# TRYPSIN SPLITTING AND DENATURATION OF β-LACTOGLOBULIN

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LINDERSTRØM-LANG, Hotchkiss and Johansen[1] have stressed the importance of denaturation in the proteolytic splitting of globular proteins. They suppose that the process genuine⇌denatured is forced in the direction of the right-hand side of the equation by splitting of the D-form, both forms gradually disappearing; and they suggest that the peptide bonds as such are masked in native globular proteins, and first 'appear' on denaturation.

More recently, Linderstrøm-Lang, Jacobsen *et al.* have found further support for the correctness of this view (unpublished investigations on volume changes in hydrolytic splitting). An interesting paper by Lundgren[2] similarly confirms the theory convincingly. By experiments with ultra-centrifugation and electrophoresis, he showed that active papain catalyses the denaturation of thyroglobulin before its splitting.

While working with other problems, it was observed that β-lactoglobulin is much more easily split by trypsin when a small quantity of urea, which in itself has only a very slight denaturing effect on the protein, is added. It was therefore thought to be of interest to investigate further the way in which trypsin acts in such comparatively weakly denaturing systems.

*Experimental.* It was found that the processes occurring (denaturation and splitting of peptide bonds) can be followed most easily and instructively by observing the changes in optical rotation. In all the experiments presented here, the concentration of lactoglobulin was 2·17 per cent and the temperature was 30° C. Ammonium–ammonia buffer systems were used, and *p*H was controlled by use of the glass electrode. The trypsin concentrations indicated are based on moist filter-cake. (Percentage trypsin times 0·41 gives actual enzyme concentration in all cases.) By use of three-branched mixing tubes, protein, enzyme and denaturing agent were kept separate until the moment of mixing. The process of denaturation was checked by estimating the proportion of protein insoluble in a salt-buffer mixture of previously given composition[3].

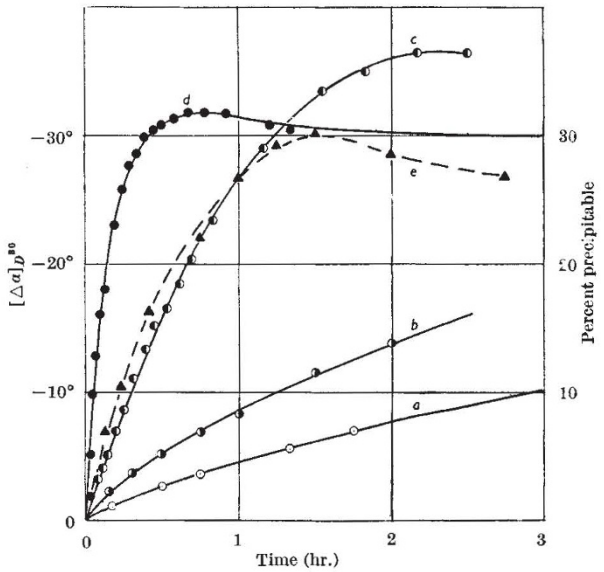


Fig. 2
*a*, Without trypsin, *p*H 9·25
*b*, 0·001 per cent trypsin, *p*H 9·25
*c*, 0·01 ,, ,, ,, ,,
*d*, 0·1 ,, ,, ,, ,,
*e*, See text

