mycelium of *Heliscus submersus*. Boiling water appears to be the most efficient in extracting 17 amino acids whilst only 10 amino acids could be detected in the 70% ethanol extract.

Subsequent to this work, THORP[11] showed that methyl ethyl ketone/6 *N* HCl (MEK/HCl, 10:1) not only proved to be an efficient desalting medium in which to resuspend impure amino acid residues, but proved to be a very satisfactory free amino acid extractant. This was done by homogenizing freeze-dried mycelium in cold MEK/HCl for 15 min. This gave a salt-free solution of 16 amino acids from mycelium of *Tetracladium setigerum* which gave very clear chromatograms by the method described.

MEK/HCl was found unreliable for the extraction of tyrosine and leucine, which are readily extracted by boiling water, complementary to this, boiling water was found poor for the extraction of histidine, arginine and taurine, all of which are extracted readily under the conditions described by MEK/HCl.

The results indicate that no single method is absolutely satisfactory for the extraction of free amino acid pools from fungal mycelium but a combination of extracts obtained by boiling water, and MEK/HCl should extract the widest possible range of free amino acids contained in the mycelium used.

Résumé. En comparant plusieurs techniques pour extraire l'amino-acide libre de la mycose fongique on a observé de grandes différences entre elles. L'eau bouillante et la méthyléthylcétone HCl se sont montrés les solvents les plus efficaces et donnant chromatogrammes les plus clairs.

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The Effect of Glucose, Glucose Monophosphates and Inorganic Phosphate on the Tryptic Digestion of Phosphorylase b

Our earlier work[1] showed that both allosteric activator AMP and inhibitor ATP protect phosphorylase b (α-1,4-glucan:orthophosphate glucosyltransferase, EC 2.4.1.1) against tryptic digestion. As is known, phosphorylase b is also inhibited allosterically by glucose and Glc-6-P. In the present study we have examined the effect of these inhibitors on the tryptic digestibility of phosphorylase b. In addition, the effect of Glc-1-P and inorganic phosphate (P_i), the substrates of the enzyme, were also studied.

Materials and methods. 4-times recrystallized rabbit-muscle phosphorylase b was prepared according to FISCHER and KREBS[2]. It was further purified by passing it through a column of Sephadex G-100 immediately before use. The gel column was equilibrated with 0.05 *M* *Tris*-HCl buffer at pH 7.2. Tryptic digestion was carried out at 37 °C, buffered in 0.05 *M* *Tris*-HCl at pH 7.2. The final concentration of the constituents in the incubation mixture (total volume 3.5 ml) were: mercaptoethanol 0.01 *M*; trypsin 3.6×10^{-7} *M*; and phosphorylase b 3.1×10^{-6} *M* or casein 1.43 mg/ml. Various concentrations of glucose, Glc-6-P, Glc-1-P and P_i are indicated in the figures. The reaction was stopped after 20 min incubation by adding 1.5 ml of 10% trichloroacetic acid. After centrifugation the acid soluble protein residues were determined spectrophotometrically at 280 nm. In the case of the fluorimetric investigations, the digestion was carried out in the constant temperature compartment of an Opton spectrofluorometer ZFM 4C. At various incubation times the emission and excitation spectra of the samples were estimated.

Results and Discussion. Various concentrations of glucose and Glc-6-P yielded a significant inhibition of tryptic digestion of phosphorylase b (Figure 1). As Figure 1 shows, Glc-6-P had a greater inhibitory effect than glucose. Glc-1-P and P_i were found, however, to have no influence on the tryptic digestion of the enzyme. That the above-mentioned compounds had an effect on phosphorylase b and not on trypsin was verified by the fact that the rate of tryptic hydrolysis of casein remained unaffected by these compounds at the concentrations used.

The tryptic digestion of the enzyme led to a decrease in the fluorescence intensity and to a slight shift of the fluorescence maximum from 340–350 nm (Figure 2A). The excitation spectrum of the protein did not alter during the digestion. The protective effect of Glc-6-P against the tryptic digestion of phosphorylase b can also be detected fluorimetrically (Figure 2B). Similar results were obtained also with glucose.

It is known that after treatment of proteins with 8 *M* urea, i.e. after all the tryptophan residues of the protein

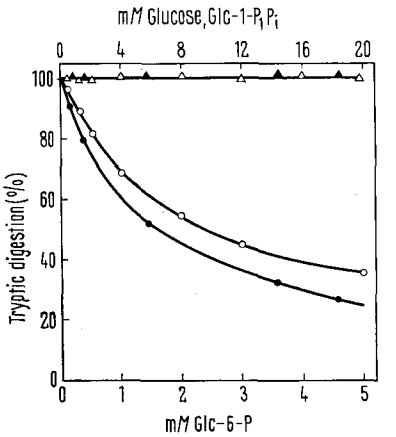

Fig. 1. Effect of glucose (○—○), Glc-6-P (●—●), Glc-1-P (△—△) and inorganic phosphate (P_i) (▲—▲) on the tryptic digestion of phosphorylase b.

[1] L. MUSZBEK, S. DAMJANOVICH and B. CSABA, Biochem. biophys. Acta *167*, 464 (1968).

