Studies on the actin-actin bonding

Since the discovery by KUSCHINSKY AND TURBA, that polymerisation of actin can be inhibited by Salyrgan[1], the mechanism of this inhibition has remained unsolved. For a closer approach to the problem the number of SH groups reacting with Salyrgan in G- and F-actin has been measured.

In our experiments actin, purified[2] with Mg^{++}, was stirred with 20 M Salyrgan/10^5 g protein at room temperature for 30 minutes, pH 6.5–7.0. It was precipitated isoelectrically and washed repeatedly. After dissolution it was denatured with 5 M guanidine hydrochloride at 0°, and the released SH groups were titrated according to EDELHOCH *et al.*[3]. As in G-actin, after denaturation with guanidine-HCl, free SH groups did not appear, we present in Table I only the SH groups that remained in 57,000 g F-actin after treatment with Salyrgan.

KOMINZ *et al.* found that 57,000 g actin protein give 4.2 cystein residues[4]. TSAO AND BAILEY reported that N-ethylmaleimide does not inhibit polymerisation, at the same time it reacts with only half of the total SH, both in G-actin and in F-actin[5]. These data, together with the results of the table above, show clearly that out of the four SH groups of G-actin, there are only two which are necessary for the transformation of G-actin to F-actin.

TABLE I

REMAINING SH GROUPS IN 57,000 g F-ACTIN AFTER TREATMENT WITH SALYRGAN

Preparation No.	*M SH*
1	2.05
2	2.10
3	1.92

KUSCHINSKY AND TURBA were the first to show that F-actin may be depolymerised with Salyrgan as well[6]. The detailed study of this depolymerisation revealed that it depends to a great extent on the pH[7]. While at pH 7.0 the depolymerisation —compared with that of the control—is 15%, at pH 8.0 it amounts to 64% and at pH 9.0 to 76%. The rate of depolymerisation does not depend practically on the added amount of Salyrgan; it is controlled by pH. The reaction is reversible and depends on time and temperature. We also observed that in completely depolymerised F-actin at pH 9.0, Salyrgan reacts with all the SH groups. The most probable explanation of this phenomenon may be that depolymerisation and simultaneously the mercaptide-forming reaction occur only if the SH groups, bound at pH 7.0 due to some previous reaction, are set free. It is reported in the literature that a hydrogen bond may be formed in peptides between SH and NH_2 groups[8–11].

In order to clarify the role of amino groups, actin was treated with specific amino group reagents. After acetylation with acetic anhydride all the amino groups are bound—without the reaction of tyrosine residues—and simultaneously F-actin is completely depolymerised[7]. Furthermore, if actin (1.35 mg/ml) is incubated for one and half minutes with formaldehyde at room temperature, pH 7.5–8.0, F-actin depolymerises, resp. G-actin does not polymerise on addition of 0.1 volume of 1 M KCl + 0.01 M $MgSO_4$. Both reactions are reversible if the 2.25% content of formaldehyde is removed by isoelectric precipitation from actin (Table II).

TABLE II

EFFECT OF FORMALDEHYDE ON THE VISCOSITY OF FIBROUS AND GLOBULAR SOLUTIONS OF ACTIN

	$\log \eta_{rel} \times 1000$	
	before isoelectric precipitation	*after isoelectric precipitation*
F-actin	249	248
F-actin + formaldehyde	35	242
G-actin + KCl + $MgSO_4$	255	250
G-actin + formaldehyde + KCl + $MgSO_4$	48	238

It was found, that ATP—which can be considered, according to STRAUB AND FEUER, as the prosthetic group of actin[12]—decreases considerably the degree of depolymerisation by Salyrgan, formaldehyde and urea (Fig. 1).

This proves the specificity of depolymerisation brought about by the binding of both SH and NH_2 groups. Moreover, the protection of urea depolymerisation indicates that the depolymerising effect of urea is localised upon the specific combining site also and it breaks the supposed hydrogen bond between SH and NH_2 groups.

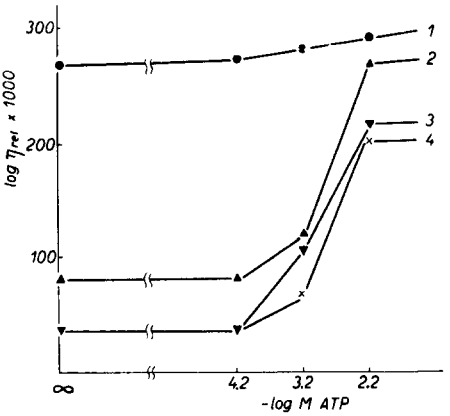

Fig. 1. Protecting effect of ATP against depolymerisation by Salyrgan, formaldehyde and urea.

