## Aminotransferase Activity of Thermal Polylysine

Thermal polyanhydro-α-amino acids (proteinoid), which have been synthesized under the conditions of the primitive Earth and which contain 18 amino acids common to protein, exhibit aminotransferase activities[1]. The most active proteinoid was that with a high proportion of lysine. From activity tests of more simple copolymers of lysine with other amino acids and of homopolymers, it could be derived that the most active form of polyanhydro-α-amino acids was thermal polylysine[1].

For this study several batches of thermal polylysine[2] were prepared individually under aseptic conditions[3] and tested for aminotransferase activity in a system containing 0.1 m*M* $CuSO_4$, 0.1 m*M* urea and 0.1 m*M* α-ketoglutaric acid (KGA) adjusted to pH 7.0 with BRITTON-ROBINSON buffer (tenfold concentration). The incubation was accomplished in a way that excluded microbial contaminations[3,4]. The incubation temperature was 37.5 °C unless otherwise described. After a given incubation period, 18.5 mg of EDTA was added to the transamination system, subsequently the sample was adjusted to pH 1 and desalted through a Lewatit S 100 ion exchange column (gift from Farbenfabriken Bayer, Leverkusen, Germany). The effluent containing glutamic acid was concentrated to dryness in a flash evaporator and used for quantitative amino acid assay on an automatic device[5].

More than 10 individually prepared batches of thermal polylysine showed, with no exception, aminotransferase activity as expressed by the formation of glutamic acid (Table I). Most of the preparations formed a gel-like mass when suspended in water or buffered solutions. They were insoluble in mineral and organic acids, alkalies, and organic solvents. One of the batches (LSD-3), however, was soluble in water and buffered solutions. The molecular weight of this preparation was estimated in the analytical ultracentrifuge to be 200,000.

Figure 1 demonstrates the course of the transamination reaction depending on the time of incubation.

Kinetic studies of this reaction have shown that it obeys MICHAELIS-MENTEN kinetics as expressed by the LINEWEAVER-BURK plot (Figure 2). The MICHAELIS con-

Table I. Formation of glutamic acid by transamination reaction catalyzed by thermal polylysine

| Polymer or control | Glutamic acid μ*M* |
|---|---|
| (a) Thermal polylysine (LSD-1) + $CuSO_4$ + KGA + urea | 3.45 |
| (b) urea + $CuSO_4$ + KGA | 0.00 |
| (c) LSD-1 + urea + KGA | 0.00 |
| (d) LSD-1 + $CuSO_4$ + KGA | 0.00 |
| (e) LSD-1 + $CuSO_4$ | 0.00 |
| (f) LSD-1 + KGA | 0.00 |
| (g) LSD-1 + urea | 0.00 |
| (h) LSD-1 | 0.00 |
| (i) Thermal polylysine (LSD-2) + $CuSO_4$ + KGA + urea | 3.72 |
| (k) Thermal polylysine (LSD-3), water-insoluble + $CuSO_4$ + KGA + urea | 4.02 |
| (l) Thermal polylsine (LSD-3), water-soluble, m.w. 200.000, + $CuSO_4$ + KGA + urea | 3.29 |
| (m) Thermal polylsine (LSD-4) + $CuSO_4$ + KGA + urea | 3.37 |
| (n) Thermal polylsine (LSD-5) + $CuSO_4$ + KGA + urea | 5.27 |
| (o) Thermal polylsine (LSD-6) + $CuSO_4$ + KGA + urea | 4.62 |
| (p) Thermal polylysine (LSD-7) + $CuSO_4$ + KGA + urea | 4.61 |
| (q) Thermal polylysine (LSD-8) + $CuSO_4$ + KGA + urea | 3.94 |
| (r) Thermal polylysine (LSD-9) + $CuSO_4$ + KGA + urea | 3.28 |
| (s) Thermal polylysine (LSD-10) + $CuSO_4$ + KGA + urea | 3.89 |

Experimental conditions: 10 mg polylysine, 0.1 m*M* $CuSO_4$ 0.1 m*M* KGA and 0.1 m*M* urea; incubation at 37.5 °C for 2 h.

