SHORT COMMUNICATIONS

BBA 63245

Nature of enzyme–coenzyme interactions in liver alcohol dehydrogenase

It has been shown[1] that changes in absorption spectra occur on interaction of liver alcohol dehydrogenase with NADH, NAD^+ and adenosine diphosphate ribose (ADP-Rib). The binding of ADP-Rib is competitive with the coenzymes[2] and probably involves multiple sites of interaction with the protein[3]. The difference spectrum reported by THEORELL AND YONETANI[1] for the interaction of ADP-Rib and liver alcohol dehydrogenase has a positive peak at 281 mμ, crosses the zero axis at 273 mμ, has a negative shoulder at 262 mμ, and a negative trough at 255 mμ. The origin of this difference spectrum has not hitherto been elucidated.

We have measured the difference spectrum obtained by changing the environment of ADP-Rib from pH 6.5 to 1.8 and this is shown in Fig. 1. This difference spectrum has a peak at 283 mμ and crosses the zero axis at 271 mμ, showing considerable similarity with the difference spectrum obtained on interaction of ADP-Rib with liver alcohol dehydrogenase. The negative shoulder at 262 mμ obtained for the

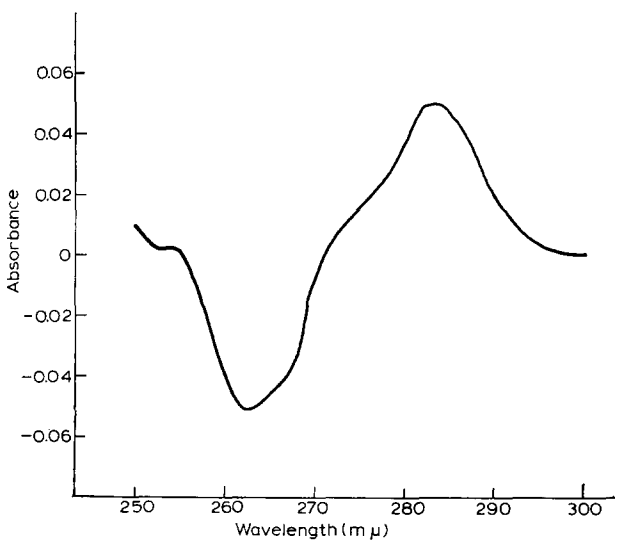

Fig. 1. Difference spectrum of ADP-Rib obtained by altering the environmental pH from 6.5 to 1.8. A matched pair of split compartment cells having a path length on each side of the septum of 0.5 cm were filled by pipetting 1.0 ml of ADP-Rib solution (100 μM in aqueous 0.02 M NaCl adjusted to pH 6.5) into one compartment and 1.0 ml of HCl solution (aqueous, pH 1.7, approximately 0.02 M) into the other compartment of each cell. To establish the base line the cells were scanned against one another from 320 mμ to 250 mμ using the 0–0.1 slidewire on the Cary Model 14 spectrophotometer. The contents of the cell in the sample compartment were then mixed and the difference spectrum recorded directly. Finally the contents of the reference cell were mixed and the spectrum of the two mixed cells determined to ensure that the base line was reproduced. The pH of the solutions was determined before and after mixing on a Radiometer type TTT1a pH meter.

Abbreviation: ADP-Rib, adenosine diphosphate ribose.

enzyme corresponds to the negative trough at 263 mμ shown in Fig. 1. The negative trough at 255 mμ obtained with the enzyme and ADP-Rib corresponds to the shoulder at 254 mμ in Fig. 1. Quantitatively, the magnitude of the spectral changes in the model system are very similar to those obtained for the enzyme in the region 280–285 mμ.

It is clear that there is a remarkable similarity between Fig. 1 and the difference spectra for liver alcohol dehydrogenase and ADP-Rib. This similarity is even more marked for liver alcohol dehydrogenase and NAD^+ *plus* caprate[1] except that with this latter difference spectrum there is an additional shoulder at 299 mμ, which might well be due to a conformational change in the protein.

It appears, therefore, that interaction of the adenine ring with liver alcohol dehydrogenase produces similar spectral changes to the protonation of the ring. It is noteworthy that the difference spectrum in Fig. 1 has a shoulder at approximately 275 mμ. It is possible that the peak at 283 mμ and the shoulder at 275 mμ are due to contributions from the different tautomers protonated at N-1 and N-3 of the 6-aminopurine ring, respectively. This shoulder is also present in the difference spectrum of the enzyme and NAD^+ *plus* caprate although it is not present in the difference spectrum of the enzyme and ADP-Rib. For ADP-Rib it is possible that the ratio of the tautomeric forms produced by the protein is different from that produced by acid.

Although it is not possible to define the exact nature of the protein side chain, the fact that the binding constant of ADP-Rib and liver alcohol dehydrogenase varies very little between pH 6 and pH 9 excludes carboxyl and imidazole side chains. Tyrosine is excluded because additional spectral changes to those observed would be expected. The group of the protein which is involved is probably, therefore, an arginine, lysine or cysteine side chain in the acid form.

We wish to thank the Science Research Council for a personal grant to one of us (R.F.) and the Wellcome Trust for a grant for the purchase of a Cary Model 14 spectrophotometer.

Department of Biochemistry,
University College,
London (Great Britain)

R. F. FISHER
A. C. HAINE
A. P. MATHIAS
B. R. RABIN

1 H. THEORELL AND T. YONETANI, *Arch. Biochem. Biophys.*, 106 (1964) 252.
2 T. YONETANI, *Acta Chem. Scand.*, 17 (1963) S 96.
3 H. THEORELL, *Bull. Soc. Chim. Biol.*, 46 (1964) 1533.

Received December 14th, 1966