Incubation: with Salyrgan: 15 min 37°; 10 M Salyrgan/10^5 g protein; 1.6 mg/ml protein.
with formaldehyde: 5 min at room temperature; 1.8% formaldehyde; 1.6 mg/ml protein.
with urea: 30 min at room temperature; 15% urea; 1.75 mg/ml protein.
control: 15 min 37°, resp. 30 min at room temperature; 1.6 mg/ml protein.

Buffer: 0.05 M borate pH 8.0. Ionic strength: 0.12. (1) control; (2) with Salyrgan; (3) with urea; (4) with formaldehyde. Viscosity measurements were carried out at 0° in an Ostwald viscosimeter as specified in our laboratory.

In order to understand more clearly the depolymerisation protecting effect of ATP and ATP-actin bonding, we have compared the protecting effect of ATP-forming compounds ($6 \cdot 10^{-3}$ M) against various depolymerising substances (Table III).

TABLE III

PROTECTING EFFECT OF ATP-FORMING COMPOUNDS ON VARIOUS DEPOLYMERISATIONS OF F-ACTIN

(The numbers represent: $\log \eta_{rel}$ in per cent of untreated controls)

	Depolymerising substance		
	Salyrgan	*Formaldehyde*	*Urea*
F-actin	28	29	28
F-actin + ATP	94	90	81
F-actin + AMP	98	60	48
F-actin + IMP	96	63	47
F-actin + adenosine	100	55	40
F-actin + adenine	74	30	30
F-actin + Na_2HPO_4	28	30	28
F-actin + $Na_4P_2O_7$	29	50	30
F-actin + $Na_5P_3O_{10}$	28	46	29

Experimental conditions: Salyrgan 10M/10^5 g protein, 37°, 15 min; formaldehyde 1.5% room temperature, 5 min; urea 15%, room temperature, 15 min.
1.6 mg/ml protein, 0.05 M borate buffer pH 8.0, ionic strength: 0.12.

As can be seen from the results, the protecting effect of ATP against the mercaptide-forming agent is completely replaced by AMP, IMP and adenosine, and to a great extent by adenine. As far as the protection of the depolymerising effect of formaldehyde and urea is concerned, ATP proves to be the most active; in the case of formaldehyde, anorganic pyro- and triphosphate are also effective.

In the presence of Mg^{++} the protecting effect of ATP against Salyrgan and potassium iodide is increased[13]. Mg^{++} and ATP together inhibit competetively the Salyrgan depolymerisation[13]. This statement means that ATP combines with the bound SH groups of F-actin and as can be seen from Table III the combination occurs from the adenine part.

On the basis of our results we may assume that the hydrogen bond between SH and NH_2 groups plays a primary role in the combination of actin-actin parts. It remains however unsolved whether this amino group belongs directly to the protein or to the nucleotide of actin.

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The biogenesis of tropane alkaloids

In recent times tracer techniques have been used in research on the biogenesis of alkaloids. No doubt these methods will give new and important facts. The results, however, should be carefully interpreted.

One of the most important reports on this subject comes from LEETE, MARION AND SPENSER[1]. They fed ornithine-2-^{14}C to *Datura stramonium* plants and isolated radio-active hyoscyamine from the whole plant. The isolated scopolamine, however, was inactive. To explain this result they suggested that scopine is synthesized by another mechanism from another precursor, *viz.* hydroxyornithine via hydroxyproline.

Although the existence of two separate synthesizing mechanisms for these alkaloids has been proposed on other grounds[2] it seems to me highly improbable that these two related alkaloids should be synthesized along different pathways. Moreover, another explanation of the above mentioned results—in my view a more likely one—can be given.

LEETE *et al.* placed the plants in 500 ml feeding solution containing 10 mg ornithine with a total activity of $2.9 \cdot 10^6$ dis/min. After 7 days, when the plants were harvested, the activity remaining in the solution was $0.01 \cdot 10^6$ dis/min, corresponding with 0.035 mg ornithine.

Their results may be fully explained when one assumes that scopolamine is the first alkaloid synthesized from ornithine, but is in a short time transformed into hyoscyamine.

At first the active ornithine competes favourably with the present ornithine, due to its concentration and the radio-activity will turn up in scopolamine, as well as in other metabolic products. After some time the radio-activity of the scopolamine produced per unit of time, will decrease as a result of the decrease in the concentration of ornithine in the feeding solution. Now, assuming that the scopolamine is constantly transformed into hyoscyamine, the radio-activity of the scopolamine fraction will gradually decrease and it is quite possible that, at the moment of analysis, practically all the radio-activity of the alkaloid fraction will be found in the hyoscyamine.

An indication that in fact ornithine is a precursor of both alkaloids was obtained in experiments with sterile root cultures of *Atropa belladonna*, which will be published in due time.

Feeding ornithine resulted in an increase in alkaloid content from 0.4% on dry weight in controls, to 0.8% and a chromatographic analysis showed that both alkaloids had increased.

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