Subunit Interactions in Hybrids of Native, Carboxypeptidase-Treated and Citraconylated Rabbit Muscle Aldolase

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(Received 10 October 1973)

1. The kinetic properties of hybrids of native (or carboxypeptidase-treated) and citraconylated rabbit muscle aldolase are compared with those of equivalent mixtures of the parental enzymes. 2. In the hybrids, the native subunits function slightly less well than in the homotetramer, but the citraconylated subunits have enhanced activity. 3. Subunits of carboxypeptidase-treated aldolase behave essentially as expected in a hybrid environment, but the citraconylated subunits do not show the same enhancement of activity found in the hybrids of native and citraconylated enzyme. The apparent affinity for fructose 1,6-diphosphate of the citraconylated subunits in hybrids of carboxypeptidase-treated and citraconylated aldolase is increased. 4. These results are interpreted in terms of a substrate-induced conformational difference between native and carboxypeptidase-treated aldolase. 5. This conformational change can take place within a single native subunit in the hybrids and does not require a similar conformational change to occur simultaneously in the other three subunits.

Hybrids of structural variants of oligomeric enzymes have proved valuable in studies of subunit interactions, and a suitable variant for hybridization can sometimes be produced by chemical modification of a native enzyme. Gibbons & Perham (1974) have described the use of *N*-citraconylated derivatives of rabbit muscle aldolase (EC 4.1.2.13) in hybridization experiments with the native enzyme. In their experiments, the kinetic properties of the unfractionated mixture of hybrids and parental species produced by denaturing and annealing a mixture of native and citraconylated aldolase indicated that the activity of each type of subunit in the hybrid environment is affected by the presence of the other type. However, a study of the isolated hybrids is desirable in order to investigate these effects in detail. When unfractionated citraconylated aldolase was used, the hybrids with native enzyme were difficult to resolve from each other, and before hybrids could be isolated it seemed likely that it would be necessary to carry out a preliminary fractionation of the citraconylated aldolase by chromatography on DEAE-cellulose (cf. Meighen & Schachman, 1970).

Although no co-operative interactions of rabbit muscle aldolase with its substrates are known, Mehler & Cusic (1967) have suggested that on binding fructose 1,6-diphosphate the enzyme undergoes a conformational change which increases the activity to a value greatly above that with fructose 1-phosphate. Removal of the C-terminal tyrosine residue of rabbit muscle aldolase leaves the activity with fructose 1-phosphate almost unchanged but specifically decreases the activity with fructose 1,6-diphosphate to about the same value as that with

fructose 1-phosphate (Drechsler *et al.*, 1959). Thus carboxypeptidase digestion produces a derivative which on binding fructose 1,6-diphosphate probably does not undergo any kinetically significant conformational change.

The present paper describes the kinetic properties of molecular hybrids formed between native or carboxypeptidase-modified rabbit muscle aldolase and *N*-citraconylated derivatives of the native enzyme. The results are interpreted in terms of interactions between the subunits of such hybrid enzymes and throw some light on the structural and functional consequences of the limited digestion of aldolase with carboxypeptidase.

Materials and Methods

Hybridization of native, carboxypeptidase-treated and citraconylated aldolase

Hybrids of native and citraconylated aldolase were prepared by denaturation of suitable mixtures of parental forms in strong solutions of guanidine hydrochloride followed by removal of the denaturant by dialysis as described by Gibbons & Perham (1974). Essentially the same method was used to make hybrids of carboxypeptidase-treated aldolase and citraconylated aldolase. However, it is necessary to inactivate the carboxypeptidase A present in the carboxypeptidase-treated preparation before mixing it with native enzyme. This was achieved by pre-incubation of the carboxypeptidase-treated derivative in the denaturing solvent for 10 min. Carboxypeptidase A treated in this way was inactivated and failed

to recover any activity after removal of the denaturant, as judged by its inability to affect the kinetic properties of native aldolase.