[2] E. H. FISCHER and E. G. KREBS, in *Methods in Enzymology* (Eds. COLOWICK and KAPLAN; Academic Press, New York 1962), vol. 5, p. 369.

have been brought into an aqueous hydrophilic environment, all the fluorescence spectra of proteins become the same (maxima at 350 nm)[3]. As tryptic digestion causes a similar change in the micro-environment of tryptophan residues, probably this change is responsible for the observed long-wave shift of the fluorescence spectrum. The decrease of the fluorescence intensity shown in Figure 2 may also be due to this change, since the quantum yield of phosphorylase b is reduced by urea. A disturbance in the energy of migration may also play a role.

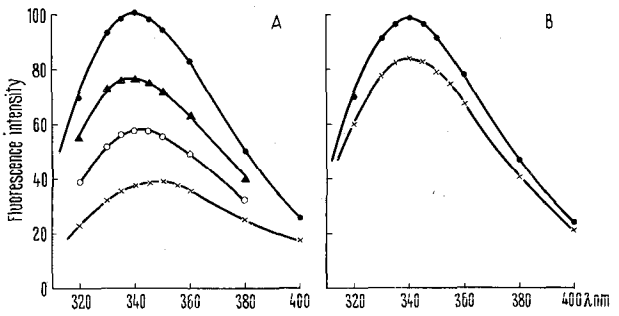

Fig. 2. Changes in the fluorescence spectrum of phosphorylase b during tryptic digestion. (A) Without Glc-6-P; (B) with 3.6 m*M* Glc-6-P. The spectra were determined after 0-min (●—●), 10-min (▲—▲), 20-min (○—○) and 50-min (×—×) incubation. The fluorescence was excited at 290 nm.

As is known, phosphorylase b is inactive in the absence of AMP. Earlier data indicate an effect of AMP on the conformation of the enzyme[1,4,5]. The demonstrated protective effect of ATP[1], glucose and Glc-6-P against tryptic digestion strongly suggest that allosteric inhibitors do not favour the native inactive state of the enzyme, but, like AMP, cause conformational changes in phosphorylase b. Since substrates tested by us did not influence the tryptic digestibility of phosphorylase b, the protective effects demonstrated seem to be specific of allosteric transitions.

Zusammenfassung. Glukose und Glc-6-P hemmen die Trypsinhydrolyse von Phosphorylase b konzentrationsabhängig, was darauf hinweist, dass die allosterischen Inhibitoren von Phosphorylase b die native Konfiguration des Enzyms verändern.

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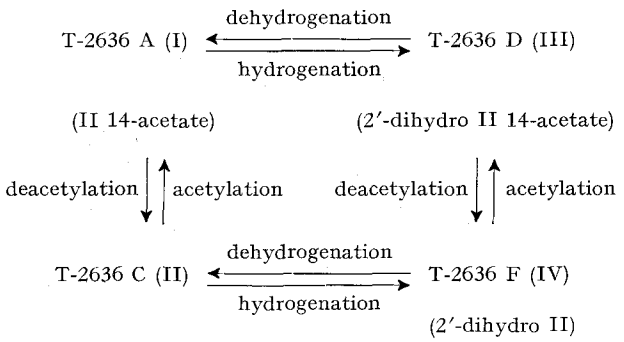
Interconversion of T-2636 Antibiotics Produced by *Streptomyces rochei* var. *volubilis*

In a previous paper[1,2], we reported the isolation and structures of T-2636 antibiotics produced by *Streptomyces rochei* var. *volubilis*[3]. The structural relations of these antibiotics are as follows.



In this paper we describe the enzymatic interconversion of these 4 antibiotics. The enzymatic deacetylation occurred when (I) was treated with rat liver homogenate[4] or the enzymes from the streptomyces, *Aspergillus sojae*[5], *Asp. niger* and *Trametes sanguinea*[6]. The acetyl group at C_{14} of (I) was deacetylated enzymatically[7].

The activity of the enzyme obtained from the fermented broth of the streptomyces by fractional precipitation with ethanol (30–60%) followed by chromatography on DEAE cellulose was remarkably much stronger than other enzymes when (I) was used as substrate.

The activity of the enzyme is optimal at pH 7 and at 40 °C, and can be kept stable in an aqueous solution at 33 °C for 30 min in the range of pH 4.0 to 9.0, but treatment of the enzyme at pH 3 and pH 10 under similar conditions leads to 25% and 85% decrease of the activity, respectively. By heating the solution at 80 °C for 10 min at pH 7, 75% inactivation is observed. The activity is inhibited (60%) by $10^{-3}M$ of $NaAsO_2$.

The enzymatic reaction is reversible as well. When ethyl acetate, ethyl formate and ethyl propionate were used as acyl donor, (II) was converted by the enzyme to (I)[7], (II) 14-formate $C_{26}H_{33}NO_8$, mp 175–177 °C (dec.), $[\alpha]_D^{22} - 259$ (c = 1.0, MeOH), UV: λ_{max}^{EtOH} 227 nm (ε = 49,200) and (II) 14-propionate, respectively. The acyl groups introduced at C_{14} were determined by the NMR- and IR-spectra.

Deacetylation of (II) 8,14-diacetate and (II) 8,14-dipropionate by the enzyme gave the corresponding 8-acetate and 8-propionate in good yield, respectively. The enzyme deacylates the acyl groups at C_{14} of the substrates selectively.

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