groups being located on opposite sides of the plane through the three middle atoms, as shown in Fig. 1. The dihedral angles, SXS/XSS (X=S, Se, or Te), are 82° and 84° in I, 83° and 85° in II, and 78° and 89° in III. The middle sulphur-sulphur bonds, between two divalent atoms, are 2.021 ± 0.007 Å and 2.036 ± 0.007 Å in I, the selenium-sulphur bonds are 2.153 ± 0.004 Å and 2.181 ± 0.003 Å in II, and the tellurium-sulphur bonds are 2.364 ± 0.009 Å and 2.370 ± 0.007 Å in III. The weighted mean of the six terminal sulphur-sulphur bonds, between a divalent and a sulphonate sulphur atom, is 2.118 Å; the calculated standard deviations of these bond lengths are 0.006 Å in I, 0.004 Å in II, and 0.009 − 0.011 Å in III.

Further details of the structures will be published later.

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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-^{32}P (Refs. 1−4). From an alkaline hydrolysate of ^{32}P-labelled NDP kinase * from Jerusalem artichoke, Norman *et al.*[2] isolated 3-^{32}P-phosphohistidine. It was recently demonstrated in this laboratory that alkaline hydrolysates of ^{32}P-labelled bovine-liver and erythrocytic NDP kinase contained not only 3-^{32}P-phosphohistidine but also 1-^{32}P-phosphohistidine and N-ε-^{32}P-phospholysine.[4,5] The main part of the covalently bound ^{32}P-phosphate of the hydrolysates was, however, recovered as two unidentified fractions denoted as X and Y.[6] The type of linkage of ^{32}P-phosphate in Fraction X has been investigated in the present work.

The reason why three different phosphoamino acids were obtained from a single phosphorylenzyme is not known. In order to rule out that one or more of them is formed during alkaline inactivation, an experiment with acid inactivation was performed. Finally the pH-dependence of the stability of the phosphoryl linkage of the non-inactivated phosphorylated enzyme was examined.

In order to investigate the phosphoryl linkage of Fraction X, 2 mg of purified bovine-liver NDP kinase were incubated with AT^{32}P as previously described.[5] From the alkaline hydrolysate of the ^{32}P-labelled enzyme, Fraction X was prepared by electrophoresis at pH 8.25 (Ref. 6), and hydrolyzed in 3 M KOH for 3 h at 100°C. When the hydrolysate was chromatographed on Dowex 1 together with synthetic 1-phosphohistidine, 3-phosphohistidine, and N-ε-phospholysine,[5] a large ^{32}P-labelled component was eluted together with 1-phosphohistidine. Fractions, corresponding to each of the three reference phosphoamino acids, were pooled, and rechromatographed on separate columns of Dowex 1 under identical conditions. The identity of the main labelled component with 1-^{32}P-phosphohistidine was finally established by paper electrophoresis and paper chromatography in altogether seven systems, as previously described.[5,7]

Of the total radioactivity of the hydrolysate of Fraction X, 9 % was obtained as 1-^{32}P-phosphohistidine, 0.3 % as 3-^{32}P-phosphohistidine, and no detectable amounts (less than 0.05 %) as N-ε-^{32}P-phospholysine. The yield of 1-^{32}P-phosphohistidine suggests that the ^{32}P-phosphate of Fraction X is bound as 1-^{32}P-phosphohistidine. The small amounts of 3-^{32}P-phosphohistidine isolated may represent transformed 1-^{32}P-phosphohistidine.[8]

On further alkaline hydrolysis Fraction X was found to give rise to Fraction Y, which is in agreement with previous work on rat-liver cell sap.[6] Thus, it may be suggested that the ^{32}P-phosphate of Fraction Y is also bound as 1-^{32}P-phosphohistidine. The two fractions

* Abbreviation: NDP kinase, nucleoside diphosphate kinase.

represented 45 % of the radioactivity of the original hydrolysate, which in addition contained a considerable amount of ^{32}P-orthophosphate. Consequently, the main 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.[5] The radioactive components were identified [5] as N-ε-^{32}P-phospholysine, 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^{-5} M AT^{32}P in a final volume of 4 ml. The sample was chromatographed on a column (1.3×38 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 nmoles 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 0.5 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_2SO_4, 1 M acetic acid, 0.1 M acetate buffer, pH 4.0 and 5.0, 0.1 M triethanolamine-acetic acid buffer, pH 7.2 and 8.7, 0.1 M $NaHCO_3-Na_2CO_3$ 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.[4] 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 nmoles of ^{32}P-phosphate per mg of enzyme.

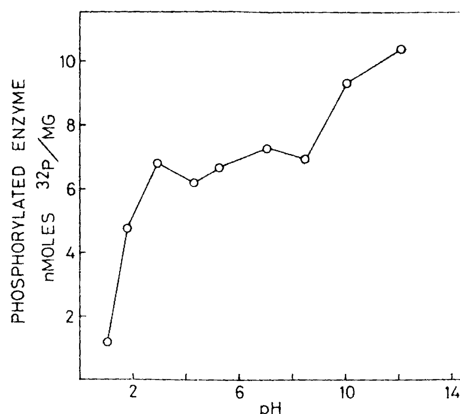

Fig. 1. Stability of ^{32}P-labelled NDP kinase at different pH values. For details, see the text.

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.[6,8,9] There was, however, no support for acyl phosphate or thiophosphate linkages, since the pH profile of both acetyl phosphate [10] and butyl thiophosphate [11] 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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The Occurrence of Atranorin in *Letharia vulpina* (L.) Vain.

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At an altitude of 6500–7500 ft the dense and umbrageous forests of Yosemite National Park, California, USA, are decorated by 1/2–1 ft long tails of a golden coloured lichen. During an accidental visit to the park one of us (N.A.S.) collected some large specimens from the surroundings of Glacier Point and Mariposa Grove, where this lichen is very abundant. Most of this material was subjected to a chemical examination, whilst a small specimen was sent to Professor Eilif Dahl for determination. Dr. Dahl kindly informed us that the lichen was *Letharia vulpina*, a lichen which is not unknown in Southern Norway, although it is usually of a much smaller size. The Scandinavian *L. vulpina* occurs as 1–2 inch asymmetrical tufts on wooden roofs, fences and the like. As far as we know it does not occur as free-hanging, long tails under shading twigs in the way typical of some Scandinavian *Alectoria* species. The distribution of *L. vulpina* in Scandinavia is restricted to a small area with a pronounced continental climate, and its local occurrence to very open places.

In agreement with literature [1] vulpinic acid was found as the major constituent, but in addition a small amount of colourless crystals was seen. Eventually they were identified with atranorin. For details, see Experimental.

Atranorin is a very common lichen acid,[1] 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 [2] 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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