Fractionation of hybrid and parental enzyme mixtures by DEAE-cellulose chromatography

Protein mixtures (up to 30 mg) dissolved in 50 mM-Tris - HCl - 2 mM-Tren* - 3 HCl - 2 mM-2-mercaptoethanol, pH 8.5 (at 20°C), were pumped at 80 ml/h on to a column (1 cm × 6 cm) of DEAE-cellulose equilibrated with the same buffer at room temperature (24°C). In these conditions, the native enzyme (and the carboxypeptidase-treated native enzyme) did not stick to the column and was collected as a 'break-through' fraction. Citraconylated aldolase with about 40% of its amino groups blocked (Gibbons & Perham, 1974) and hybrids of native and citraconylated enzyme were completely adsorbed to the DEAE-cellulose. After washing the column with several column volumes of buffer to remove all native enzyme, the adsorbed protein was eluted at room temperature with a linear gradient from 0 to 0.75 M-NaCl in 50 mM-Tris-HCl-2 mM-Tren-3 HCl-2 mM-2-mercaptoethanol, pH 8.5 (at 20°C), over a volume of 200 ml. Tren was included as a heavy-metal-chelating agent in preference to EDTA, which would stick very firmly to DEAE-cellulose and would not allow rapid equilibration. Since Tren, which is positively charged at pH 8.5, does not stick to DEAE groups, the same column could be reused many times. The column was eluted at 20 ml/h and 1.3 ml fractions were collected. Native aldolase and its derivatives were completely stable over the period required to complete the fractionation, and control samples of parental forms of the enzyme dissolved in the chromatography buffer were kept at room temperature for the duration of the fractionation procedure.

Other techniques

The citraconylation of aldolase and other techniques and materials used were exactly as described by Gibbons & Perham (1974).

In the range of 0-50% modification of amino groups in aldolase, citraconylation is restricted to amino groups and the extent of reaction is therefore given as the percentage blocking of amino groups (Gibbons & Perham, 1974). The native enzyme contains approx. 28 amino groups/subunit (Anderson *et al.*, 1969).

* Abbreviations: Tren, triaminotriethylamine; N, subunit of native rabbit muscle aldolase; K, subunit of carboxypeptidase-treated aldolase; C, subunit of citraconylated aldolase.

Results

Preparation and fractionation of citraconyl-aldolase

Rabbit muscle aldolase (320 mg) was treated with a 1.7 molar ratio of citraconic anhydride to protein amino groups to give a preparation with about 38% of the amino groups blocked and having 7% of the activity of the native enzyme in the standard assay with fructose 1,6-diphosphate (Gibbons & Perham, 1974). This preparation was subjected to chromatography on DEAE-cellulose to decrease its heterogeneity. Fig. 1 is the elution profile obtained.

A single asymmetric peak of protein emerged, the specific activity of which diminished gradually across the gradient. Single fractions near the peak (containing about 20 mg of protein) were used for the hybridization experiments that follow. Near the peak the extent of citraconylation, measured by the trinitrobenzenesulphonic acid assay for free amino groups (Gibbons & Perham, 1970, 1974), varied by about 1% per fraction (the limit of the assay). Individual fractions from the DEAE-cellulose column were not significantly less diffuse on cellulose acetate electrophoresis than unfractionated citraconylated aldolase. Nonetheless, by using this fractionated material it was subsequently possible to resolve the hybrids of native and citraconylated enzyme by chromatography on DEAE-cellulose. This improvement in resolution may best be attributed to the chromatographic fractionation of citraconylated aldolase according to the distribution of citraconyl substituents over the surface of the protein rather than to fractionation merely on the basis of the total number of substituents per molecule.

