Ternary Complex Formation of Fatty Acids and Fatty Acid Amides with Horse Liver Alcohol Dehydrogenase-Coenzyme Complexes

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In 1955 Theorell *et al.*[1] concluded from reaction velocity constant measurements that formate reacts with the ADH—DPN ** complex but not with ADH—DPNH and suggested that it enters into the binding site for ethanol in ADH—DPN. More recent studies by Theorell and Winer [2] have shown that while formate has a small effect on D_{red}, it exerts a very strong effect on D_{ox} and suggested that the effect of formate depends on the formation of a ternary ADH—DPN-formate complex.

Since the fluorometric method appeared to offer a very rapid procedure for scanning the effects of potential inhibitors on both D_{ox} and D_{red} it was decided to investigate the effects of other saturated fatty acids. All fatty acids studied (C_1—C_{20}) form ternary complexes with ADH—DPN and compete with the ethanol binding site. It is possible to calculate the dissociation constants describing the effects of ADH-fatty acid, $K_{E,I}$, ADH—DPN-fatty acid, $K_{EO,I}$, and ADH-fatty acid-DPN, $K_{EI,O}$. The formation of fluorescent ternary complexes of ADH—DPNH-amides is also reported and it is suggested that these complexes, just as the fluorescent lactic dehydrogenase-DPNH-anion complexes recently described by Winer and Schwert [3], are similar in structure to the activated complex involved in the conversion of ADH—DPNH-aldehyde to ADH—DPN-alcohol.

Experimental. Fluorometric measurements of emission spectra and the calculation of dissociation constants have been previously described [2,4] and were made at 23.5°C in 0.1 ionic strength phosphate buffer, pH 7.03. Pure crystalline ADH was prepared as described earlier [3]. DPN and DPNH were commercial products from the Sigma Chemical Company. Fatty acids, C_1—C_8, were reagent grade chemicals further purified by distillation under suitable pressures; C_{10}—C_{20} acids were gifts of Professor E. Stenhagen and solubilized after lyophilization of the potassium salts. Amides were Eastman Kodak products.

Discussion and results. Long chain saturated fatty acids exhibit very strong ternary complex formation with ADH—DPN. The dissociation constant for the ternary complex, ADH—DPN-fatty acid, $K_{EO,I}$, decreases from 2 800 μM for formate to 0.7 μM for C_{15} fatty acid. While the dissociation constant for fatty acid and ADH, $K_{E,I}$, decreases from 40 000 μM for formate to 6 μM for C_{15} acid, the dissociation constant reflecting the effect of DPN on the ADH-fatty acid complex, $K_{EI,O}$, is rather constant at about 5 μM to C_{11} acid and then increases to 2—7 times this value from C_{11}—C_{15}. The ability of the fatty acids to form complexes of the type EI and EIO disappears abruptly from C_{16} and upwards but the dissociation constant reflecting the formation of ternary complex, $K_{EO,I}$, remains constant at about 25 μM from C_{16}—C_{20}. The formation of ADH—DPN-fatty acid results in a further quenching of the ADH—DPN emission spectra measured at 350 mμ when activated at 289 mμ. The acids show no effect on the ADH—DPNH emission spectra when measured and activated at the same wave lengths. From initial velocity measurements of the enzymatic reaction obtained kinetically, the acids compete with ethanol for the same binding site.

The corresponding fatty acid amides form fluorescent ternary complexes with ADH—DPNH but not with ADH—DPN. A representative emission spectrum with acetamide in shown in Fig. 1. The curve labelled ADH—DPNH-acetamide was obtained when acetamide at a final concentration of 0.2 M was added to ADH and DPNH present at the concentrations used for the other curves. While the wave length of maximum emission for ADH—DPNH is shifted to 450 mμ, the wave length for the ternary complex, ADH—DPNH-acetamide, is shifted to 440 mμ.

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** Abbreviations. ADH—DPN and ADH—DPNH, ½ molecule of horse liver alcohol dehydrogenase (E) and oxidized (O) or reduced (R) diphosphopyridine nucleotide, respectively; D_{ox}, the dissociation constant of ADH—DPN; D_{red}, the dissociation constant of ADH—DPNH; I, fatty acid or fatty acid amide.

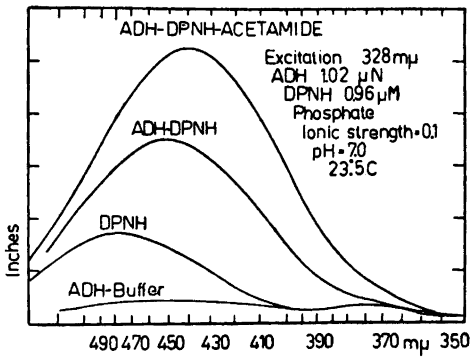

Fig. 1.

Similar wave length shifts have been observed with the lactic dehydrogenase-DPNH-carboxylic acid ternary complexes [3]. The lower amides, through *iso*butyramide, fluoresce more intensely than the ADH—DPNH complex, but higher molecular weight amides, though actually quenching, to varying extents, the fluorescence of ADH—DPNH, still exhibit the wave length shift. By analogy with kinetic experiments with formate [1], it is presumed that the very strong ternary complex formation observed with the longer chain amides has its effect on the "off" velocity of the ternary complex. The dissociation constants of the ternary complex, ADH—DPNH-amide, $K_{ER,I}$, and the ADH-amide complex, $K_{E,I}$, vary from 5 000 μM and 50 000 μM, respectively, for acetamide to 11 μM and 160 μM, respectively, for *n*-hexamide. $K_{EI,R}$ varies from 0.05 μM for acetamide to 0.02 μM for *n*-hexamide. From emission spectral data at 350 mμ the amides show a further quenching of the ADH—DPNH fluorescence when the ternary complex is formed. No effect, however, is seen with the ADH—DPN complex. As indicated by initial velocity measurements, the amides compete for the aldehyde binding site.

Ternary complex formation with the longer chain amides and acids is a logical consequence of the substrate specificity studies on liver ADH with different aldehydes and alcohols recently reported by one of us (A.D.W.) [6]. If it is considered that aldehydes and amides, and alcohols and acids, in this system, function as structural analogs, there is strong suggestion that the ADH—DPNH-amide and ADH—DPN-fatty acid complexes, in which no net reaction can occur, represent abortive complexes formed between ADH, DPNH, aldehyde and ADH, DPN, alcohol.

The complete data will be given in a forthcoming paper in this journal.

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Isolation of Palustric Acid from the Oleoresins of North-European Pine and Spruce by Partition Chromatography

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Partition-chromatographic analyses of the resins of North-European (Scotch) pine, *Pinus silvestris*, and (Norway) spruce, *Picea abies*, have shown that these contain palustric acid. Palustric acid was discovered in 1954 by Loeblich, Baldwin and Lawrence in the gum and wood rosins of two American pine species, *Pinus palustris* and *Pinus caribaea* [1]. The acid has subsequently been isolated from the rosin of a further American pine, *Pinus elliotti*, and from gum rosin, wood rosin and tall oil rosin isolated from this species [2]. The acid has also been found as a component in tall oil rosin from North-European pine (Bruun and Gåsland [3,4]), but has not yet been isolated from the natural rosins of North-European tree species or other non-Ameri-