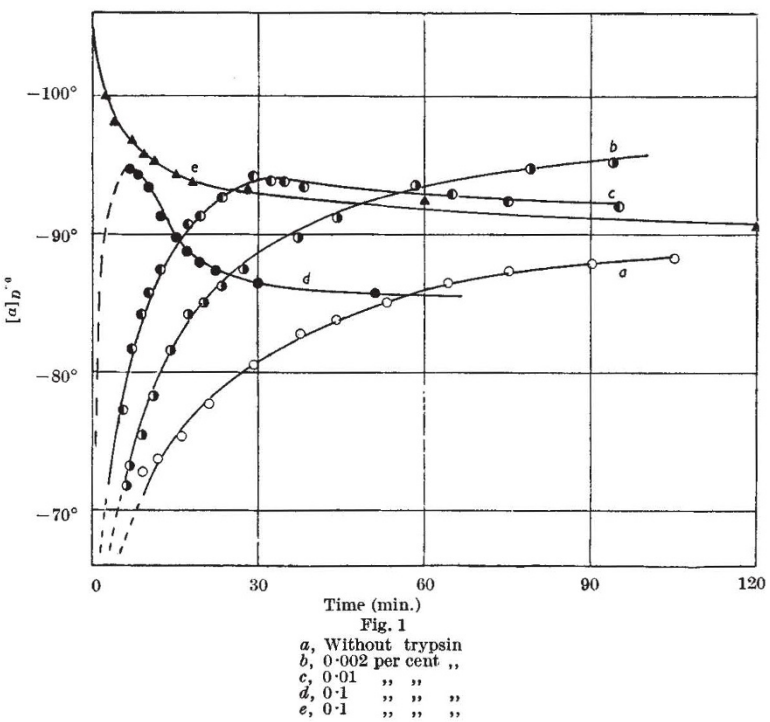


Fig. 1
*a*, Without trypsin
*b*, 0·002 per cent ,,
*c*, 0·01 ,, ,,
*d*, 0·1 ,, ,, ,,
*e*, 0·1 ,, ,, ,,

Curve *a*, Fig. 1, shows how a progressive denaturation of β-lactoglobulin is accompanied by a considerable increase in lævorotation, while curve *e* shows that the splitting of completely (urea-) denatured β-lactoglobulin is accompanied by a comparatively small decrease in rotation. Curve *a* refers to the following system: 2·17 per cent lactoglobulin, 19 per cent urea, 1/10 *N* ammonium–ammonia, *p*H 8·25. Curves *b*, *c* and *d* show that the addition of trypsin to this system has a pronounced 'catalytic' effect on the rate of denaturation. On addition of 0·1 per cent trypsin, the process is especially illuminating. In this case the denaturation is extremely rapid and almost complete, and is 'followed' by splitting of the denatured protein (compare with curve *e* in Fig. 1).

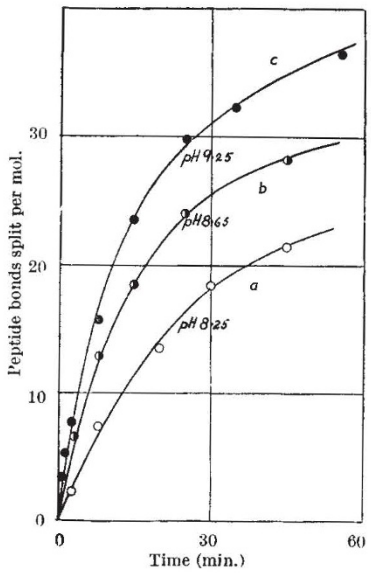


Fig. 3
*a b* and *c*, with 0·1 per cent trypsin

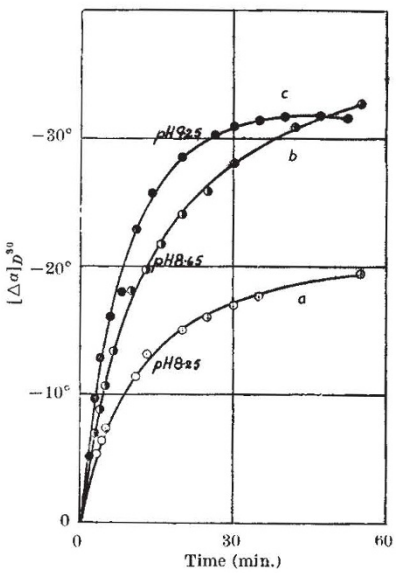


Fig. 4
*a b* and *c* with 0·1 per cent trypsin

The splitting of native β-lactoglobulin (in systems without urea) is accompanied by an increase in lævo-rotation (see, for example, curve *a*, Fig. 4), and according to the preceding paragraph it seems natural to regard this as an indication that the process of denaturation (with a large increase in rotation) precedes the splitting of peptide bonds (accompanied by a relatively small decrease in lævo-rotation).