Evidence for a three-membered hybrid set formed between native and citraconylated aldolase

Various proportions of native aldolase and a chromatographic fraction of citraconylated aldolase (35% blocked) were mixed, then denatured and annealed as described by Gibbons & Perham (1974) and the product was subjected to cellulose acetate-strip electrophoresis (Plate 1). Three discrete electrophoretic intermediates were found in the denatured-annealed mixtures but not in control mixtures that had not been denatured. This is the number of hybrid species expected with parental species each composed of four identical subunits (Shaw, 1964). The relative amounts of the hybrid and parental forms are suggestive of random assortment of native and citraconylated subunits into tetramers during annealing, although no accurate study of this has been made. In this electrophoretic system the native enzyme had zero mobility and, relative to the citraconylated enzyme, the electrophoretic mobilities of the hybrids were 0.29 (N₃C₁), 0.55 (N₂C₂) and 0.79 (N₁C₃). Replacement of a native subunit by a citraconylated

EXPLANATION OF PLATE I

Cellulose acetate-strip electrophoresis of denatured-annealed mixtures of native and 35% citronylated aldolase at pH6.5
The method used was that described by Gibbons & Perham (1974). C: citronylated enzyme; N: native enzyme; 1,2,3: mixtures with C/(C+N) ratios of 0.71, 0.45 and 0.22 respectively. The dashed line marks the origin.

Fig. 1. DEAE-cellulose chromatography of 40% citraconylated aldolase

Conditions were as described in the Materials and Methods section for chromatography of hybrids except that a larger column (2cm×6cm) was used and the volume of the gradient was 500ml. A 330mg portion of enzyme was used. Elution was at 80ml·h⁻¹ and 4.5ml fractions were collected. Specific activity with fructose 1,6-diphosphate is expressed relative to that of the unfractionated material. —, E_{280} ; ●---●, specific activity.

subunit therefore increases the mobility of the tetrameric enzyme by about 0.25, as would be expected for equal increments of charge. However, the changes in mobility diminish slightly as the number of citraconylated subunits increases, perhaps because the frictional drag which is due to a citraconylated subunit is greater than that due to a native subunit. In support of this idea, it has been shown that the sedimentation coefficient of tetrameric rabbit muscle aldolase falls gradually as the extent of citraconylation is increased (Gibbons & Perham, 1970) and that the structure of citraconylated aldolase is distorted in comparison with the native enzyme (Gibbons & Perham, 1974). All these effects could be due to a slight swelling of the enzyme as a result of citraconylation.

Purification of native and citraconylated aldolase hybrids

The hybrids of native and citraconylated aldolase were separated from each other and from the parental species by ion-exchange chromatography on DEAE-cellulose. The compositions of the mixtures of hybrids and parental forms produced by denaturation and annealing of various mixtures of parental forms were such that useful amounts of all five species could not be purified from a single mixture. The N_3C_1 and N_2C_2 hybrids were therefore purified from a

mixture produced by hybridizing 71% of the native enzyme and 29% of (37%) citraconylated aldolase (Fig. 2), whereas the N_2C_2 and N_1C_3 hybrids were isolated from a mixture produced by hybridizing 26% of the native enzyme and 74% of (39%) citraconylated aldolase. The peaks of protein eluted from DEAE-cellulose were always sharper at the leading edge of the peak, with the result that the second of two adjacent peaks was usually contaminated by the first, as may be seen from the specific-activity profile of the second peak in Fig. 2. Also, the separation of adjacent peaks declined as the number of citraconylated subunits in the species increased. Thus it was necessary to rechromatograph some of the peaks on the same column to obtain completely pure preparations.

Criteria of purity and identity of the hybrids

Two criteria were used to check the purity of the hybrids, (1) a constant specific activity across a peak after chromatography on DEAE-cellulose and (2) analytical electrophoresis on cellulose acetate strips. All the kinetic measurements were made with completely pure preparations. The identities of the hybrids were obvious from their electrophoretic and chromatographic properties, in the light of similar work by Penhoet *et al.* (1967) and Meighen & Schachman (1970). To confirm these assumed

Fig. 2. Elution profile on DEAE-cellulose chromatography of a mixture of 71% native aldolase and 29% of (37%) citraconylated aldolase after denaturation and annealing

The column was developed as described in the Materials and Methods section. Fructose 1,6-diphosphate aldolase activity was measured in the standard assay. —, E_{280} ; ●—●, specific activity relative to that of the native enzyme.

subunit compositions, the contents of native subunits in two of the hybrids were estimated by measuring the number of free amino groups per molecule of tetramer by using the trinitrobenzenesulphonic acid assay (Gibbons & Perham, 1970, 1974). After correction for reaction of thiol groups with trinitrobenzenesulphonic acid as explained by Gibbons & Perham (1970), the ratios of native to total subunits in the N_3C_1 and N_2C_2 hybrids were found to be 0.76 and 0.43 respectively, which is in reasonable agreement with the expected values.

