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INHIBITION OF CHICKEN ERYTHROCYTE AMP DEAMINASE BY TETRAIODOFLUORESCEIN COMPOUNDS

MASATAKA YOSHINO[a] and YASUHIKO KAWAMURA[b]

[a]*Department of Biochemistry, Yokohama City University School of Medicine, Yokohama 232, and* [b]*Central Laboratory for Clinical Investigation, Nagoya University Hospital, Nagoya 466 (Japan)*

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Summary

A kinetic study has been performed on the inhibition of the chicken erythrocyte AMP deaminase (AMP aminohydrolase, EC 3.5.4.6) reaction by tetraiodofluorescein and Rose Bengal. These dyes inhibited the enzyme by decreasing its affinity for the substrate without affecting the maximum velocity. Kinetic analysis has shown the inhibition constants for tetraiodofluorescein and Rose Bengal to be 350 and 55 μM, respectively, and the presence of 4 binding sites of the enzyme for the inhibitors per enzyme molecule. These results suggest that the fluorescein dyes mimic the AMP binding at the catalytic center of the enzyme, which can be formed by the "dinucleotide fold".

The importance of AMP deaminase (AMP aminohydrolase, EC 3.5.4.6) in the purine nucleotide cycle [1,2], interconversion of adenine, inosine and guanine nucleotides [3—6] and stabilization of adenylate energy charge [7] in various tissues makes this enzyme an interesting subject for enzymological and physicochemical studies from the regulatory point of view. In previous papers we have reported some regulatory properties of AMP deaminase from various tissues [8—12], and allosteric inhibition of the chicken erythrocyte enzyme by blue dextran, which forms a complex with the enzyme [13]. Blue Dextran chromophore [14] and the dye tetraiodofluorescein [15] have been shown to bind to dehydrogenases or kinases at a position coincident with that of the adenosine portion of NAD or ATP, and appears to be common inhibitors of these enzymes. It is, thus, reasonable to assume that tetraiodofluorescein dyes are specific inhibitors of AMP deaminase, which also react at the same site of the Blue Dextran chromophore, and this assumption was verified in the present paper. The adenosine binding

sites of AMP deaminase was suggested to be formed by the "dinucleotide fold".

AMP deaminase was purified from chicken erythrocytes as described previously [8]. The enzyme activity was measured by estimating the production of ammonia colorimetrically using Nessler's reagent [11].

The effect of various fluorescein compounds on the activity of AMP deaminase was examined. The enzyme was inhibited by tetraiodofluorescein and Rose Bengal (tetraiodotetrachlorofluorescein) (Eastman Kodak), which have halogen atoms in their xanthene rings. However, of the xanthene derivatives tested, the following dyes without halogen atoms showed no effects: xanthene, xanthone, xanthydrol, 3′,6′-dichlorofluoran (fluorescein chloride), 2′,7′-dichlorofluorescein and fluorescein sodium.

Plots of reaction velocity as a function of AMP concentration gave a sigmoid curve, as demonstrated previously [10]. With the addition of tetraiodofluorescein or Rose Bengal the AMP saturation curve became more sigmoidal in shape, suggesting these ligands to be allosteric inhibitors (Fig. 1)

The effect of increasing concentrations of Rose Bengal and tetraiodofluorescein on the enzyme activity was examined. Plots of the reaction velocity against inhibitor concentration gave sigmoid curves, suggesting the presence of cooperativity with respect to the binding of the inhibitors, tetraiodofluorescein and Rose Bengal. The cooperativity of inhibitor binding was enhanced by increasing the AMP concentration. This allosteric system lends itself to the convenient method of analysis described by Blangy et al. [16] using a function called the "quotient function" $\bar{Q}$, which is the ratio of the amount of the enzyme existing in the R state to that in the T state. We may write

$$\bar{Q} = \frac{\bar{R}}{1-\bar{R}} = \frac{v}{V'-v} = C \cdot \frac{1}{(1+\beta)^n}$$

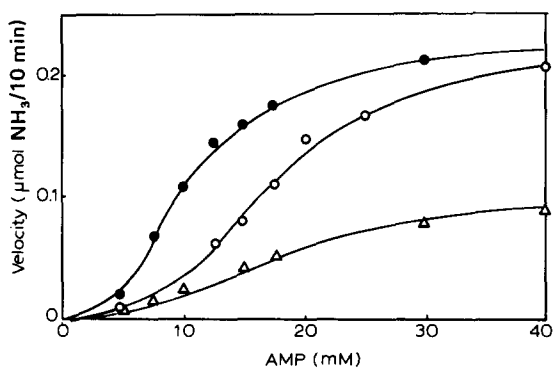

Fig.1. Effect of AMP concentration on the velocity of the AMP deaminase reaction in the absence or presence of fluorescein dyes. The reaction mixture contained 40 mM Tris-HCl buffer (pH 7.1), 0.8 mg/ml bovine serum albumin, various concentrations of AMP and fluorescein dyes and the enzyme in a final volume of 0.25 ml. The reaction was carried out at 37°C for 10 min and was stopped by the addition of 0.1 ml Nessler's reagent. The resulting color was measured at 430 nm in a final volume of 1 ml. The activity was determined in the absence (•) or presence of 0.06 mM (○) Rose Bengal and 0.3 mM tetraiodofluorescein (△).

