Enhancement of Enzymatic Catalysis of Cross-Linked Dextran in the Presence of Non-Ionic Polymer

The specific interactions between some macromolecular substances may be enhanced in media containing non-ionic water-soluble polymers. It has been shown previously that an enhancement of certain antigen-anti-body interactions occurs in the presence of dextrans[1-8] and polyethylene glycols[9]. A similar enhancement in reactivity was recently observed in an enzyme system[10]. The enzyme employed was α-amylase, the activity of which was increased in the presence of dextran during the hydrolysis of a synthetic highmolecular weight cross-linked blue starch polymer[11]. Furthermore, in this system it was shown that in addition to enhancement of enzymatic activity, the inhibition by specific antibody could also be demonstrated[10].

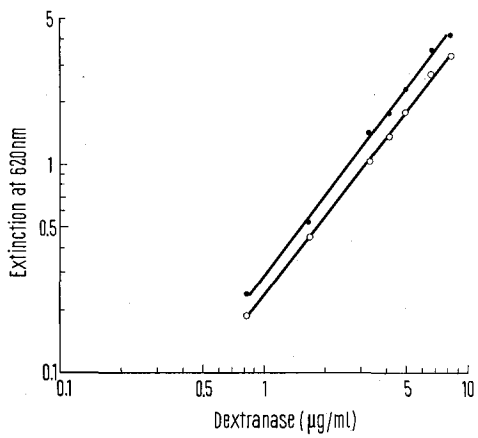

Fig. 1. Hydrolysis of blue dextran polymer (25 mg/ml) with indicated amounts of dextranase for 30 min at 45 °C. ●—●, in the presence of polyethylene glycol; ○—○, in the absence of polyethylene glycol.

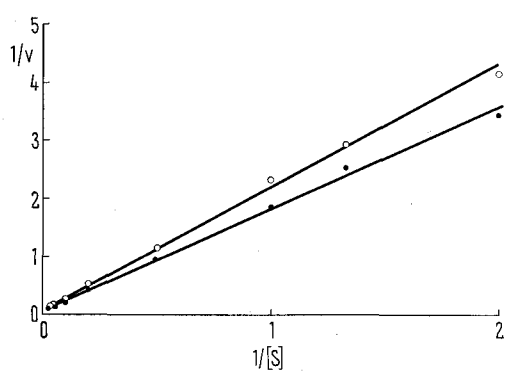

Fig. 2. Lineweaver-Burk plot. Dextranase activity (of dextranase preparation, 4.17 µg/ml) throughout 60-fold increase of substrate concentration (from 0.5–30 mg/ml of reaction mixture). The reaction was run at 45 °C for 30 min. ●—●, in the presence of 4% of polyethylene glycol; ○—○, in the absence of polyethylene glycol.

The present study describes another enzymatic system, namely dextran-dextranase, which can be influenced by the presence of non-ionic polymer of polyethylene type. The substrate used was a cross-linked and coloured dextran, the synthesis of which is described elsewhere[12].

Dextranase (Worthington, 150 U/mg protein) at a given concentration was pre-incubated in water bath at 45 °C. Aliquots of suspended blue dextran polymer in 0.1 *M* potassium phosphate buffer, pH 6.0 (containing 0.02% sodium azide) were used. To some tubes polyethylene glycol compound 20 *M* (Union Carbide) was added to give a final concentration of 4%. After a given time of incubation at 45 °C, the reaction was terminated by the addition of 0.5 ml of 0.5 *M* sodium hydroxide. The coloured supernatant was separated from the unhydrolyzed polymer by centrifugation. The extinction of supernatant was measured in a Zeiss PMQ II spectrophotometer at 620 nm.

Figure 1 shows the hydrolysis of blue dextran polymer by dextranase in the presence and in the absence of polyethylene glycol. In the presence of polyethylene glycol, there is a definite increase in the enzymatic activity at all levels of dextranase used. The hydrolysis of various amounts of blue dextran polymer by a constant amount of enzyme, in the presence and in the absence of the non-ionic polymer, is shown in a Lineweaver-Burk plot (Figure 2). Both curves (with different slopes) intercept the 1/v-axis at the same point. The increase in the Michaelis constant suggests that, in the presence of polyethylene glycol, there is an increase in the affinity between the enzyme and substrate. The changes in apparent K_m values of dextranase in the presence and in the absence of various non-ionic polymers using dextrans cross-linked to different degrees, will be reported on at a later date.

Zusammenfassung. Die enzymatische Spaltung von vernetztem und gefärbtem Dextran mit Dextranase wird in Gegenwart von neutralem (nicht ionogenem) Polymer-Polyethyleneglykol erhöht.