Kinetic properties of the hybrids of native and citraconylated enzyme

The kinetics of both native and citraconylated aldolase are unchanged after denaturation and annealing (Gibbons & Perham, 1974). The kinetic behaviour of the hybrids of native and citraconylated aldolase should therefore be the appropriate linear combination of the parental activities, if the subunits express their activity independently. This assumption was supported by the results of control experiments in which kinetics of simple mixtures of the parental forms were examined. These kinetics were in excellent agreement with those predicted from a knowledge of the kinetics of the parental forms.

The kinetics expected for non-interacting subunits and the experimentally determined kinetics of the native-citraconylated enzyme hybrids are compared in Figs. 3 and 4. The two Figures represent different experiments, but the parental kinetics in the

two cases are so alike that the results are comparable. The specific activities of the enzymes were calculated by using an extinction coefficient at 280 nm of $E_{1\text{cm}}^{1\text{mgml}^{-1}} = 0.96$, since Gibbons & Perham (1974) have observed that citraconylation of up to 50% of the protein amino groups causes no change in the extinction at 280 nm. All the hybrids showed significant divergence from the kinetics predicted for them. At low substrate concentrations their activity was low, but the rate of change of activity with increasing fructose 1,6-diphosphate concentration was always greater than expected and at high substrate concentration the activity of the hybrids exceeded the predicted values.

A separate set of measurements was used to determine the apparent K_m of the native subunits for fructose 1,6-diphosphate in the N_3C_1 and N_2C_2 hybrids. The highest substrate concentration used for these determinations was $33\text{ }\mu\text{M}$. At this concentration of substrate, the citraconylated subunits would not be expected to show any significant activity, since the K_m of the citraconylated enzyme for fructose 1,6-diphosphate is very high ($>400\text{ }\mu\text{M}$) compared with that of the native enzyme (Gibbons & Perham, 1974). Indeed, the double-reciprocal plots obtained were all straight and gave K_m values of $1.4\text{ }\mu\text{M}$ (native enzyme), $1.3\text{ }\mu\text{M}$ (N_3C_1 hybrid), and $1.8\text{ }\mu\text{M}$ (N_2C_2 hybrid), which are not significantly different from each other. To observe the activity of the citraconylated subunits in a hybrid environment, the expected activity of the appropriate number of native subunits (calculated from that of the native

Fig. 3. Kinetics of fructose 1,6-diphosphate aldolase activity of two hybrids formed between native aldolase and 37%-citraconylated aldolase, N_3C_1 (●) and N_2C_2 (○)

The symbols $\square$ (N_4) and $\blacksquare$ (C_4) represent the kinetics of the parental enzymes. The predicted kinetics for the N_3C_1 and N_2C_2 hybrids (continuous lines) were calculated from the kinetics of the parental species assuming the subunits to be independent.

Fig. 4. Kinetics of fructose 1,6-diphosphate aldolase activity of the N_2C_2 (○) and N_1C_3 (●) hybrids formed between native aldolase and 39%-citraconylated aldolase

The symbols $\square$ (N_4) and $\blacksquare$ (C_4) represent the kinetics of the parental enzymes. The predicted kinetics for the N_2C_2 and N_1C_3 hybrids (continuous lines) were calculated from the kinetics of the parental species assuming the subunits to be independent.