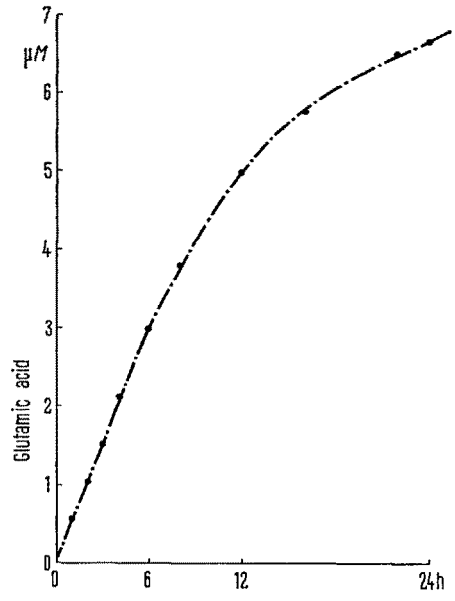


Fig. 1. Effect of thermal polylysine on rate of transamination in aqueous solution as expressed by formation of glutamic acid from α-ketoglutaric acid and urea as the $NH_2$-group donor.

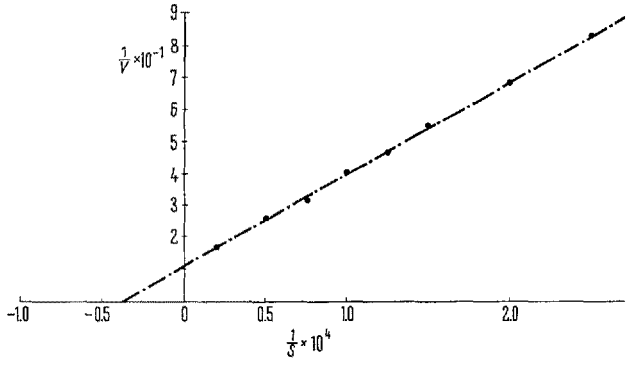


Fig. 2. LINEWEAVER-BURK plot of transamination reaction in the presence of thermal polylysine in aqueous solution.

[1] G. KRAMPITZ, S. DIEHL and T. NAKASHIMA, Naturwissenschaften *54*, 516 (1967).
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[3] G. KRAMPITZ and H. HARDEBECK, Naturwissenschaften *53*, 81 (1966).
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stant ($K_m$) for this reaction at pH 7.00 and 37.5 °C is $2.86 \times 10^{-4} M$. The catalytic constant for water-insoluble preparations is probably more favorable. Calculated for LSD-3 the catalytic constant is 0.6 $M$ glutamic acid per min per 1 $M$ polylysine. A correlation between molecular weight and aminotransferase activity can be derived from studies on partial hydrolysates of thermal polylysine (Figure 3). While lysine (monomer) and total hydrolysates of thermal polylysine are completely inactive, activity increases with the reciprocal degree of hydrolysis. From these results we conclude that water-insoluble preparations of thermal polylysine having the highest activity compared with water-soluble preparations should have a higher molecular weight than LSD-3.

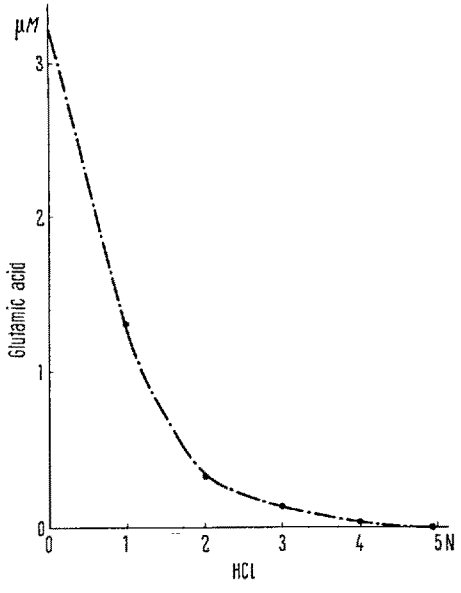


Fig. 3. Inhibition of transamination reaction catalyzed by partial hydrolysis of polylysine. Experimental conditions: 20 mg thermal polylysine, 0.1 m$M$ KGA and 0.1 m$M$ $CuSO_4$; incubation at 37.5 °C for 2 h. 20 mg of thermal polylysine were hydrolyzed for 24 h by 1, 2, 3, 4, and 5 $N$ HCl at 109 °C prior to the transamination trial.