In order to elucidate this further and to see if, under these conditions, there is a similar interaction between the enzyme and the alkaline environment, the stability of β-lactoglobulin in this medium was investigated. At *p*H 8·25 a slow denaturation was found (measured by increasing lævo-rotation and insolubility in the salt-buffer mixture previously described), and the speed was found to increase with rising *p*H. Curve *a*, Fig. 2, shows this alkali denaturation at *p*H 9·25, and by analogy with Fig. 1 it is further shown how increasing concentrations of trypsin catalyse this process. Control experiments with heat-treated trypsin (0·01 per cent) gave a curve exactly like curve *a*.

The dotted curve in Fig. 2 shows the percentage of total protein precipitated at different times by the salt-buffer mixture; the curve has been constructed from analysis of samples removed at intervals from a system corresponding to curve *c* of Fig. 2 (0·01 per cent trypsin). The curve gives the direct proof that, under these conditions, more denatured protein is produced than the enzyme can break down immediately to unprecipitable products.

Fig. 3 shows how the rate of splitting increases with rising *p*H values (three values given). On the basis of the experiments described above, the probable explanation for this is that the pure alkali denaturation, and consequently the alkali denaturation catalysed by trypsin, proceeds more quickly as the medium becomes more alkaline (Fig. 4).

According to Northrop[4], the degree of ionization of the protein substrate determines the optimum *p*H for splitting, an explanation which Bergmann and Fruton[5] from their investigations on the breakdown of synthetic substrates have described as inadequate.

In the case of β-lactoglobulin, alkali denaturation will, of course be of great importance in determining the optimum *p*H (this will naturally also be dependent on the stability of the trypsin and as additional experiments seem to show, among other things also on the concentration of the trypsin and on the temperature chosen). The observation[6] that the optimum *p*H for the trypsin splitting of denatured protein is lower than for breakdown of the same in the native state may support the view given above. So far it is uncertain if alkali and acid stability have the same importance for the proteolytic hydrolysis of other native proteins.

It was thought to be interesting to compare β-lactoglobulin with trypsin-resistant proteins such as native serum albumin and egg albumin, which are easily split in the denatured state. In contrast to lactoglobulin, these are not easily denatured by alkali (standing several days at *p*H 9). Neither was trypsin found to have any catalytic effect on the denaturation of egg albumin in a weakly denaturing system (25 per cent urea, *p*H 9·25), and both conditions for splitting were thus missing here.

The results given here are in agreement with the hypothesis proposed by Linderstrøm-Lang *et al.* for denaturation as the initial process in trypsin splitting. They demonstrate, however, that the mechanism of this process is more complicated than these investigators had supposed, and a further analysis cannot be given before additional experimental material is available. I am indebted to Prof. Linderstrøm-Lang for valuable criticism.

[1] Linderstrøm-Lang, K., Hotchkiss, R. D., and Johansen, G., *Nature*, **142**, 996 (1938).
[2] Lundgren, H. P., *J. Biol. Chem.*, **138**, 293 (1941).
[3] Jacobsen, C. F., and Korsgaard Christensen, L., *Nature*, **161**, 30 (1948).
[4] Northrop, J. H., *J. Gen. Physiol.*, **5**, 263 (1922).
[5] Bergmann, M., and Fruton, J. S., "Advanc. Enzymol.", **1**, 63 (1941).
[6] Oppenheimer, C., *Die Fermente*. Supp. **1**, 687 (1936).

# NUCLEAR INTERACTIONS OF THE PARTICLES PRODUCED IN COSMIC RAY BURSTS

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We wish to report the preliminary results of an investigation to study nuclear bursts, carried out recently with a counter-controlled cloud chamber at the Laboratorio della Testa Grigia (3,500 m.).

By a 'nuclear burst' we mean any event in which a particle (ionizing or not), by interacting with a nucleus, gives rise to new ionizing particles, generally different in nature and in energy. In this definition