N_4 tetramer) was then subtracted from the observed hybrid activity to produce plots such as that shown in Fig. 5. As expected, at low substrate concentrations (1–100 μM -fructose 1,6-diphosphate) there is a deficit of activity, presumably corresponding to a lowered V_{max} activity of the native subunits (cf. Figs. 3 and 4). As the fructose 1,6-diphosphate concentration increases above 10 μM , the shape of the specific-activity profile for the citraconylated subunits in the hybrids resembles that of the citraconylated enzyme,

Fig. 5. Specific activity of the citraconylated subunits of the N_2C_2 hybrid (continuous line) calculated from the data of Fig. 4

The activity of citraconylated enzyme (dashed line) is included for comparison.

although the citraconylated subunits show elevated activities at high substrate concentrations. Assuming that the increase in hybrid activity over the range 10 μM –23 mM-fructose 1,6-diphosphate is entirely due to the citraconylated subunits, it is possible to estimate the relative activity of a citraconylated subunit in a hybrid environment and in the citraconylated tetramer. The native activity was measured at a substrate concentration of 13 μM -fructose 1,6-diphosphate (10 times the K_m for the native enzyme) and the activity of the citraconylated subunits at the highest concentration of fructose 1,6-diphosphate used (23 mM) which almost saturates the citraconylated enzyme. Estimates for the V_{max} values of the native and citraconylated subunits in the various hybrids obtained in this way are given in Table 1.

Table 1. Estimated V_{max} values of the native and citraconylated subunits in aldolase hybrids

Values are all normalized to the same protein concentrations.

Hybrid	Fructose 1,6-diphosphate	
	Native subunits (% of V_{max} of native enzyme)	Citraconylated subunits (% of V_{max} of citraconylated enzyme)
N_3C_1	97	200
$N_2C_2^*$	86	175
N_1C_3	81	130

* Average of data from Figs. 3 and 4.

Fig. 6. Fructose 1,6-diphosphate aldolase kinetics of hybrids of carboxypeptidase-treated aldolase and 36%-citronylated aldolase

The points (●) are experimental values and the continuous lines were calculated from the kinetics of the parental enzymes (not shown here), assuming the subunits to be independent.

Effect of removal of the C-terminal tyrosine residue on subunit interactions in aldolase

A carboxypeptidase-treated derivative of rabbit muscle aldolase prepared as described by Gibbons & Perham (1974) showed 7% of the fructose 1,6-diphosphate aldolase activity of the native enzyme in the standard assay. Like the native enzyme, the carboxypeptidase-treated enzyme was found to be reversibly denatured in 4M-guanidine hydrochloride with complete recovery of its kinetic properties. Polyacrylamide-gel electrophoresis at pH9 in the presence of 9.5M-urea (Davis, 1964) demonstrated that the carboxypeptidase-treated enzyme had exactly the same pattern of two closely spaced bands as the native enzyme (Chan *et al.*, 1967; Anderson *et al.*, 1969). Thus the carboxypeptidase-treated enzyme was expected to behave like the native enzyme on DEAE-cellulose chromatography at pH8.5.

Hybrids of carboxypeptidase-treated aldolase and 36%-citronylated aldolase were prepared and purified in the same way as the hybrids of native and citronylated enzyme. Carboxypeptidase digestion does not affect the K_m of aldolase for fructose 1,6-diphosphate and the kinetics of the carboxypeptidase-treated derivative resemble those of the native enzyme in all but the value of V_{max} . (Mehler & Cusic, 1967). A slight inhibition is observed with high concentrations of fructose 1,6-diphosphate with both native and carboxypeptidase-treated enzymes. The measured kinetic properties of the K_3C_1 and K_2C_2 hybrids are shown in Fig. 6 in comparison with the kinetics expected for the appropriate linear