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Effect of Alkoxyglycerols on the Serum Ornithine Carbamoyl Transferase in Connection with Radiation Treatment

Alkoxyglycerols occur in small quantities in many natural products. In the haemopoietic organs of mammals, particularly the bone marrow, they are relatively abundant. They also occur in relatively high concentrations in human mother's milk[1-4]. They occur most abundantly in nature in the liver oil of certain species of shark[3,4]. The general formula for alkoxyglycerols is $CH_2OH \cdot CHOH \cdot CH_2O \cdot R$, where R is a longchain aliphatic radical. The

Table I. S-OCT (nanomoles) increase after irradiation (Controls)

	Initial level 0	Days after start of radiation treatment			
		1	4	6	8
Arithmetic mean:	1.3	2.2	4.6	3.2	2.4
Range	0.4–3.6	0.3–9.9	0.3–26.0	0.6–12.5	0.4–10.9
Geometric mean:	1.1	1.2	2.7	2.2	1.3
S.D.	0.2	0.3	0.3	0.3	0.2

Table II. S-OCT (nanomoles) increase after irradiation and treatment with alkoxyglycerols

	Initial level 0	Days after start of radiation treatment			
		1	4	6	8
Arithmetic mean:	1.2	1.6	2.3	1.6	1.3
Range	0.3–3.0	0.1–11.0	0.2–12.9	0.4–3.8	0.1–2.3
Geometric mean:	1.0	1.0	1.4	1.4	1.0
S.D.	0.2	0.3	0.3	0.3	0.2

most common natural alkoxyglycerols are the saturated batyl and chimyl alcohols (with 18 and 16 carbon atoms, respectively, in the side chain) and the unsaturated selachyl alcohol with 18 carbon atoms in the side chain. The alkoxyglycerols occur most frequently as fatty acid esters[1].

The alkoxyglycerols have proved to be of medical interest. To some extent they prevent leucopenia and thrombocytopenia[1]. The administration of alkoxyglycerols to patients with cancer of the uterine cervix results in higher survival rates than if radiation treatment alone is given[1,5]. Furthermore the alkoxyglycerols act as growth factors: they promote the growth of *Lactobacillus lactis*[1].

In all experiments, preparations from the liver oil of Greenland shark have been used. These oils contain up to 50% of alkoxyglycerol esters (main component selachyl alcohol).

In this study we try to elucidate some of the effects of the alkoxyglycerols by performing ornithine carbamoyl transferase (OCT) analyses. OCT is synthesized in the liver, where it is involved in the synthesis of urea. It has been shown that OCT in serum (S-OCT) rises in connection with liver injury. However, S-OCT may also rise without such injury being present. Such a rise occurs in certain clinical states involving an increased breakdown of protein[6]. It has been shown that S-OCT is elevated in conjunction with radiation[7]. The OCT-activities were determined by the incubation of serum with citrulline carbamoyl ^{14}C in arsenate buffer according to the modification of REICHARD[8]. 50 µmoles per 1 ml of incubation mixture were used. The results are expressed in nanomoles (nm) $^{14}CO_2$ liberated by 0.5 ml serum on 2 h incubation under standard conditions.

S-OCT analyses were made on 20 women with cancer of the uterine cervix in connection with the first radium implant. Analyses were performed immediately before the first implant and 1, 4, 6 and 8 days afterwards. The results are compiled in Table I. The most marked increase was observed on the 4th and the 6th day. As the S-OCT-values are exponentially distributed[6], the standard deviations are calculated from the logarithms of the S-OCT-values.

Similar analyses have now been performed on 20 patients with cancer of the uterine cervix who, unlike the series above, were treated prophylactically with alkoxyglycerols for 8 days before the radiation therapy with 0.6 g/day. The results are given in Table II. It will be seen that the S-OCT values were lower when alkoxyglycerols had been given prophylactically than when the patients only received the radiation treatment. Taking the mean of the logarithms of the S-OCT values for the different days and forming the antilogarithms (i.e. the geometrical means), it will be seen that these remain almost constant from day 0 to day 8 for the patients treated with alkoxyglycerols (Table II) and practically the same as the initial value, which is 1.2 nm.

Previous investigations have shown that alkoxyglycerols act as growth factors, promoting the growth of irradiated rats as well as the growth of *L. lactis*. One reason why S-OCT does not rise on radiation treatment of the alkoxyglycerol patients may be that the protein synthesis in the liver is stimulated, thereby compensating for the breakdown of proteins that occurs as a result of radiation treatment alone.

Résumé. L'ornithine carbamoyl transférase du sérum (S-OCT) augmente après un traitement aux rayons. L'augmentation la plus marquée a été observée le 4ème jour après le commencement de la radiothérapie. Nous n'avons toutefois observé qu'une petite augmentation de S-OCT lorsque des alcoxyglycérols ont été administrés prophylactiquement et pendant le traitement aux rayons.

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