cytochrome $a^{2+}a_3^{3+}$ and cytochrome $a^{3+}a_3^{2+}$. Interactions also occur in the reduction of ferrihaemoglobin (Rossi-Fanelli *et al.*, 1964), but in the opposite direction ($n > 1$). Moreover, haemoglobin interactions involve the penta-co-ordinate ferrous form, whereas cytochrome aa_3 interactions seem to involve the ferric species.

In an alternative model of cytochrome aa_3 interactions (Wilson *et al.*, 1972), the redox titration is thought to distinguish cytochromes a and a_3 , forming the unique intermediate species cytochrome $a^{3+}a_3^{2+}$. This model requires strong interactions involving ferrocycytochrome a_3 , and demands interaction-dependent changes in assigned extinction coefficients as well as in the redox potentials. Choice between the two models is presently uncertain, but they agree in suggesting that the behaviour of cytochrome oxidase cannot be interpreted in terms of fully independent cytochromes a and a_3 but must involve co-ordinated activity of the unit now designated as cytochrome aa_3 (Muijsers *et al.*, 1968).

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The Mechanism of Phosphoglucomutase from *Micrococcus lysodeikticus*

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Unlike rabbit muscle phosphoglucomutase, which has a phosphoenzyme mechanism (Ray & Roscelli, 1964; Britton & Clarke, 1968), the mutase from *Micrococcus lysodeikticus* yielded converging plots when the reciprocal of the initial velocity was plotted

against the reciprocal of the glucose 1-phosphate concentration at fixed concentrations of glucose 1,6-diphosphate and vice versa, and it could not be isolated as a phosphoenzyme (Hanabusa *et al.*, 1966). These findings were considered to exclude a phosphoenzyme mechanism and to indicate a sequential addition of cofactor and substrate with an intermolecular transfer of phosphate from glucose 1,6-diphosphate to the substrates. The interpretation of these experiments, however, is equivocal, and the enzyme has therefore been investigated by the flux approach (Britton & Clarke, 1968).

Tests for induced transport at intermediate substrate concentrations (1 mM) showed co-transport with ^{32}P -labelled substrates but no induced transport with ^{14}C -labelled substrates, and at chemical equilibrium ^{14}C -labelled substrates exchanged twice as rapidly as ^{32}P -labelled substrates. These results excluded an intramolecular transfer of phosphate, were quantitatively in agreement with a phosphoenzyme type of mechanism and were only compatible with an intermolecular transfer of phosphate from glucose 1,6-diphosphate if there were a very slow isomerization of the free enzyme or enzyme-glucose 1,6-diphosphate complex. Identical results at low concentrations of substrates (158 μM) excluded the last possibility, which could only have produced the lack of co-transport seen with ^{14}C -labelled substrates if the K_m for glucose 1-phosphate had been over 20 times lower than the measured value ($1.3 \times 10^{-4} \text{ M}$). With ^{14}C -labelled substrates at high concentrations (26.3 mM) there was a lack of induced transport, which indicated that any isomerization of the phosphoenzyme must have a rate constant in excess of about 10^6 s^{-1} .

These results show that the phosphoglucomutases from rabbit muscle and *M. lysodeikticus* share a common phosphoenzyme mechanism. The very rapid isomerization of the phosphoenzyme suggests that both enzymes are related mechanistically and perhaps evolutionarily to glucose 1,6-diphosphatase.

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