sum of independent subunits. Between 1 and 10 μ M-fructose 1,6-diphosphate the hybrids have slightly greater activity than expected, although the effect is very small. As the substrate concentration increases, however, the activity of the hybrids rises faster than expected, but at the highest fructose 1,6-diphosphate concentrations again approaches the predicted value. The divergence of observed and predicted kinetics at intermediate substrate concentrations is greater for the K_3C_1 hybrid than for the K_2C_2 hybrid. If the citronylated subunits behaved as in the hybrids of native and citronylated enzyme, the observed and expected activities would continue to diverge from each other as the fructose 1,6-diphosphate concentration increased. However, instead of the change in the V_{max} of the citronylated subunits found with the native-citronylated enzyme hybrids, for the hybrids of carboxypeptidase-treated enzyme and citronylated enzyme there is a change in the apparent affinity for fructose 1,6-diphosphate of the citronylated subunits. The concentration of fructose 1,6-diphosphate required to cause the enzymic activity of a hybrid to rise from the plateau value observed between 10 and 100 μ M-fructose 1,6-diphosphate to half the value at the highest substrate concentration used (23mM, which virtually saturates the citronylated enzyme) should be a measure of the affinity of the citronylated subunits for substrate, assuming that the affinity of the carboxypeptidase-treated subunits remains the same. For the citronylated enzyme this concentration is 3.2mM, but for the K_3C_1 hybrid it is decreased by a factor of three and for the K_2C_2 hybrid by a factor of 1.3. It is difficult to tell whether similar changes of affinity of the citronylated subunit obtain for the hybrids of native and citronylated enzyme, since the effect would be largely masked by the changes in V_{max} values which undoubtedly occur.

Discussion

Interactions between the subunits of an oligomeric protein can occur in a number of ways. Binding of a ligand to a specific site in one subunit may stabilize (Monod *et al.*, 1965) or induce (Conway & Koshland, 1968) a conformational change in the protein that results in the alteration of the properties of specific sites on different subunits. Moreover, the activity of a subunit will depend in part on the correct folding of the peptide chain in adjacent subunits in an oligomer. This is thought to take place when the wrongly folded gene products of two mutant alleles complement and combine to give a functional aggregate (Crick & Orgel, 1964). Interactions between the subunits of a hybrid molecule might be expected to be of the latter type. The influence of binding other subunits in increasing the activity of a 'matrix-bound' isolated monomer has been demonstrated for aldolase

by Chan (1970) and for glycogen phosphorylase by Feldman *et al.* (1972).

In hybrids of native and citraconylated rabbit muscle aldolase, the subunits do not express the activity they show in the corresponding homotetramers. The native subunits in hybrids of native and citraconylated aldolase perform slightly less well than in the native enzyme. However, the subunits of carboxypeptidase-treated aldolase are at least as active in hybrid combination with citraconylated subunits as in the homotetramer. Conversely, citraconylated subunits are altered in their kinetic behaviour when hybridized with subunits of native or carboxypeptidase-treated aldolase. These results complement and extend those of Meighen & Schachman (1970) and Brenner-Holzach & Leuthardt (1972), who used inactive derivatives of aldolase and showed that the specific activity of the native subunits in hybrids was close to normal.

Citraconylated subunits in native-citraconylated enzyme hybrids recover almost all the activity of a native subunit at high substrate concentration. This conclusion supports the idea that the catalytically essential amino groups of aldolase are not readily available to citraconic anhydride, as suggested by Gibbons & Perham (1974) on the basis of a detailed study of the kinetics of citraconylated derivatives of aldolase. The effect of native subunits in increasing the $V_{\max}$ value of citraconylated subunits in a hybrid of native and citraconylated aldolase increases with the number of native subunits in the tetramer (Table 1). This is in accord with the suggestion (Gibbons & Perham, 1974) that contact of the citraconylated subunits with native subunits in a hybrid tetramer decreases the distortion of the citraconylated subunits so that they resemble native subunits more closely [cf. the theory of interallelic complementation referred to above (Crick & Orgel, 1964)].

