Evidence of the Presence of 1-Phosphohistidine as the Main Phosphorylated Component at the Active Site of Bovine Liver Nucleoside Diphosphate Kinase

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Nucleoside diphosphate kinase (ATP: nucleoside diphosphate phosphotransferase, EC 2.7.4.6) obtained from various sources has been shown to be phosphorylated during incubation with adenosine triphosphate. The activity of this enzyme was used to assay the presence of phosphate. From an alkaline hydrolysate of 32P-labelled NDP kinase, Norman et al. isolated 32P-phosphohistidine. The isolation of this compound indicates that the enzyme is phosphorylated at the active site. The isolated 32P-phosphohistidine was found to be phosphorylated at the active site of the enzyme.

The isolation of 32P-phosphohistidine from the alkaline hydrolysate of the enzyme further supports the hypothesis that the enzyme is phosphorylated at the active site. This finding is significant because it provides evidence for the involvement of a phosphorylation event in the catalytic mechanism of the enzyme. The isolation of 32P-phosphohistidine from the alkaline hydrolysate of the enzyme further supports the hypothesis that the enzyme is phosphorylated at the active site. This finding is significant because it provides evidence for the involvement of a phosphorylation event in the catalytic mechanism of the enzyme.
represented 45% of the radioactivity of the original hydrolysate, which in addition contained a considerable amount of $^{32}$P-orthophosphate. Consequently, the major part of the $^{32}$P-phosphate of the phosphorylated enzyme seems to exist, at least after alkaline inactivation, in the form of 1-$^{32}$P-phosphohistidine.

In order to investigate whether the alkaline inactivation was a prerequisite for obtaining the three phosphoamino acids, incubation of bovine-liver NDP kinase was instead interrupted by 0.1 M HCl. After isolation of $^{32}$P-labelled enzyme and hydrolysis in alkali, the sample was investigated with respect to acid-labile phosphoamino acids. The radioactive components were identified $^5$ as N-$\varepsilon$-$^{32}$P-phospho-lysine, 1-$^{32}$P-phosphohistidine, and 3-$^{32}$P-phosphohistidine, which accounted for 0.8, 1.6, and 0.9%, respectively, of the total radioactivity of the hydrolysate. These values are in good agreement with those obtained after inactivation with alkali $^5$ (0.9, 2.5, and 0.7%, respectively). The result suggests that the alkaline inactivation is not essential for binding of the phosphoryl groups to the enzyme.

The question whether the $^{32}$P-phosphate is present as N-phosphoamino acids in the non-inactivated phosphorylated enzyme as well is important, although difficult to answer unequivocally. To get some information on this subject a pH-stability test on the non-inactivated phosphorylated enzyme was performed. 0.060 mg of bovine-liver NDP kinase was mixed with $10^{-4}$ M ATP$^{32}$P in a final volume of 4 ml. The sample was chromatographed on a column (1.3 x 38 cm) of Sephadex G-50, which was eluted with 0.01 M triethanolamine-acetic acid buffer, pH 7.4. The enzyme fraction was found to contain about 20 nmole of $^{32}$P-phosphate per mg of enzyme protein.

Human albumin (AB Kabi, Stockholm, Sweden) was added as carrier protein to the phosphorylated enzyme to a final concentration of 0.6 mg/ml. 0.5 ml of the mixture was added to 1 ml of each of the following solutions: 0.05 M and 0.5 M H$_2$SO$_4$, 1 M acetic acid, 0.1 M acetic buffer, pH 4.0 and 5.0, 0.1 M triethanolamine-acetic acid buffer, pH 7.2 and 8.7, 0.1 M NaHCO$_3$—Na$_2$CO$_3$ buffer, pH 10.3, and 0.05 M NaOH. Incubation was performed for 30 min at 40°C, and was interrupted by the addition of alkali as previously described. However, unlabelled orthophosphate was added instead of unlabelled ATP in order to dilute any $^{32}$P-orthophosphate adsorbed to the enzyme. Phosphorylated enzyme was separated from $^{32}$P-orthophosphate by chromatography on Sephadex G-50 (Ref. 4). The amount was determined and expressed in nmole of $^{32}$P-phosphate per mg of enzyme.

As shown in Fig. 1, the phosphoryl linkages were labile to acid and stable to alkali. Such stability is compatible with that of nitrogen-bound phosphoryl groups. $^1$ $^5$ $^9$ There was, however, no support for acyl phosphate or thiophosphate linkages, since the pH profile of both acetyl phosphate $^1$ and butyl thiophosphate $^1$ differs from that shown in Fig. 1.

The data in the present work thus suggest that the main part of the $^{32}$P-phosphate of the $^{32}$P-labelled bovine-liver NDP kinase is bound as 1-$^{32}$P-phosphohistidine.

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Fig. 1. Stability of $^{32}$P-labelled NDP kinase at different pH values. For details, see the text.


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Atranorin is a very common lichen acid, but to our knowledge it has not previously been found in lichens producing vulpinic acid type substances.

The possibility existed that *L. vulpina* growing in North America might be different in acid content from *L. vulpina* growing in Scandinavia. To test this possibility a sample of the lichen was obtained from Höljes, Sweden. Atranorin was found as in the North American sample. Thus, although the two types of *L. vulpina* differ remarkably in both morphology and ecology, they agree as far as the dominating lichen acids are concerned.

Experimental. Air dried and ground *L. vulpina* (64 g) collected under *Abies magnifica* Murr. was extracted with ether for 24 h in a Soxhlet extractor. On thin layer chromatograms on silica gel in benzene-chloroform 1:1 and anisaldehyde as spraying agent several spots were obtained. Two of them were identified as due to the presence of vulpinic acid and of atranorin.

The ether solution was concentrated twice to deposit a mixture of coloured and colourless crystals. The coloured crystals were removed by hand, whilst the colourless crystals remained. The former were shown to be identical with vulpinic acid by comparison with an authentic sample. The latter were crystallised from acetone, m.p. 194–195° (108 mg). The material gave a negative Beilstein test, and there was no depression of m.p. on admixture with authentic atranorin. Their IR spectra in KBr, obtained with a Perkin-Elmer Model 21 spectrometer, were essentially identical.

A sample of *L. vulpina* from Höljes, Sweden, (36 g) was treated as above, except that vulpinic acid was removed by treatment with chloroform in the cold. Colourless crystals (11 mg) remained on the filter, m.p. 193–194° after one crystallisation from acetone. There was no depression on admixture with authentic atranorin and the IR spectra in KBr were essentially identical.

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