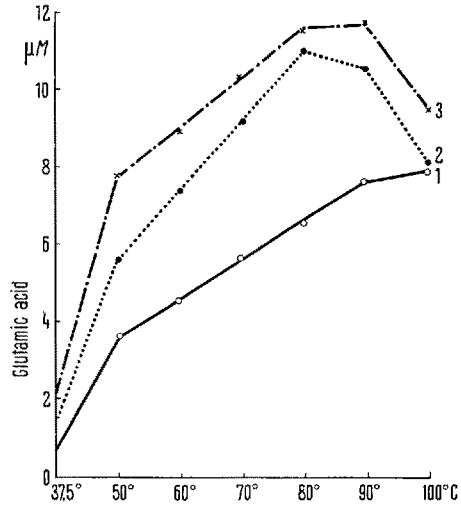


Fig. 4. Influence of the temperature of incubation on the rate of transamination reaction expressed by the formation of glutamic acid. Experimental conditions: 10 mg thermal polylysine, 0.1 m$M$ KGA, and 0.1 m$M$ $CuSO_4$. Incubation at the given temperatures for 1 h (1), 2 h (2), and 3 h (3).

Thermal polyanhydro-α-amino acids that catalyze the hydrolysis of *p*-nitrophenyl acetate can be almost totally inactivated by heating in buffered solutions[6]. Thermal polylysine, however, does not lose its aminotransferase activity, even when it is heated in buffered solutions at 90 °C for 2 h.

Studies on the influence of temperature effect on the said transamination system revealed that its activity is increased by elevated temperatures (Figure 1).

Modification of amino groups of thermal polylysine by trinitrobenzenesulfonic acid[7], dinitrofluorbenzene[8] and acetylation[9] caused complete inactivation of the polymer.

Several batches of poly-L-lysine, HBr, synthesized by the LEUCHS method, did not show any aminotransferase activity in the system used. We conclude from these results that the transamination activity of thermal polylysine depends on the degree of polymerization, that is to say the molecular weight, the shape of the macromolecule and, in connection with both, on the number of available amino groups.

Glutamic acid formed in the transamination reaction described was 50% L and 50% D as tested by use of L-glutamic acid decarboxylase and D-amino acid oxidase[10].

Furthermore, derivatives of urea were tested for their influence on the transamination reaction. Modifications of the carbonyl group of urea had an inhibitory effect on the formation of glutamic acid, while modifications of the amino groups were far less effective in this respect (Table II).

Irradiation of thermal polylysine in a dry state with an UV-lamp (2570 Å and 3650 Å) for 30 min did not change the activity of the polymer.

Table II. Formation of glutamic acid by transamination reaction from urea and urea derivatives by thermal polylysine

| | Glutamic acid µ$M$ |
|---|---|
| Urea | 3.29 |
| Thiourea | 0.25 |
| Methylurea | 3.04 |
| Acetylurea | 3.04 |
| Biuret | 2.58 |
| *N*-Methylacetamide | 1.98 |

Experimental conditions: 0.1 µ$M$ LSD-3, water-soluble, 0.1 m$M$ urea or derivative, 0.1 m$M$ $CuSO_4$ and 0.1 m$M$ KGA; incubation at 37.5 °C for 2 h.

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These results could explain at least in part how, under the conditions of the primitive Earth, the first enzymes could have come into existence, and how they could have survived their exposure to elevated temperatures and strong UV-light[11,12].

