Short Communications

SC 2231

The apparent high reactivity of some amino groups of bovine serum albumin

In the course of preparing dinitrophenylated proteins containing a small number of DNP groups/mole it was observed that some of the amino groups of bovine serum albumin reacted far more rapidly with fluorodinitrobenzene than did those of a number of other proteins. The product showed a typical ε-DNP-lysine spectrum with a maximum at 360 mμ and a shoulder at about 410 mμ. When the reaction was followed spectrophotometrically the upper curve shown in Fig. 1 was obtained. From this it was clear that the initial rapid formation of DNP-amino groups slowed down after a few minutes and that the reaction continued for at least 15 min longer at a constant slow rate, comparable with that of FDNB with chymotrypsinogen or lysozyme. In order to estimate approximately the number of groups involved in the rapid reaction, the initial rate was corrected for the non-specific reaction using the rate constant determined from the later part of the curve. The resulting curve approached a limiting absorbancy of about 0.5. The theoretical figure for two DNP groups/mole bovine serum albumin is 0.52. Assuming that the remaining 58 amino groups are about equally reactive, an approximate enhancement of reactivity of the two groups of 500-fold was calculated from the initial and final slopes of the original curve, allowing for the decreased concentration of FDNB at the end of the reaction. This apparent high reactivity of the two amino groups could be due to either: (a) a direct effect on their reactivity of some unspecified nature; (b) a lowering of their pK so that they were predominantly in the free-base form in 0.1 M $NaHCO_3$ (pH 8.3); (c) an increase in the local concentration of FDNB in the neighbourhood of the amino group due to adsorption on a non-polar binding site. The last explanation, involving

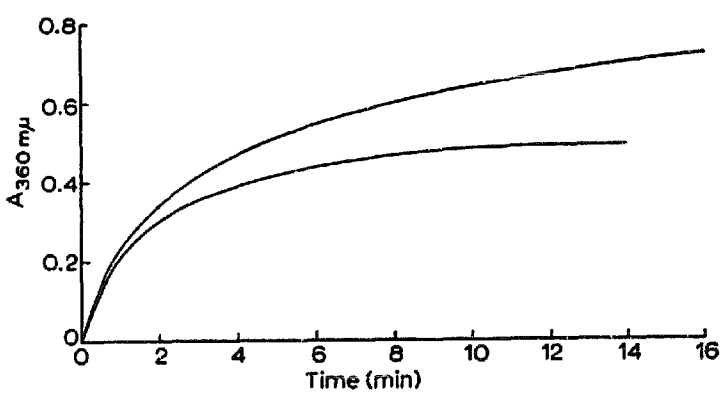

Fig. 1. Time course of reaction of FDNB with bovine serum albumin. 0.029 mM albumin (Armour, crystalline) (1.9 mg/ml) and 0.21 mM FDNB in 0.1 M $NaHCO_3$. The reaction was followed at 360 mμ in 0.5-cm cuvettes using a Unicam SP 700 recording spectrophotometer. The reference cell contained the 0.21 mM FDNB in 0.1 M $NaHCO_3$. Upper curve, experimental; lower curve, corrected for non-specific reaction (see text).

the close proximity of a cationic ammonium group to a non-polar region would accord well with a number of theories of the binding of non-polar anions by bovine serum albumin, which postulate the presence of such sites[1]. The following experiments support this hypothesis. The rate was considerably reduced by low concentrations of

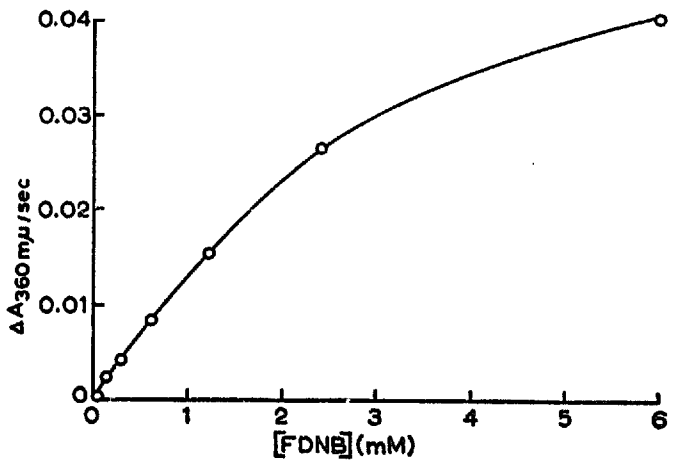

Fig. 2. Effect of FDNB concentration on initial velocity of reaction with bovine serum albumin. 0.11 mM albumin in 0.1 M $NaHCO_3$. The reactions were initiated by adding 2–50 μl of an alcoholic solution of FDNB, contained in a Carlsberg constriction pipette, to 2 ml of albumin solution in a 1-cm cuvette. The slight inhibitory effect of the alcohol on the reaction did not affect the general conclusions.

oleate and caproate. The reaction with 0.015 mM serum albumin was 30% inhibited by 0.03 mM oleate or 0.45 mM caproate and 70% inhibited by 0.08 mM oleate. In view of this inhibitory activity of fatty acid anions on the reaction it should be remembered that commercial preparations of bovine serum albumin may contain small amounts of firmly bound fatty acid[1], and that if this were removed more "reactive" amino groups might be unmasked. It is also of interest that *N*-acetyl-L-tryptophan (0.02 mM) which is firmly bound (K = 0.005 mM), probably to the terminal α-NH_2 group[2], had no effect on the rate. If the hypothesis of a non-polar binding site for FDNB is correct, then the relation between initial rate of reaction and FDNB concentration should not be linear but should follow a Michaelis-Menten type of relationship with the rate approaching a maximum value as the binding sites become saturated. The results in Fig. 2 show this to be qualitatively correct. A plot of 1/[FDNB] against $1/v$ gave a straight line, from which a K_m of about 5 mM and a V_{max} of 0.034 sec^{-1} could be calculated.

Further experiments are clearly necessary before the relation between the anion binding sites and the reactive amino groups can be regarded as firmly established, but it appears likely that it should be possible to label 2 or possibly 3 of these sites specifically using FDNB.

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[2] R. H. MCMENAMY AND J. L. ONCLEY, *J. Biol. Chem.*, 233 (1958) 1436.

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