Essential —SH Groups in Liver Ketohehexokinase

PREPARATIONS of liver ketohehexokinase (ATP: D-fructose 1-phosphotransferase) phosphorylating fructose at C-1 have been obtained mainly by Leuthardt and Testa^{1,2}, Staub and Vestling³, Hers⁴, Kuyper⁵ and Parks⁶. Recently, Cadenas and Sols⁷ obtained a preparation from intestinal mucosa. The importance of the —SH groups of the enzyme has not been investigated, except by Kuyper, whose results were rather negative⁵.

Our ketohehexokinase preparation was obtained from beef liver, according to Parks's method, omitting the second fractionation with methanol, introducing small modifications and lyophilizing for better conservation. This preparation phosphorylates 11 μ moles of fructose/mg protein nitrogen in 20 min, under the conditions set up by Hers⁴. It also phosphorylates sorbose at a velocity of 57 per cent of that of fructose. The Michaelis constant for fructose is 4.6×10^{-4} M.

With this preparation we have tested for —SH groups with four reagents.

The *p*-chloromercuribenzoate is a strong inhibitor of the ketohehexokinase. At 8×10^{-5} M concentration, the whole enzymatic activity disappears. This inhibition is partially reverted by cysteine 2×10^{-4} M and the recuperation reaches up to 90 per cent with cysteine 2×10^{-3} M. If the unbound inhibitor is removed by dialysis, the same effect is observed.

N-ethylmaleimide, another effective —SH reagent, is also a powerful inhibitor of the enzyme, as is shown in Fig. 1.

Fig. 1. Inhibition of ketohehexokinase by *N*-ethylmaleimide, *o*-iodosobenzoate and *p*-chloromercuribenzoate. Reaction mixtures: 0.5 ml. enzyme solution with or without inhibitor, 0.1 ml. fructose 0.4 per cent, 0.1 ml. ATP 0.05 M, 0.1 ml. MgAc₂ 0.05 M, 0.3 ml. KAc 4 M, 0.1 ml. NaF 0.5 M, at 37° C, 20 min. ■, *o*-Iodosobenzoate; ▲, *N*-ethylmaleimide; ●, *p*-chloromercuribenzoate

o-Iodosobenzoate as well inhibits the enzyme. The inhibition is about 50 per cent at 6×10^{-5} M and reaches 100 per cent at 2.5×10^{-4} M. Cysteine does not produce reactivation even at a concentration of 10^{-2} M.

It has been reported⁵ that iodoacetate does not inhibit ketohehexokinase. However, if the enzyme solution is previously maintained in the presence of 0.02 M iodoacetate at 37° C for some time, the enzymatic activity becomes clearly inhibited. This inhibition increases with the time of previous incubation.

If iodoacetic acid is recrystallized several times, some diminution of the inhibitory effect is observed. However, the moniodoacetate action cannot be attributed to the possible presence of iodine. In fact, iodine is an inhibitor of the enzyme, but only at concentrations higher than 10^{-5} N, which cannot be found when the recrystallized moniodoacetic acid is used. The inhibitory effect of iodoacetic acid is not due to the possible decomposition products formed from it during the time of previous incubation, nor can it be attributed to a thermal inactivation of the enzyme. Neither is the pH appreciably modified during the previous incubation.

These results seem to offer sufficient evidence for the presence of essential —SH groups in the ketohehexokinase. The behaviour with moniodoacetate can be explained by a lower reactivity between the sulphhydryl groups and this inhibitor.

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Isolation of a Polar Aldosterone-like Substance from the Urine and Adrenal Incubates of Patients with Arterial Hypertension

A CONJECTURED role of disordered sodium-retaining function of the adrenal cortex in the genesis of essential hypertension has been largely founded on indirect evidence^{1,2}, including the rationale of low-sodium intake^{3,4} and of natriuretic agents^{5,6} in therapy; and on direct steroid measurements^{7,8}. These latter have shown hyper-excretion of the mineralocorticoid, aldosterone (11 β ,21-dihydroxy-20-oxo-pregn-4-ene-18-al), and its major metabolite, tetrahydroaldosterone (3 α ,11 β ,21-trihydroxy-20-oxo-5 β -pregnane-18-al), in uncomplicated as well as complicated forms of this disease. These observations have favoured the hypothesis that essential hypertension may represent, in effect, a special form of hyperadrenocorticism. Correlation of normal aldosterone regulation with the renin-angiotensin mechanism in the kidney^{9,10} has further focused attention on possible regulatory abnormalities of aldosterone in the pathogenesis of arterial hypertension.

Investigations here of mineralocorticoid function in essential hypertensives have led to the isolation from urine and adrenal incubates of an uncharacterized substance, designated as X_2 , which may be chemically and physiologically related to aldosterone but differing from aldosterone in that it is chromatographically more highly polar (? hydroxylated) in character. The isolation of this substance, and of three other highly polar metabolites (X_1 , THX_1 and THX_2), occurred fortuitously as a result of the application of reversed-phase partition chromatography¹¹ to the concurrent measurement of aldosterone and tetrahydroaldosterone in urine. This chromatographic modality has been considered of particular value for the separation of relatively polar C_{19} and C_{21} steroids which, because of retarded mobilities, are poorly resolved in conventional liquid-liquid systems. Our interest in this unknown substance was intensified by its absence in the urine of normal subjects under basal conditions. The results of our biosynthetic studies have also stimulated this research. An adrenal site of biogenesis of X_2 has been suggested by the incorporation of ¹⁴C-acetate into this fraction during *in vitro* incubations of surviving adrenals surgically removed from one patient with essential hypertension (3.0 μ g/g tissue/3 h) and from another patient with Cushing's syndrome due to adrenocortical hyperplasia (2.1 μ g/g tissue/3 h). A relationship of the excretion of this metabolite to stress and to cardiovascular disease was early seen on its discovery in the urine of a subject who, while being studied by us during the course of intense psychological stress, developed sinus tachycardia, mild hypertension, and retinal hemorrhages.

The mobilities of this metabolite isolated from the urine of hypertensives by reversed-phase partition chromato-