azi-1,2-dideoxy-D-*glycero*-L-*manno*-octitol, a photoaffinity label for β-D-galactosidase from *E. coli*

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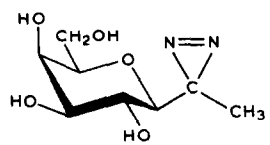
The active site of β-D-galactosidase from *E. coli* (EC 3.2.1.23) has been the target of affinity labelling experiments[1–9]. Two amino acids, possibly of relevance in enzymic catalysis, have been identified[6,9] by degradation of the covalently modified protein, and the character of the amino acids has been deduced[3] from the chemical stability of the introduced label. Affinity labelling is carried out usually with potential alkylating reagents which need properly positioned reactive partners at the substrate binding sites. With very reactive analogues[5], preliminary kinetic studies cannot be carried out because the binding sites become irreversibly blocked. Another disadvantage can be the chemical instability of the covalent modification, since the label may be lost by hydrolysis during protein fragmentation and work-up procedures.

Photoaffinity reagents normally do not have these disadvantages, since they are stable under ordinary incubation conditions and react neither with the buffer medium nor with the receptor protein. Therefore, binding studies can be carried out. On irradiation, the reagent yields a highly reactive intermediate, such as a carbene or nitrene, which indiscriminately reacts with the nearest group or groups, even with a hydrocarbon side-chain[10]. We have synthesised several sugar derivatives which are potential photoaffinity labels[11]. Especially useful for photochemical and steric reasons are compounds that carry a diazirine group, since they allow photoaffinity labelling to be carried out efficiently using low-energy u.v. light that causes no damage to the receptor protein. Introduction of a diazirine group does not excessively alter the bulk or polarity of the ligand and normally does not prevent binding[12,13]



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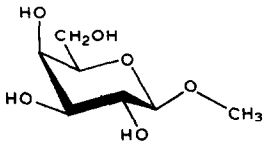

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0008-6215/87/$ 03.50

3,7-Anhydro-2-azi-1,2-dideoxy-D-*glycero*-L-*manno*-octitol[11] (**1**) sterically resembles methyl β-D-galactopyranoside (**2**), which is a substrate for β-D-galactosidase. The diazirine group in **1** is located in the same position as the glycosidic oxygen in **2**, which means that it will come into close contact with the group in the enzyme which normally initiates glycoside cleavage by protonating O-1. Moreover, **1** is a good competitive inhibitor of the hydrolysis of *o*-nitrophenyl β-D-galactopyranoside (oNPG) by β-D-galactosidase with an inhibition constant (K_i) of 0.75mM [a 0.05M NaK phosphate buffer (pH 6.8) containing mM $MgCl_2$ at 30° was used with zero, 0.23, 0.46, 0.69, 0.92, 1.38, and 1.84mM **1**, and determination of the *o*-nitrophenol released with an Eppendorf photometer at 405 nm]. Isopropyl 1-thio-β-D-galactopyranoside (IPTG)[14] and 2,6-anhydro-D-*glycero*-L-*manno*-heptitol[15,16] have K_i values of 0.087 and 19mM, respectively. The binding of a 3,7-anhydro-2-azi-1,2-dideoxyoctitol may not always be satisfactory, as shown[17] by the rather high K_i value of 80mM for 3,7-anhydro-2-azi-1,2-dideoxy-D-*glycero*-D-*gulo*-octitol and β-D-glucosidase from sweet almonds. When a solution (6 μg/mL) of β-D-galactosidase in the above buffer at 4° was irradiated in the presence of 7.5 and 15mM **1** for 10 min, using a Rayonet RPR 100 reactor equipped with 16 RPR 3500 Å lamps and filtering out light below 350 nm, the enzyme was irreversibly inactivated to extents of 29% and 39.4%, respectively. In the absence of **1**, this treatment had little effect on enzyme activity and, in the presence of the competitive inhibitor IPTG (85mM), the enzyme was protected from photodeactivation by **1**. There was no significant additional effect above 15mM **1**. This degree of deactivation under conditions of enzyme saturation is good considering the normally predominating intramolecular reactions which stabilise the carbene[18].

Apparently, the carbene derived from **1** on irradiation is in a position to react with a nearby group in the active site of the enzyme, thereby becoming covalently attached to the protein. On storage of a solution of the deactivated enzyme in buffer, there was no re-activation during 24 h, which reflects the stability of the chemical modification. Work is in progress on the photoaffinity labelling of β-D-galactosidase with radioactively labelled **1** in order to locate the position of photomodification of the enzyme.

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