# FRUCTOSE 1,6-BISPHOSPHATE ALDOLASE FROM LIVER: THE ABSOLUTE CONFIGURATION OF THE INTERMEDIATE CARBINOLAMINE

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## 1. Introduction

The classical studies of Rose et al. [1,2] on the stereochemistry of the aldolase reaction have shown that the enzyme controls the orientation of the aldehyde approaching the active site as well as which face (face si) of the enolate ion is attacked.

We complete here the description of the configuration of the enolate ion by proposing that the lysyl amino group of the active site [3] attacks the 'face si' of the C-2 group of fructose bisphosphate with the intermediate formation of the (2*R*)-carbinolamine.

## 2. Materials and methods

Fructose bisphosphate aldolase (D-fructose 1,6-bisphosphate D-glyceraldehyde 3-phosphate lyase, EC 4.1.2.13) from beef liver was prepared according to the procedure of Chappel et al. [4]. Only form II (spec. act. 0.8 I U/mg protein) was used in these experiments. Aldolase activity was measured spectrophotometrically [5]. Protein was measured assuming an absorbance of 0.89 at 280 nm for a 1 mg/ml solution of liver aldolase [6]. [U-$^{14}$C]Fructose bisphosphate was purchased from the Radiochemical Centre, Amersham, England.

$N^6$-β-Glycerollysine was prepared according to the procedure of Speck et al. [7]. $N^6$ (2-Deoxy-2-glucitol) L-lysine (glucitollysine) and $N^6$ (2-deoxy-2-mannitol) L-lysine (mannitollysine) were prepared by condensation of bromobutylhydantoin with either mannitolamine or glucitolamine. The procedure was the same as for glycerollysine except that condensation was performed in methanol.

Mannitollysine, glucitollysine and glycerollysine were separated by chromatography on a Dowex 50-X8 ($Na^+$-form) 200–400 mesh 1.1X80 cm column following the procedure of Moore and Stein [8].

The radioactive protein derivative was precipitated by the addition of 436 mg solid ammonium sulfate/ml of solution. The precipitate was dissolved in 1 ml of M NaCl, dialysed against 0.15 M NaCl and against water. Radioactive determinations were made in a Packard Tri-Carl scintillation counter in 10 ml of Bray solution [9]. Protein hydrolysis was performed at 110°C for 48 h in evacuated sealed tubes.

The hydrolysate was dried under reduced pressure in a rotary evaporator, dissolved and dried three times from water and finally dissolved in 2 ml of 0.1 M sodium citrate buffer, pH 3.42.

## 3. Results and discussion

Liver aldolase (9 mg) was treated for 7 min at 2°C with 0.64 mM [U-$^{14}$C]fructose bisphosphate (specific radioactivity 3100 cpm/nmol) and 17 mM $NaBH_4$ in the presence of 0.1 M sodium acetate buffer, pH 5.3. After treatment, catalytic activity was reduced to 30% and the radioactive substrate (192 000 cpm) was irreversibly bound to the enzyme.

The protein was hydrolysed in 6 N HCl and the hydrolysate was submitted to chromatography on Dowex 50-X8 ($Na^+$-form). Two radioactive peaks (fig.1) were eluted accounting for 12% and 68% respectively of the radioactivity (192 000 cpm) placed on the column. The first peak (23 000 cpm) was coincident with the authentic standard of glucitollysine and the second peak (130 000 cpm) was