where $\bar{R}$ represents the fraction of the enzyme molecules in the R conformation, and v is the velocity obtained in the presence of the inhibitor. V' is the maximum velocity which can be reached in the presence of a given concentration of the substrate, if the protein is entirely in the R conformation, and β is the normalized concentration of the inhibitor, that is, $[I]/K_T$; K_T is the microscopic dissociation constant of the inhibitor for the T state of the enzyme. C is the constant. It is, therefore, convenient to plot $\sqrt[n]{(V'-v)/v}$ against tetraiodofluorescein or Rose Bengal concentration, assuming that there are 4 independent sites for these dyes per molecule of the enzyme in the T conformation. As shown in Fig. 2, all the inhibition curves were converted to straight lines converging on the abscissa at the same point, $-K_T$ (fluorescein dyes). This suggests that there are indeed 4 independent sites for these dyes per molecule. From these data, K_T for tetraiodofluorescein and Rose Bengal was calculated to be 350 and 55 μM, respectively. If a number of sites other than 4 is assumed for these dyes, the functions deviate from linearity and do not meet on the abscissa at the same point. Thus, the most probable number of sites for fluorescein dyes is 4.

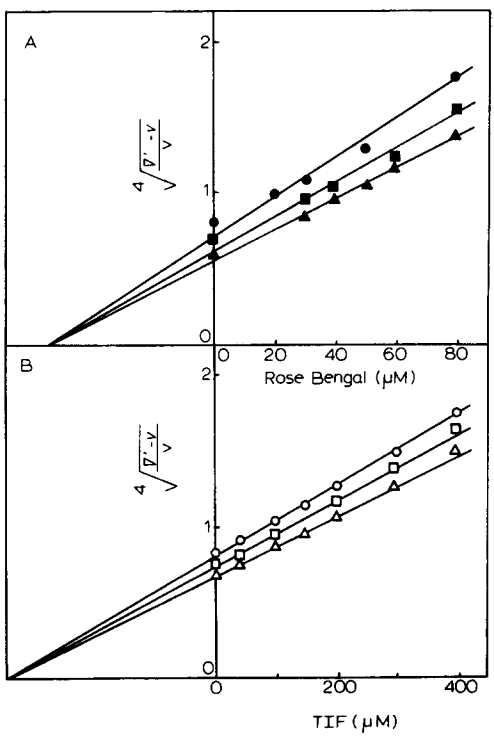

Fig.2. Effect of Rose Bengal and tetraiodofluorescein (TIF) concentration on the velocity of the AMP deaminase reaction at various concentrations of AMP. The reaction mixture was similar to that in Fig. 1. The data were expressed as variation of the "quotient function" Q with respect to the inhibition by fluorescein dyes assuming four binding sites for the dyes. (A) Inhibition by Rose Bengal. (B) Inhibition by tetraiodofluorescein. The concentrations of AMP were as follows: ● and ○, 13.5 mM; ■ and □, 15.0 mM; ▲ and △, 17.5 mM.

A number of organic dyes have recently proven useful in studying the interaction of ligands with proteins. Of particular interest is the finding on the specific interaction of fluorescein dyes [15] and Blue Dextran chromophore [14] with dehydrogenases or kinases. Blue Dextran has been reported to bind and to inhibit several enzymes with remarkably high affinity. Extensive studies on Blue Dextran chromatography performed by Thompson et al. [14] have revealed that Blue Dextran chromophore binds to the NAD-sites or the "dinucleotide fold" of dehydrogenases. This chromatography has been, thus, applicable to rapid screening of a wide range of proteins for the occurrence of the dinucleotide fold. Tetraiodofluorescein and its derivative, Rose Bengal have also been shown to be an adenosine analogue and to bind effectively to several dehydrogenases and kinases [15,17—20]. X-ray crystallographic studies on the interaction of lactate dehydrogenase (EC 1.1.1.27) with the fluorescein dye [15] have shown that tetraiodofluorescein binds to the adenosine binding site of the coenzyme binding domain in the enzyme. In the previous paper we have reported that Blue Dextran inhibits chicken erythrocyte AMP deaminase in an allosteric manner, and presented some evidence for the formation of a complex between Blue Dextran and the enzyme. These data prompted us to assume that tetraiodofluorescein or Rose Bengal could inhibit AMP deaminase, which interacts with Blue Dextran chromophore, by mimicing the adenosine of AMP. As demonstrated in this paper, only two fluorescein dyes containing halogen atoms in their xanthene rings, tetraiodofluorescein and Rose Bengal, inhibited AMP deaminase in an allosteric manner: kinetic analysis has revealed that the number of binding sites for the fluorescein dyes is 4, which is identical with that of the active sites of the enzyme [10]. It is reasonable to assume that tetraiodofluorescein and Rose Bengal can interact with the enzyme at the adenosine binding sites which are formed by the "dinucleotide fold": the fold is commonly observed in dehydrogenases and some kinases.

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