TABLE I

PURIFICATION OF CM-CELLULASE FROM HEPATOPANCREAS OF LITTORINA SP.

Purification step	*Total protein (mg)*	*Specific activity (Hultin units/mg protein)*	*Total enzyme units*	*Purification*	*Recovery of enzyme (%)*
Acetone precipitation	604	720	434 000	(1)	100
Column Bio-Gel P-30	72	4 010	288 000	5.6	66.4
Column Bio-Gel P-200 Peak IV	7.4	12 100	89 500	16.8	20.6
Repeated Column Bio-Gel P-200	0.7	50 800	35 560	70.6	8.2

migrated as a single band on disk electrophoresis in a polyacrylamide gel[9] and was shown to be homogeneous according to ultracentrifugation data.

The above mentioned action of metal ions for crude cellulase was also maintained for the purified enzyme, except Ag^+ which inhibited it at a final concentration of 10 mM.

The purification of other glucosidases is in progress.

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The effect of ^{60}Co γ-irriadation on the tryptic digestion of phosphorylase *b*

Tryptic digestion is a useful method for studying the steric conformational changes of proteins[1]. In the present work we investigated the effect of different allosteric effectors on the tryptic digestion of phosphorylase *b* (α-1,4-glucan:orthophosphate glucosyltransferase, EC 2.4.1.1) before and after ^{60}Co γ-irradiation. This represents a logical extension of our earlier experiments which indicated a higher radiation sensitivity for allosteric than for catalytic sites[2,3].

The procedures for the preparation and purification of rabbit muscle phos-

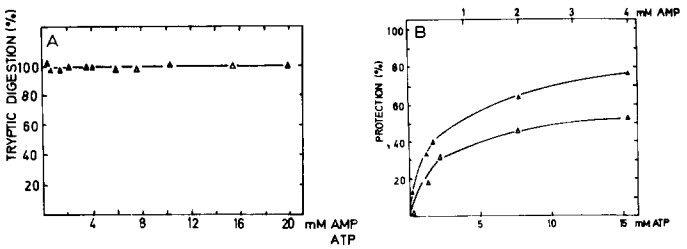

Fig. 1. A. The tryptic digestion of casein in the presence of various concentrations of AMP (▲—▲) and of ATP (△—△). B. Effect of AMP (▲—▲) and ATP (△—△) on the tryptic digestion of phosphorylase *b*.

phorylase *b* were as described earlier[2]. A twice-crystallized preparation of trypsin, and casein (Hammersten) were purchased from Reanal Chemical Co., Budapest. Tryptic digestion was carried out at 37°, buffered by 0.05 M Tris–HCl at pH 7.3. The final concentrations of the constituents (total volume 3.5 ml) were: cysteine, 3 mM; trypsin, $3.6 \cdot 10^{-7}$ M; and phosphorylase *b*, $3.1 \cdot 10^{-6}$ M or casein, 1.43 mg/ml. The different concentrations of AMP, ATP, Glc-6-*P* and Glc-1-*P* are indicated in Figs. 1 and 2. The reaction was stopped after 20-min incubation by adding 1.5 ml of 10% trichloroacetic acid. The precipitate of the remaining proteins was centrifuged and the nucleotides were removed by repeated washing with 3.33% trichloroacetic acid. The acid-insoluble protein residues were dissolved in 0.1 M NaOH for spectrophotometric determination at 280 mμ. The degree of digestion was expressed in terms of the difference between undigested and digested samples.

As is shown in Fig. 1A and Table I, neither the nucleotides (AMP, ATP) nor the glucose phosphates (Glc-6-*P*, Glc-1-*P*) had any effect on the tryptic digestion of casein. These results indicated that the above-mentioned compounds did not influence directly the effect of trypsin.

Various concentrations of AMP, ATP and Glc-6-*P* yielded a significant inhibition of tryptic digestion of phosphorylase *b* (see Fig. 1B and Table I). However, 20 mM Glc-1-*P* did not decrease the action of trypsin on the substrate. The most effective

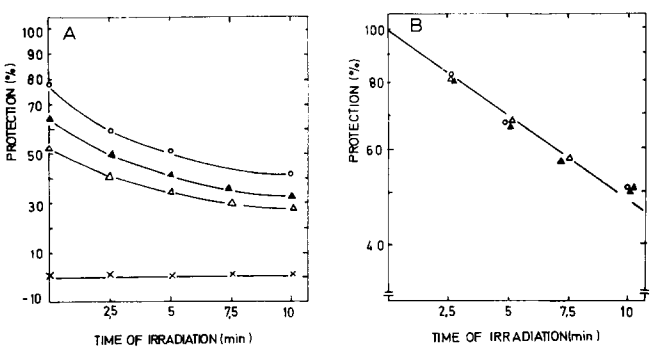

Fig. 2. A. Effect of ^{60}Co γ-irradiation on the tryptic digestion of phosphorylase *b*. 1-min irradiation from the Cobalt gun was equal to 3390 R according to the Fricke dosimeter. ×—×, irradiated phosphorylase *b* digested without effectors; ○—○, protective effect of 4.6 mM Glc-6-*P*; ▲—▲, protective effect of 2.0 mM AMP; △—△, protective effect of 15.3 mM ATP added after irradiation. B. The above effects in a semilogarithmic plot, if the protection of various non-irradiated controls is taken as 100%.

protective compounds proved to be the AMP and Glc-6-*P*. A significantly higher ATP concentration was necessary to produce a similar protection. These findings indicate that the allosteric effector AMP, which has been known to be more effectively bound than ATP, is a better protective agent in this system.

The ^{60}Co γ-irradiation of phosphorylase *b* had no influence on tryptic digestibility of protein in the absence of additions (Fig. 2A). However, the ability of allosteric effectors to protect phosphorylase *b* against tryptic digestion was greatly influenced. Fig. 2B demonstrates the decreasing protective effect of allosteric effectors: the per cent of protection of the non-irradiated controls is plotted as a function of the irradiation dose. A semilogarithmic plot manifested an exponential connection between the decrease of protection and the irradiation doses; moreover, it showed that the decreasing protective effect of the three different effectors depended, equally, only on the dose of irradiation. These data are in agreement with our earlier findings that ionising radiation-induced damage of phosphorylase *b* involves largely an effect on the allosteric sites with loss of ability to bind the effectors[2].

TABLE I

PROTECTIVE EFFECT OF Glc-1-*P* AND Glc-6-*P* AGAINST THE TRYPTIC DIGESTION OF CASEIN AND PHOSPHORYLASE *b*

Sugar phosphates	*Concn. (mM)*	*Protection (%)*	
		Casein	*Phosphorylase b*
Glc-1-*P*	20.0	0.0	0.0
Glc-6-*P*	4.6	0.0	78.2

Regarding the radiosensitivity of the ability of allosteric effectors to protect an enzyme against the tryptic digestion, described above, agreement exists between our earlier results and the present findings[2,3]. The data suggest that allosteric transitions or feed-back sensitivity were rather sensitive to ionising radiation even at doses which hardly influenced the original molecular conformation of the enzyme.

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