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SHORT COMMUNICATION

STIMULATORY EFFECT OF TRIS BUFFER ON A WHEAT EMBRYO AMINO ACID INCORPORATING SYSTEM

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Abstract—Tris buffer at low levels stimulates *in vitro* amino acid incorporation in a wheat embryo system. Results of experiments in which tris buffer and K^+ concentrations were varied, suggest that the tris "effect" is due to its cationic function.

A NOT uncommon observation is that tris (hydroxymethyl) amino methane (tris) reagent is inhibitory to several enzyme systems when present in a high concentration.[1–4] During recent studies of TMV–RNA mediated, amino acid incorporation in a wheat embryo system[5] we have also observed inhibition by a high concentration of tris buffer (see Fig. 1). However, further study of this effect revealed the novel observation that, at lower concentrations, tris buffer was highly stimulatory. The present communication documents these observations and suggests a possible explanation.

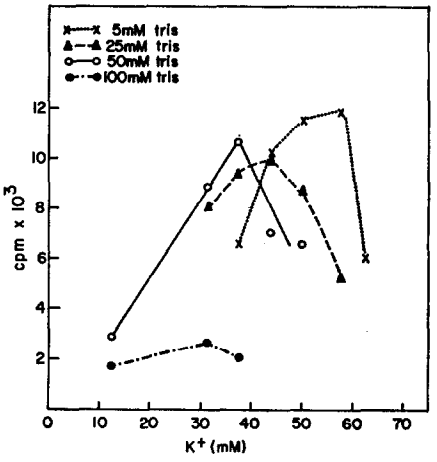

FIG. 1. K^+ REQUIREMENT FOR AMINO ACID INCORPORATION AT VARIOUS LEVELS OF TRIS BUFFER.

Table 1 presents data showing the effect of tris buffer on the incorporation of ^{14}C-leucine into protein. The salt regime used for these incubations was 37·5 mM K^+ and 4 mM Mg^{2+},

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with the concentration of tris buffer being varied at two values of pH. It can be seen that at each pH value there is an optimal tris concentration. Both above and below the optimum there is considerable decrease of activity. Table 1 also shows that an essentially similar situation exists with triethanolamine (TEA), indicating that the effect is not exclusively that of a primary amine. The pH of the reaction mixtures was maintained by a buffer concentration of 1·5 mM. Thus, the stimulatory effect was independent of buffering capacity.

TABLE 1. THE EFFECT OF VARYING LEVELS OF CATIONIC AMINE BUFFERS ON AMINO ACID INCORPORATION

	Counts per minute*			
	Tris		TEA	
mM	pH 7·7	pH 8·1	pH 7·7	pH 8·1
5	1678	2602	—	—
12·5	2401	4460	2933	4558
25	—	5987	—	—
50	6462	6827	3750	5332
100	—	844	—	—
125	—	—	97	78

* Results expressed as counts per minute of ^{14}C-leucine incorporated into a hot TCA insoluble fraction after 30 min incubation at 30° of the reaction mixture, described in the Experimental section.

A suggestion as to the nature of the tris effect evolved from the observation that the wheat embryo system is particularly sensitive to monovalent cations. Thus, it appeared possible that the tris effect might be due to its cationic component. This was examined in the experiment shown in Fig. 1. In this case both the K^+ and tris (pH 8·1) concentrations were varied. It may be seen that as the K^+ concentration is increased, the tris optimum decreases. Thus, at 38 mM K^+, 50 mM tris is almost twice as active as 5 mM tris, while at 50 mM K^+ the situation is exactly reversed. On a more practical level the data show that for each level of tris used there is a different K^+ optimum, with inhibition on either side of this optimum. As the tris concentration is decreased there is an increasing K^+ concentration required for maximum incorporation. At the highest levels of tris (100 mM) the system is strongly inhibited.

These data, therefore, suggest that the tris buffer may function as a cationic component. In addition, there are other, more complex, aspects. Low K^+ levels (12·5 mM) and high tris levels (50 and 100 mM) stimulate relatively little incorporation, indicating that tris can only provide a limited part of the cationic component. Similar observations were made with triethanolamine buffer (results not presented).

The observation that amine buffers react as cations in a complex manner may be of interest to the general problem of the mechanism of cationic function in enzymatic reactions. For the moment, however, the more obvious conclusion is that the buffer component should be examined quantitatively and not assumed to be an inactive controller of pH, particularly in systems sensitive to monovalent ions.

EXPERIMENTAL

Preparation of the *in vitro* amino acid incorporating wheat embryo system has been described elsewhere.[5] 0·4 ml of reaction mixture contained: ^{14}C-leucine (0·125 μc and 0·474 mμM); TMV-RNA (10 μg); tRNA

(16 μg); washed ribosomes (250 μg RNA); ATP (1 mM); an ATP generating system (8 mM sodium creatine phosphate and 16 μg creatine phosphate kinase); GTP ($2{\cdot}5 \times 10^{-5}$ M); Cleland's reagent (0·224 mM); Mg^{2+} (4 mM); K^+ (37·5 mM); supernatant, S-100 (95 μg protein).

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