If the only difference between native and carboxypeptidase-treated rabbit muscle aldolase were in the value of $V_{\max}$, one would expect that the activity of the citraconylated subunits in hybrids of carboxypeptidase-treated enzyme and citraconylated enzyme would resemble that in hybrids of native and citraconylated enzyme. However, at high substrate concentration the change in the activity of citraconylated subunits in hybrids of carboxypeptidase-treated and citraconylated enzyme is small compared with that observed for hybrids of native and citraconylated enzyme. The increase in affinity for fructose 1,6-diphosphate of the citraconylated subunits in hybrids of carboxypeptidase-treated and citraconylated enzyme presumably can also be accounted for in terms of 'complementation', the distortion of the active sites of the citraconylated subunits being lessened in the hybrid environment. Although it is not demonstrated by these results, the affinity for fructose 1,6-diphosphate of citra-

conylated subunits in hybrids of native and citraconylated aldolase may be similarly affected.

To explain the demonstrated differences in the enhancement of activity of citraconylated subunits seen in the two types of hybrid, there must be a conformational difference between native and carboxypeptidase-treated subunits that alters the inter-subunit binding, at least in the presence of saturating concentrations of substrate. According to Mehler & Cusic (1967), there is a conformational change in rabbit muscle aldolase on binding fructose 1,6-diphosphate, which converts the enzyme from a conformation like that of carboxypeptidase-treated enzyme into a more active species. The present results support that idea and suggest further that the conformational difference in one subunit of aldolase can influence the activity of other subunits. However, since the native subunits in hybrids of native and citraconylated enzyme possess essentially full activity at substrate concentrations well below the K_m of the citraconylated subunits, it is clear that the main effect of binding fructose 1,6-diphosphate can be expressed within one subunit and does not require binding of substrate to all four subunits. In other words, a native subunit can express its full catalytic activity on binding fructose 1,6-diphosphate, regardless of the lack of an identical substrate-induced conformational change in the adjacent subunits in an aldolase tetramer. Only when the conformation of some of the subunits is distorted, as in the hybrids of native and citraconylated enzyme, is there an effect on the activity of neighbouring subunits after the binding of fructose 1,6-diphosphate to a native subunit.

I thank Dr. R. N. Perham and Dr. H. B. F. Dixon for advice. This work was supported by a grant from the Science Research Council (B/SR/9480).

References

- Anderson, P. J., Gibbons, I. & Perham, R. N. (1969) *Eur. J. Biochem.* **11**, 503-509
- Brenner-Holzach, O. & Leuthardt, F. (1972) *Eur. J. Biochem.* **31**, 423-426
- Chan, W. W.-C. (1970) *Biochem. Biophys. Res. Commun.* **41**, 1198-1204
- Chan, W., Morse, D. E. & Horecker, B. L. (1967) *Proc. Nat. Acad. Sci. U.S.* **57**, 1013-1020.
- Conway, A. & Koshland, D. E., Jr. (1968) *Biochemistry* **7**, 4011-4023
- Crick, F. H. C. & Orgel, L. E. (1964) *J. Mol. Biol.* **8**, 161-165
- Davis, B. J. (1964) *Ann. N.Y. Acad. Sci.* **121**, 404-427
- Drechsler, E. R., Boyer, P. D. & Kowalsky, A. G. (1959) *J. Biol. Chem.* **234**, 2627-2634
- Feldman, K., Zeisel, H. & Helmreich, E. (1972) *Proc. Nat. Acad. Sci. U.S.* **69**, 2278-2282
- Gibbons, I. & Perham, R. N. (1970) *Biochem. J.* **116**, 843-849

- Gibbons, I. & Perham, R. N. (1974) *Biochem. J.* **139**, 331–342
- Mehler, A. H. & Cusic, M. E., Jr. (1967) *Science* **155**, 1101–1103
- Meighen, E. A. & Schachman, H. K. (1970) *Biochemistry* **9**, 1163–1176
- Monod, J., Wyman, J. & Changeux, J.-P. (1965) *J. Mol. Biol.* **12**, 88–118
- Penhoet, E., Kochman, M., Valentine, R. S. & Rutter, W. J. (1967) *Biochemistry* **9**, 2940–2949
- Shaw, C. R. (1964) *Brookhaven Symp. Biol.* **17**, 117–129