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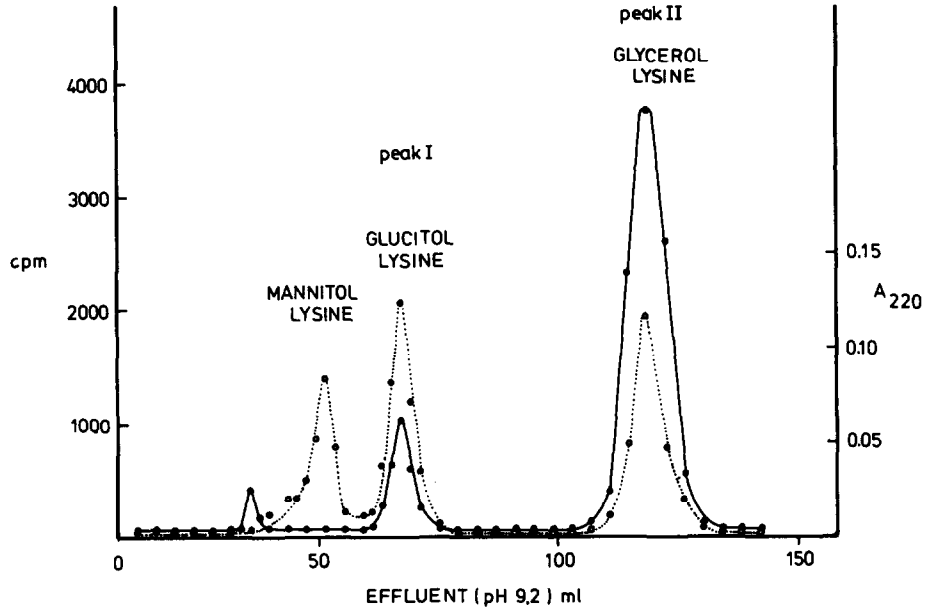
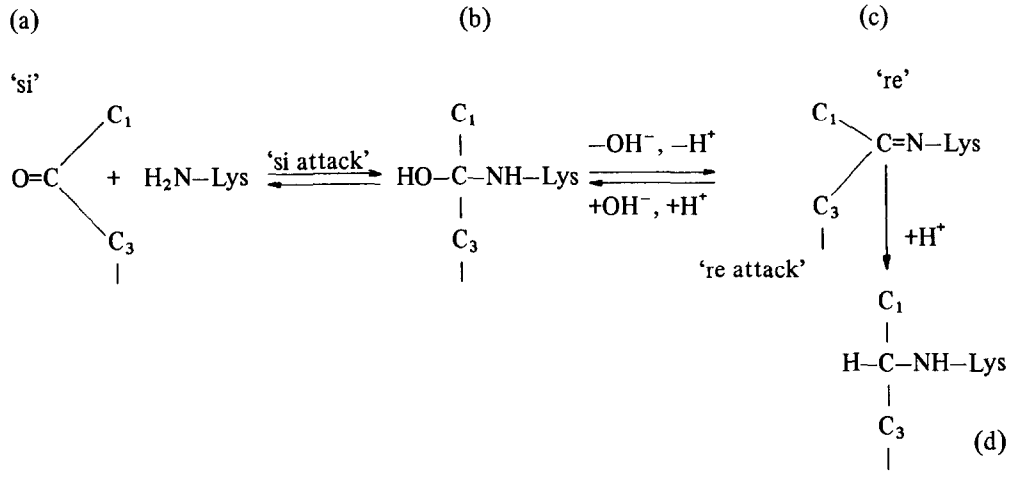



Fig.1. Column chromatography on Dowex 50-X8 of the $^{14}C$-labelled protein hydrolysate. To the protein hydrolysate, dissolved in 2 ml 0.1 M sodium citrate buffer, pH 3.42, were added as internal standards 0.3 nmol each of glucitollysine and mannitollysine and 0.6 nmol of glycerollysine. Elution was performed with 130 ml buffer, pH 3.42, followed by 47 ml 0.1 M sodium citrate buffer, pH 6.7 and finally by 0.1 M sodium bicarbonate buffer, pH 9.2. The column was operated at 25°C, flow rate was 6 ml/h, 2 ml fractions were collected. Analysis of effluent fractions was performed on 0.1 ml samples by following periodate consumption at 220 nm in 0.1 M phosphate buffer, pH 7.0. (o . . . . o). Radioactivity was measured on 0.5 ml samples. (•——•).

coincident with the authentic standard of glycerollysine. We have thus found that reduction with $NaBH_4$ of the aldolase–fructose bisphosphate complex is a stereospecific reaction. Of the two possible products, glucitollysine and mannitollysine, only glucitollysine (d) is in fact formed. This shows that $H^-$ attacks the 'face re' of the Schiff base C-2 (c).

Since it is likely that $H^-$ and $OH^-$ are coming from the same direction (i.e., from the medium) we propose



that $OH^-$ attacks the 'face re' of the Schiff base C-2 (c) yielding the (2*R*)-carbinolamine (b), also formed by reaction of the lysyl amino group of the active site with the 'face si' of the fructose bisphosphate C-2 (a). (2*R*)-Carbinolamine (b) could be also obtained by attack of the amino group of the active site on the C-2 of $\beta$ fructofuranose 1,6 bisphosphate (but not $\alpha$-fructofuranose 1,6 bisphosphate) provided the attack occurs from the side opposite to the ring oxygen.

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