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*Zusammenfassung.* Thermisch hergestelltes Polylysin beschleunigt die Übertragung von Aminogruppen des Harnstoffs auf α-Ketoglutarsäure. Das pH-Optimum dieser Reaktion, die die Anwesenheit von Cu-Ionen erfordert, liegt bei 7.00. Die Transaminierungsreaktion folgt der MICHAELIS-MENTEN-Kinetik. Thermisch hergestelltes Polylysin wird durch Erhitzen in Pufferlösungen nicht inaktiviert, während Depolymerisation oder Modifizierungen der Aminogruppen den Verlust der Aktivität zur Folge haben.

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## Secretory Responses of the Submaxillary Gland in Hypophysectomized Rats after Treatment with Thyroxine

Endocrine glands, mainly the hypophysial and thyroid glands and the gonads, are known to affect the structure of salivary glands in rodents[1-3]. Regarding glandular function, previous results indicate that hormonal factors, particularly thyroxine, are of great importance for the secretory responses of the rat's submaxillary gland to parasympathomimetics[4]. To study the role of thyroxine its effects on the threshold dose of acetylcholine and on the maximal secretory responses to stimulation of the chorda-lingual nerve or pilocarpine of the submaxillary gland were determined in hypophysectomized rats.

Twenty-five female rats were used. Hypophysectomy was performed at the age of 110–120 days in all animals. The experiments were performed 7 weeks after the operation. Thirteen rats were untreated while 12 received daily s.c. injections of a physiological dose of thyroxine[5], 6.5 μg L-thyroxine, for 3 weeks starting the treatment 4 weeks after hypophysectomy. The completeness of hypophysectomy was checked[6]. The hypophysectomy was incomplete in 6 animals. The results refer to 19 rats where microscopic examination revealed no other hypophysial cells than small pars tuberalis cells lining the pituitary stalk.

To study the secretory responses the rats were anaesthetized using chloralose (100 mg/kg) i.v. after preliminary ether. The submaxillary ducts were exposed in the neck and cannulated with small glass cannulae giving about 100 drops out of 1 ml of distilled water. Secretion appearing at the tip of the cannula was marked on a smoked drum. A series of doses of the hydrochloride of acetylcholine (0.05–5 μg/kg) was given i.v. to estimate the threshold dose. The maximal secretory response of the gland was estimated by stimulating the chorda-lingual nerve by 20 shocks/sec, which is known to cause a secretion of maximal rate[7], or by giving pilocarpine i.v. in increasing doses from 50–200 μg/kg every 30–60 sec until the maximal flow rate was reached. The maximal secretory response is expressed as μl saliva/min/gland or μl saliva/min/mg glandular tissue (dry weight). After the experiments the submaxillary glands were carefully cleaned and weighed (wet weight). The dry weight was determined after heating to 105–110 °C for 48 h.

The size of the submaxillary gland was found to be increased by about 50% in hypophysectomized rats after

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Weight of submaxillary glands and maximal secretory responses to chorda stimulation or pilocarpine in rats after hypophysectomy and in hypophysectomized rats given thyroxine

| | Glands | | | Maximal secretory responses | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | No. | Wet weight (mg) | Dry weight (mg) | Chorda stimulation | | | Pilocarpine | | |
| | | | | No. | μl/min | μl/min/mg | No. | μl/min | μl/min/mg |
| Hypophysectomy | 11 | 66 ± 3.7 | 14.1 ± 0.87 | 7 | 13 ± 1.6 | 0.89 ± 0.083 | 7 | 4.5 ± 0.30 | 0.32 ± 0.026 |
| Hypophysectomy + thyroxine | 8 | 100 ± 6.4[a] | 21 ± 1.4[a] | 4 | 40 ± 5.7[b] | 1.9 ± 0.18[a] | 7 | 10.9 ± 0.86[a] | 0.53 ± 0.049[b] |

Values are mean ± S.E.M. [a] $P < 0.001$. [b] $P < 0.01$ when compared with hypophysectomy.